New Marine Cembrane-type Diterpenoids from the Okinawan Soft Coral *Clavularia koellikeri*

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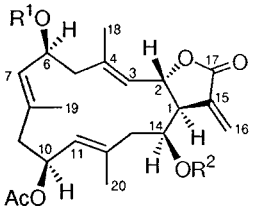
Received June 19, 2000

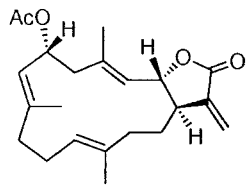
Seven new marine diterpenoids having a cembrane skeleton were isolated from the Okinawan soft coral *Clavularia koellikeri*. Their structures were determined based on the results of spectroscopic analysis and chemical conversions. Compound **1** showed cytotoxic activity against human colorectal adenocarcinoma cells (DLD-1, IC_{50} 4.2 μg/mL) and strong growth inhibition against human T lymphocytic leukemia cells (MOLT-4, IC_{50} 0.9 μg/mL).

Soft corals of the genus *Clavularia*, inhabiting the Okinawan coral reefs, are known to contain secondary metabolites with unique structures and remarkable biological activities. During the course of our investigation on biologically active substances from *Clavularia* spp.,[1] seven new cembrane-type diterpenoids (**1**−**7**) having an α-methylene γ-lactone moiety were isolated from *Clavularia koellikeri* (family Clavularidae). These compounds were found to be congeners of the kericembrenolides isolated previously from *C. koellikeri*.[2] This paper describes structure elucidations of diterpenoids **1**−**7** through spectroscopic analysis and chemical conversions.

Specimens of *C. koellikeri* collected on the coral reef of Ishigaki Island (Okinawa Prefecture, Japan) were immersed in MeOH. The MeOH extract was successively partitioned between EtOAc and H_2O to afford an EtOAc-soluble portion, and then the aqueous layer was extracted with *n*-BuOH to afford *n*-BuOH- and H_2O-soluble portions. The EtOAc-soluble portion was chromatographed on a Si gel column, eluting in turn with hexanes, hexanes−EtOAc, EtOAc, and MeOH, to afford eight fractions. Compound **1** (0.0015% yield) from the fifth and sixth fractions; compounds **2** (0.0004%), **3** (0.0002%), **5** (0.0004%), **6** (0.0002%), and **7** (0.0003%) from the seventh fraction, and compound **4** (0.0013%) from the fourth fraction were isolated by repeated purification using flash column chromatography, medium-pressure liquid chromatography (MPLC), and HPLC. Known diterpenoids (kericembrenolides,[2] stolonidiol,[3] and neodolabellenol[2,4]) were also isolated and identified.



1 : R^1 = Ac, R^2 = Ac
2 : R^1 = Ac, R^2 = H
3 : R^1 = H, R^2 = Ac



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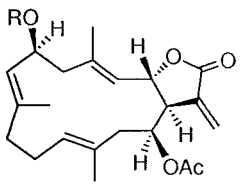
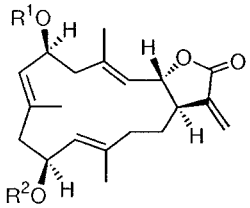


5 : R = H
15 : R = Ac



6 : R^1 = Ac, R^2 = H
7 : R^1 = H, R^2 = Ac

Results and Discussion

The molecular formula of compound **1** was found to be $C_{26}H_{34}O_8$ by HREIMS. Signals for all 26 carbons appeared in the ^{13}C NMR spectrum of **1** (Table 1). The DEPT spectrum exhibited signals for six methyls, three sp^3 methylenes, five sp^3 methines, one sp^2 methylene, three sp^2 methines, and eight sp^2 quaternary carbons. The IR spectrum of **1** showed absorptions due to the functionalities of an acetate ester (1731, 1237 cm^{-1}) and an α-methylene γ-lactone (1763, 1665 cm^{-1}). The presence of the α-methylene γ-lactone system in **1** was also demonstrated by UV absorption at 208 (ϵ 12 600) nm and signals at δ 5.66 (H-16a) and 6.35 (H-16b) ppm observed in the ^1H NMR spectrum (Table 2). The ^1H NMR spectrum of **1** also disclosed signals for three olefinic protons at δ 5.03 (H-3), 5.15 (H-11), and 5.16 (H-7) ppm; four oxymethine protons either bearing three acetates or in the γ-lactone group at δ 5.30 (H-2), 5.31 (H-14), 5.57 (H-6), and 5.67 (H-10) ppm; three methyl groups connecting with an olefin at δ 1.68 (H-19), 1.82 (H-20), and 1.88 (H-18) ppm; and three methyl groups in acetate esters at δ 2.00, 2.02, and 2.04 ppm. The ^1H−^1H COSY spectrum demonstrated correlations from H-1 to H-3, H-5 to H-7, H-9 to H-11, and H-13 to H-1. ^1H−^1H long-range correlations were also observed between H-1 and the H-16 protons, H-3 and H-18, H-7 and H-19, and H-11 and H-20, by proton-decoupling analysis. These spectroscopic findings and the degree of unsaturation (10) showed **1** to have a 14-membered cembrane-type diterpenoid skeleton with an α-methylene γ-lactone. After assignments between all the direct C−H bondings were made based on HMQC, the gross structure was determined by HMBC analysis. The correlations according to HMBC are shown in Figure 1.

The structure of **1** containing three acetoxyl groups at C-6, C-10, and C-14 was similar to that of kericembrenolide C with two acetate esters at C-6 and C-9,[2] except for the position of one acetate substituent, which was shifted from

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10.1021/np000309w CCC: $19.00
Published on Web 11/10/2000

Table 1. ^{13}C NMR Data for Compounds **1**−**7** (125 MHz, $CDCl_3$, δ ppm)

position	1	2	3	4	5	6	7
1	46.2	48.0	46.3	45.7	42.9	42.8	43.0
2	73.4	72.9	73.6	73.9	78.0	79.0	79.2
3	124.3	124.4	123.5	123.9	126.8	123.9	123.2
4	139.7	139.5	141.1	141.3	141.6	139.3	140.6
5	42.3	42.3	41.8	45.5	44.9	42.3	45.2
6	68.9	68.9	66.8	66.7	67.9	69.0	66.4
7	127.0	127.1	131.4	129.3	125.1	126.3	131.4
8	137.3	137.3	134.9	138.5	136.0	138.3	135.2
9	44.4	44.5	44.5	38.4	39.2	48.2	44.7
10	66.9	67.0	66.4	23.4	23.9	64.9	67.3
11	126.7	126.4	126.9	128.6	123.8	129.2	125.1
12	138.0	138.3	137.9	129.7	131.9	138.9	140.9
13	41.9	44.9	45.2	42.1	36.3	36.0	35.8
14	73.6	72.6	73.4	74.0	32.6	32.5	32.3
15	136.4	138.4	136.5	136.9	140.2	140.0	140.1
16	123.7	122.8	123.5	123.1	122.2	122.0	121.9
17	169.3	169.8	169.4	169.7	170.4	169.9	170.0
18	19.9	19.8	20.0	19.7	16.0	19.8	19.9
19	16.3	16.3	15.8	15.4	15.5	16.3	15.9
20	15.8	16.1	16.0	15.8	15.4	15.2	15.6
CH_3CO	20.9	21.2	20.9	21.0	21.4	21.3	21.3
CH_3CO	21.2	21.3	21.2				
CH_3CO	21.3						
CH_3CO	170.0	170.0	170.1	170.2	170.4	170.0	170.3
CH_3CO	170.1	170.2	170.2				
CH_3CO	170.1						

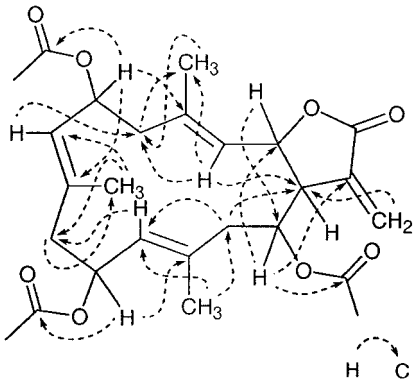


Figure 1. HMBC for **1**.

C-9 to C-10 and the presence of another acetate at C-14. The stereochemistry for the three trisubstituted olefins of **1** was determined by NOESY analysis. The NOESY spectrum exhibited correlations between H-3 and H-5, H-7, and H-9, and H-11 and H-13 to disclose the (3*E*,7*E*,11*E*) stereochemistries. The chemical shift values of the three methyl carbon signals for C-18, C-19, and C-20 (δ 19.9, 16.3, and 15.8 ppm, respectively), which appeared in the high-field area in the ^{13}C NMR spectrum also supported the all-*E* configurations. The relative configurations at C-1, C-2, and C-14 were determined by the coupling constants from the ^1H NMR data ($J_{1,2}$ = 3.4 Hz, $J_{1,14}$ = 3.7 Hz) and NOE correlations between H-1 and H-3, and H-2 and H-13, as shown in Figure 2. The relative configurations of the remaining two chiral centers at C-6 and C-10 were deduced from the following NOE analysis. NOE correlations between H-1 and H-20, H-1, and H-3 indicated that these protons (H-1, H-3, and H-20) were oriented to the same side, while NOE correlations between H-2 and H-18, H-18 and H-7 and between H-18 and H-11 demonstrated that these protons (H-2, H-7, H-11, and H-18) were oriented in the opposite direction. Considering the relationships of these protons, the relative configurations at C-6 and C-10 were determined by the NOE correlations between H-6 and H-3, H-6 and H-19, H-19 and H-10, and H-10 and H-20, as shown in Figure 2.

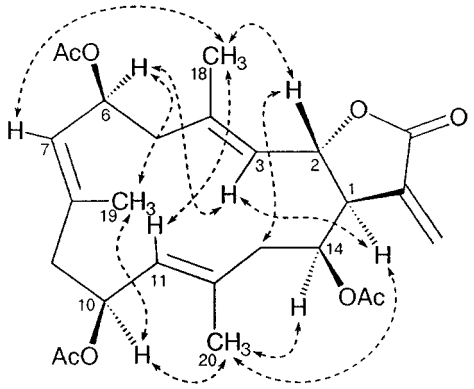


Figure 2. Key NOE correlations for **1**.

The absolute configuration of each chiral center in **1** was determined by the modified Mosher's method using 2-(2′-naphthyl)-2-methoxyacetic acid ester (2NMA ester, Scheme 1).[5] Compound **1** was treated with potassium carbonate in MeOH at room temperature to obtain triol **8**.[2] In this reaction the Michael-type addition of MeOH occurred concomitantly with the α-methylene γ-lactone moiety, to

Table 2. ^1H NMR Data for Compounds **1**−**3** (500 MHz, $CDCl_3$, δ ppm, *J* in Hz)

position	1	2	3
1	2.66 (m)	2.57 (m)	2.66 (m)
2	5.30 (dd, 3.4, 8.8)	5.33 (dd, 3.6, 8.9)	5.31 (dd, 3.9, 8.7)
3	5.03 (br d, 8.8)	5.03 (br d, 8.9)	4.93 (br d, 8.7)
5	2.17 (m)	2.17 (m)	2.16 (dd, 10.9, 13.7)
	2.62 (m)	2.62 (m)	2.53 (br d, 13.7)
6	5.57 (ddd, 5.4, 9.1, 10.0)	5.58 (ddd, 4.3, 9.0, 10.9)	4.54 (ddd, 3.9, 9.2, 10.4)
7	5.16 (br d, 9.1)	5.16 (br d, 9.0)	5.24 (br d, 9.2)
9	2.18 (m)	2.18 (m)	2.20 (br d, 12.4)
	2.61 (m)	2.61 (m)	2.64 (dd, 4.3, 12.4)
10	5.67 (ddd, 5.4, 9.0, 10.1)	5.66 (ddd, 5.3, 8.9, 10.6)	5.66 (m)
11	5.15 (br d, 9.0)	5.13 (br d, 8.9)	5.17 (br d, 8.8)
13	2.17 (dd, 8.8, 13.5)	2.20 (dd, 8.8, 13.4)	2.11 (dd, 8.7, 13.3)
	2.54 (br d, 13.5)	2.50 (dd, 3.8, 13.4)	2.64 (m)
14	5.31 (dd, 3.7, 8.8)	4.18 (dd, 3.8, 8.8)	5.31 (dd, 3.9, 8.7)
16	5.66 (d, 1.9)	5.65 (d, 2.2)	5.65 (d, 2.1)
	6.35 (d, 2.4)	6.42 (d, 2.6)	6.34 (d, 2.6)
18	1.88 (3H, br s)	1.87 (3H, br d, 0.9)	1.89 (3H, br s)
19	1.68 (3H, br s)	1.68 (3H, br s)	1.65 (3H, br s)
20	1.82 (3H, br s)	1.76 (3H, br s)	1.81 (3H, br s)
AcO	2.00 (3H, s)	2.02 (3H, s)	2.01 (3H, s)
AcO	2.02 (3H, s)	2.04 (3H, s)	2.04 (3H, s)
AcO	2.04 (3H, s)		

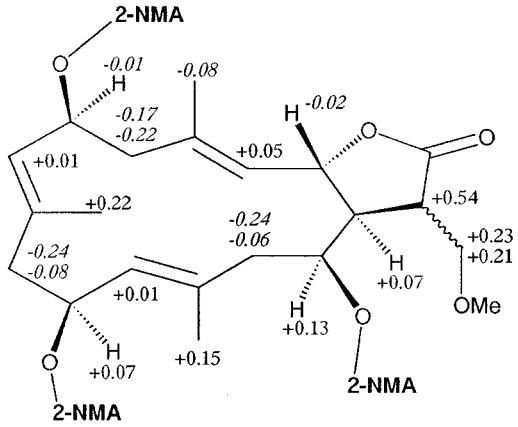
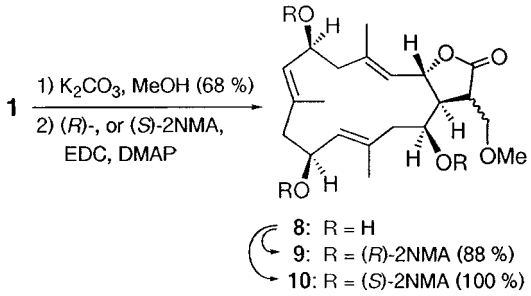


Figure 3. δΔ Values for 2-NMA ester of **1**.

Scheme 1

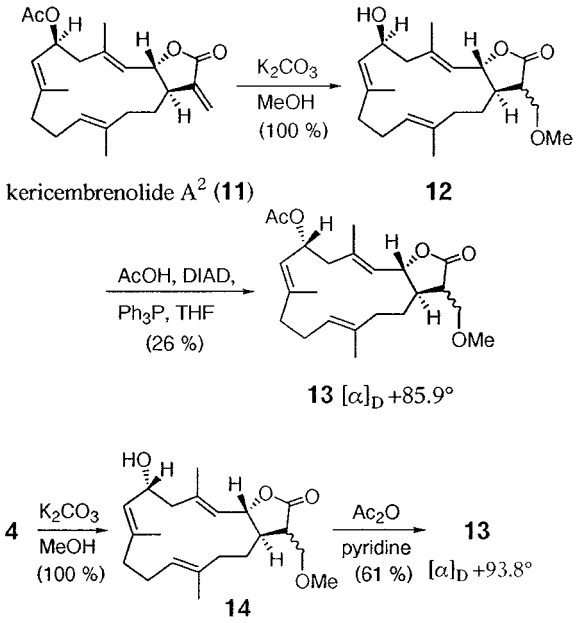


give a methyl ether at C-16 with an unknown stereochemistry at the newly arisen chiral center (C-15). Half of the amount of **8** was converted into (*R*)-2NMA triester **9**, and the other half was converted into (*S*)-2NMA triester **10**. After taking ^{1}H NMR spectra of both diastereomers, the $\delta\Delta$ [$\delta_{(R)\text{-ester}} - \delta_{(S)\text{-ester}}$] value of each corresponding proton was calculated, and they are summarized in Figure 3. Positive and negative $\delta\Delta$ values are systematically arranged at the left side and right side of each chiral center, to disclose absolute configurations of 6*S*, 10*S*, and 14*S*. These findings established the absolute configurations of **1** to be 1*S*, 2*S*, 6*S*, 10*S*, and 14*S*.

Compound **2** was shown to have the molecular formula $C_{24}H_{32}O_7$ by HREIMS. The IR and UV spectra of **2** indicated absorptions due to an α-methylene γ-lactone [ν_{max} 1759, 1665 cm^{-1}, λ_{max} 218 nm (ϵ 3000)]. The IR spectrum also exhibited acetate ester (1731, 1239 cm^{-1}) and hydroxyl (3452 cm^{-1}) functionalities. The ^{1}H NMR of **2** showed three characteristic signals at δ 1.68, 1.76, and 1.87 ppm due to olefinic methyl protons, two three-proton singlets at δ 2.02 and 2.04 ppm due to the two acetoxyl moieties, and a signal at δ 4.18 ppm assigned as the proton on the carbon bearing the secondary hydroxyl group (Table 2). After assignment of each direct C−H bonding from HMQC data, the ^{13}C NMR and ^{1}H NMR data of **2** were compared with those of **1**, indicating that the gross structure of **2** was a deacetyl analogue of **1** at C-14. The structure was confirmed by correlations observed in ^{1}H−^{1}H COSY and HMBC spectra. The absolute stereochemistry of **2** was established by the following chemical transformation. Acetylation of **2** provided the corresponding triacetate **1**, the spectral data and the optical rotation ([α]$_D$ −18.0°) of which were identical to those of natural **1**. From these results, it was concluded that the structure for **2** was the 14-hydroxy analogue of **1** with the same absolute configuration.

Compound **3** was found to have the molecular formula, $C_{24}H_{32}O_7$, the same as that of **2**, by the combination of HREIMS and ^{13}C NMR analysis. The IR and UV absorptions indicated the presence of an α-methylene γ-lactone [ν_{max} 1759, 1667 cm^{-1}, λ_{max} 211 nm (ϵ 4500)] moiety and hydroxyl (3415 cm^{-1}) and acetate (1731, 1260 cm^{-1}) functionalities. The ^{13}C and ^{1}H NMR spectra of **3** (Tables 1 and 2) were quite similar to those of **2**, except for the signal at δ 4.54 ppm in ^{1}H NMR due to a proton assigned as that at C-6 bearing a secondary hydroxyl group. 2D NMR analysis determined the gross structure of **3** to be a 6-hydroxy analogue of **1**. The absolute configuration was established by acetylation of **3** to form the corresponding triacetate. The spectral data and optical rotation value for the triacetate derived from **3** ([α]$_D$ −18.8°) were identical with those of natural **1**.

Scheme 2



The molecular formula of compound **4** was determined to be $C_{22}H_{30}O_4$ from both HREIMS and ^{13}C NMR data (Table 1). The absorptions observed in the IR and UV spectra suggested the presence of an α-methylene γ-lactone [ν_{max} 1765, 1667 cm^{-1}, λ_{max} 229 nm (ϵ 2500)] and an acetate ester (1731, 1241 cm^{-1}). Signals for three olefinic methyls at δ 1.61, 1.70, and 1.89 ppm and for one acetate at δ 2.00 ppm were observed in the ^{1}H NMR spectrum (Table 3). ^{1}H−^{1}H COSY and HMBC analysis suggested the gross structure to be the same as that of kericembrenolide A (**11**).[2] However, the spectral data of **4** and **11** were not identical. NOESY analysis supported the structure of **4** as well as the same relative configuration of **11**, except at C-6. These observations indicated **4** to be the epimer at C-6 of **11**. The chemical conversion of **11** was thus carried out to determine the absolute configuration of **4**, as shown in Scheme 2.

Methanolysis of the C-6 acetate ester in **11** with potassium carbonate in MeOH accompanied by the addition of MeOH to the α-methylene γ-lactone moiety gave the corresponding C-6 alcohol **12** as a single compound after chromatographic separation. Mitsunobu inversion[6] was effected for **12** with acetic acid, triphenylphosphine, and diisopropyl azodicarboxylate at room temperature to afford **13**. Compound **4** was similarly converted to the corresponding methyl ether **13** via **14**, as also shown in Scheme 2. The spectral data, including the optical rotation for each compound **13** obtained from **4** and **11**, were almost identical, thus establishing the structure of **4** to be 6-epikericembrenolide A (1*S*,2*S*,6*R*).

HREIMS data for compounds **5**, **6**, and **7**, as well as their ^{13}C NMR (Table 1) data, indicated the same molecular

Table 3. ^{1}H NMR Data for Compounds **4**−**7** (500 MHz, $CDCl_3$, δ ppm, J in Hz)

position	4	5	6	7
1	2.83 (m)	2.65 (ddd, 2.6, 5.3, 9.9)	2.42 (m)	2.40 (m)
2	5.33 (dd, 4.0, 8.8)	4.91 (dd, 2.6, 10.1)	4.84 (dd, 4.0, 8.9)	4.83 (dd, 4.4, 8.7)
3	4.94 (br d, 8.8)	5.15 (br d, 10.1)	5.00 (br d, 8.9)	4.92 (br d, 8.7)
5	2.16 (dd, 9.1, 15.7)	2.17 (dd, 10.7, 12.2)	2.17 (dd, 10.9, 14.9)	2.10 (m)
	2.62 (dd, 4.0, 15.7)	2.59 (dd, 5.2, 12.2)	2.59 (br dd, 4.3, 14.9)	2.63 (m)
6	4.60 (dt, 4.0, 9.1)	5.71 (ddd, 5.2, 9.9, 10.7)	5.58 (ddd, 4.3, 9.9, 10.9)	4.54 (ddd, 4.2, 9.4, 10.9)
7	5.18 (br d, 9.1)	5.00 (br d, 9.9)	5.14 (br d, 9.9)	5.16 (br d, 9.4)
9	2.15 (m)	2.00 (ddd, 4.3, 4.6, 12.5)	2.12 (dd, 8.3, 12.6)	2.20 (dd, 10.7, 11.7)
	2.24 (m)	2.25 (m)	2.66 (br dd, 5.2, 12.6)	2.62 (m)
10	2.13 (m)	2.12 (br dd, 4.3, 14.5)	4.59 (ddd, 5.2, 8.3, 10.6)	5.66 (ddd, 5.2, 8.8, 10.7)
	2.22 (m)	2.30 (ddd, 4.6, 9.9, 14.5)		
11	5.04 (br d, 5.4)	4.85 (br dd, 4.3, 9.9)	5.17 (br d, 10.6)	5.13 (br d, 8.8)
13	2.13 (m)	2.08 (m)	2.09 (m)	2.11 (br d, 14.7)
	2.39 (br d, 14.1)	2.23 (m)	2.32 (m)	2.30 (br d, 14.7)
14	5.25 (ddd, 1.9, 3.6, 12.1)	1.65 (m)	1.59 (m)	1.70 (m)
		1.68 (m)	1.68 (m)	1.88 (m)
16	5.62 (d, 2.3)	5.60 (d, 2.0)	5.57 (d, 2.2)	5.57 (d, 2.4)
	6.32 (d, 2.7)	6.26 (d, 2.2)	6.27 (d, 2.6)	6.26 (d, 2.7)
18	1.89 (3H, br d, 0.6)	1.72 (3H, br s)	1.83 (3H, br s)	1.83 (3H, br d, 0.6)
19	1.61 (3H, br s)	1.75 (3H, br s)	1.66 (3H, br s)	1.67 (3H, br s)
20	1.70 (3H, br s)	1.61 (3H, br s)	1.79 (3H, br s)	1.73 (3H, br s)
AcO	2.00 (3H, s)	2.03 (3H, s)	2.02 (3H, s)	2.04 (3H, s)

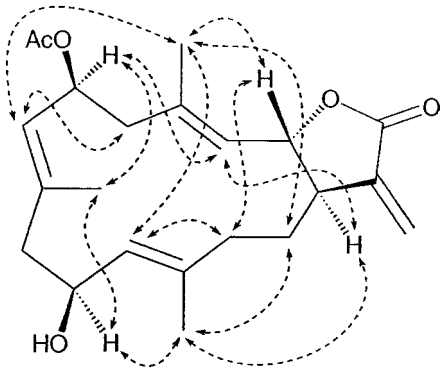


Figure 4. NOE correlations for **6**.

formula, $C_{22}H_{30}O_5$, for each compound. ^{1}H NMR (Table 3), IR, and UV spectra suggested these compounds to have cembrane-type structures, including the functionalities of one acetate, one alcohol, and three trisubstituted olefins with *E* configurations. The 2D NMR analysis for compound **5** indicated the gross structure to be 14-acetoxy-6-hydroxy-cembra-(3*E*,7*E*,11*E*,15)-tetraen-17,2-olide. Acetylation of **5** provided the 6,14-diacetate **15**. The spectral data of **15** were identical to those of kericembrenolide C,[2] thus showing compound **5** to have the absolute configuration (1*S*,2*S*,6*S*,-14*S*).

The ^{1}H and ^{13}C NMR spectra of compounds **6** and **7** were quite similar to each other. Based on the 2D NMR analysis, **6** and **7** were determined respectively to have 6-acetoxy-10-hydroxy- and 10-acetoxy-6-hydroxy-cembra-(3*E*,7*E*,-11*E*,15)-tetraen-17,2-olide gross structures. The NOESY analysis for compound **6**, as shown in Figure 4, indicated the relative configurations were 1*S**, 2*S**, 6*S**, and 10*S**. Compound **7** was found to have the same relative configurations according to the NOESY analysis. Although the absolute configurations for **6** and **7** are under investigation, these compounds are assumed to have the same stereochemistry as those of compounds **1**−**3**.

Compound **1**, obtained as the major cembranolide in the present study, was examined for cytotoxic activities in vitro for human colorectal adenocarcinoma cells (DLD-1) and human T-lymphocyte leukemia cells (MOLT-4). Compound **1** showed the proliferation of DLD-1 to inhibit completely at 0.6 μM/mL (IC_{50} 4.2 μg/mL) and that of MOLT-4 to inhibit at 0.6 μM/mL (IC_{50} 0.9 μg/mL). The known diterpenoids, kericembrenolides D and F, were also examined in the same cell line, which showed the growth inhibition for DLD-1 and MOLT-4 to be equivalent to those of **1**. Other new diterpenoids are currently under investigation.

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), a DPX-400 (^{1}H; 400 MHz), and a Varian Gemini-300 (^{1}H; 300 MHz) instrument in $CDCl_3$. ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC NMR spectra were measured with 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 (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Mass spectra were obtained with a Micromass Auto Spec spectrometer. Column chromatography was carried out on Merck Si gel 60 (70−230 mesh), and flash column chromatography was performed on Merck Si gel 60 (230−400 mesh). MPLC was carried out with a KHLC-201-43 (Kusano) apparatus using a CIG prepack column (Si gel, CPS-HS-221-05, for normal phase and ODS Si gel, CPO-HS-221-20, for reversed phase). HPLC was conducted with a YMC-Pack SIL-06 column (Si gel, SH-043-5-06, normal phase) and a YMC-Pack ODS-AM column (ODS Si gel, SH-343-5AM, reversed phase).

Animal and Material. The soft coral *C. 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 deposited 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- (72.6 g) soluble portions. An aliquot of the EtOAc-soluble portion (11.0 g) was chromatographed on a Si gel column (500 g). Stepwise elution with hexanes (300 mL), hexanes−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. Repeated separation and purification of fractions 5 and 6 (2.16 g) [eluted with hexanes−

EtOAc (1:1 and 1:2)] using Si gel flash column chromatography, MPLC (normal and reversed phase), and HPLC gave **1** (11.0 mg) along with kericembrenolides D and E.[2] The seventh fraction (0.58 g) [eluted with EtOAc] was subjected to Si gel column chromatography. Further purification by MPLC (normal and reversed phase) and HPLC afforded compounds **2** (2.9 mg), **3** (1.8 mg), **5** (2.8 mg), **6** (1.2 mg), and **7** (1.9 mg) along with stolonidiol and its acetate.[3] The fourth fraction (2.47 g) [eluted with hexanes−EtOAc (2:1)] was subjected to Si gel column chromatography to give five fractions. Further purification of fraction 2 (2.03 g) by MPLC (normal and reversed phase) and HPLC afforded compound **4** (9.6 mg) along with neodolabellenol[2,4] and kericembrenolides A, B, and C.[2]

Compound 1: colorless, viscous oil; $[\alpha]^{25}_D$ −19.5° (*c* 0.13, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 208 (4.10) nm; IR (dry film) ν_{max} 1763, 1731, 1665, 1237 cm^{-1}; ^{13}C NMR and ^1H NMR, see Tables 1 and 2, respectively; HREIMS *m/z* 474.2252 [calcd for $C_{26}H_{34}O_8$, 474.2254 $(M)^+$].

Compound 2: colorless, viscous oil; $[\alpha]^{25}_D$ −19.2° (*c* 0.19, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 218 (3.48) nm; IR (dry film) ν_{max} 3452, 1759, 1731, 1665, 1239 cm^{-1}; ^{13}C NMR and ^1H NMR, see Tables 1 and 2, respectively; HREIMS *m/z* 432.2139 [calcd for $C_{24}H_{32}O_7$, 432.2148 $(M)^+$].

Compound 3: colorless, viscous oil; $[\alpha]^{25}_D$ −35.0° (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211 (3.65) nm; IR (dry film) ν_{max} 3415, 1759, 1731, 1667, 1260 cm^{-1}; ^{13}C NMR and ^1H NMR, see Tables 1 and 2, respectively; HREIMS *m/z* 372.1926 [calcd for $C_{22}H_{28}O_5$, 372.1937 $(M - CH_3CO_2H)^+$].

Compound 4: colorless, viscous oil; $[\alpha]^{25}_D$ +102.3° (*c* 0.13, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (3.39) nm; IR (dry film) ν_{max} 1765, 1731, 1667, 1241 cm^{-1}; ^{13}C NMR and ^1H NMR, see Tables 1 and 3, respectively; HREIMS *m/z* 298.1930 [calcd for $C_{20}H_{26}O_2$, 298.1933 $(M - CH_3CO_2H)^+$].

Compound 5: colorless, viscous oil; $[\alpha]^{25}_D$ −35.6° (*c* 0.18, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 215 (3.44) nm; IR (dry film) ν_{max} 3461, 1759, 1731, 1665, 1230 cm^{-1}; ^{13}C NMR and ^1H NMR, see Tables 1 and 3, respectively; HREIMS *m/z* 314.1870 [calcd for $C_{20}H_{26}O_3$, 314.1882 $(M - CH_3CO_2H)^+$].

Compound 6: colorless, viscous oil; $[\alpha]^{25}_D$ −25.8° (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 213 (3.52) nm; IR (dry film) ν_{max} 3443, 1759, 1731, 1666, 1240 cm^{-1}; ^{13}C NMR and ^1H NMR, see Tables 1 and 3, respectively; HREIMS *m/z* 314.1873 [calcd for $C_{20}H_{26}O_3$, 314.1882 $(M - CH_3CO_2H)^+$].

Compound 7: colorless, viscous oil; $[\alpha]^{25}_D$ −44.0° (*c* 0.15, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 219 (3.48) nm; IR (dry film) ν_{max} 3417, 1760, 1731, 1667, 1241 cm^{-1}; ^{13}C NMR and ^1H NMR, see Tables 1 and 3, respectively; HREIMS *m/z* 314.1926 [calcd for $C_{20}H_{34}O_8$, 314.1882 $(M - CH_3CO_2H)^+$].

Chemical Conversion of 1 to 8. To a mixture of **1** (5.0 mg, 0.02 mmol) in MeOH (1 mL) was added potassium carbonate (5 mg) at room temperature. The reaction mixture was vigorously stirred for 1 h at this temperature, and then EtOAc (2 mL) was added. The mixture was filtered by passing over a small plug of Si gel column, the plug was rinsed twice with 2 mL of EtOAc, and the combined filtrates were concentrated under reduced pressure. The oily residue was purified by Si gel column chromatography [hexanes−EtOAc (2:1) as eluent] to obtain triol **8** (3.1 mg, 68% yield) as a colorless oil.

Compound 8: ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.65 (3H, br s, H-19), 1.74 (3H, br s, H-20), 1.83 (3H, br s, H-18), 2.63 (1H, m, H-1), 2.86 (1H, m, H-15), 3.34 (3H, s, OMe), 3.61 (1H, dd, *J* = 3.9, 9.4 Hz, H-16a), 3.65 (1H, dd, *J* = 5.8, 9.4 Hz, H-16b), 4.09 (1H, dd, *J* = 3.0, 11.7 Hz, H-14), 4.53 (1H, m, H-10), 4.59 (1H, ddd, *J* = 3.3, 8.1, 10.1 Hz, H-6), 4.93 (1H, br d, *J* = 8.8 Hz, H-3), 5.23 (1H, br d, *J* = 9.5 Hz, H-11), 5.28 (1H, dd, *J* = 6.2, 8.8 Hz, H-2). The H-5, -7, -9, -13, and OH proton signals are difficult to discern.

Esterification of 8 with (*R*)-2NMA. To a mixture of **8** (1.2 mg, 0.003 mmol), (*R*)-2NMA (2 mg, 0.011 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.4 mg, 0.003 mmol) in $CHCl_3$ (1 mL) under an argon atmosphere was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 2.0 mg, 0.010 mmol) at room temperature, and the mixture was stirred for 1 h. After removal of urea derivatives by Si gel column chromatography, the crude product was purified by HPLC [hexanes−*i*-PrOH (12:1)] to afford (*R*)-2NMA triester **9** (2.5 mg, 88% yield) as a colorless oil.

Compound 9: ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.43 (3H, br s, H-20), 1.55 (3H, br s, H-18), 1.59 (3H, br s, H-19), 2.21 (1H, m, H-1), 2.46 (1H, m, H-15), 3.45 (1H, dd, *J* = 4.6, 11.7 Hz, H-16a), 3.61 (1H, dd, *J* = 6.2, 11.7 Hz, H-16b), 4.90 (1H, br d, *J* = 11.2 Hz, H-3), 4.91 (1H, br d, *J* = 6.1 Hz, H-11), 4.96 (1H, br d, *J* = 11.1 Hz, H-7), 4.99 (1H, dd, *J* = 6.1, 11.2 Hz, H-2), 5.09 (1H, dd, *J* = 3.9, 11.4 Hz, H-14), 5.60 (1H, m, H-10), 5.58 (1H, m, H-6). The H-5, -9, and -13 proton signals are difficult to discern.

Esterification of 8 with (*S*)-2NMA. To a mixture of **8** (1.2 mg, 0.003 mmol), (*S*)-2NMA (2.0 mg, 0.11 mmol), and DMAP (0.4 mg, 0.003 mmol) in $CHCl_3$ (1 mL) under an argon atmosphere was added EDC (2.0 mg, 0.010 mmol) at room temperature, and the mixture was stirred for 1 h. After removal of urea derivatives by Si gel column chromatography, the crude product was purified by HPLC [hexanes−*i*-PrOH (12:1)] to afford (*S*)-2NMA triester **10** (3.2 mg, 100% yield) as a colorless oil.

Compound 10: ^1H NMR ($CDCl_3$, 400 MHz) δ ppm 1.28 (3H, br s, H-20), 1.57 (3H, br s, H-19), 1.63 (3H, br s, H-18), 1.92 (1H, m, H-15), 2.14 (1H, m, H-1), 3.22 (1H, dd, *J* = 4.6, 11.8 Hz, H-16a), 3.39 (1H, dd, *J* = 5.7, 11.8 Hz, H-16b), 4.84 (1H, br d, *J* = 9.0 Hz, H-3), 4.90 (1H, br d, *J* = 10.1 Hz, H-11), 4.95 (1H, br d, *J* = 11.0 Hz, H-7), 4.95 (1H, m, H-14), 5.00 (1H, dd, *J* = 5.6, 9.0 Hz, H-2), 5.53 (1H, ddd, *J* = 4.7, 9.1, 10.1 Hz, H-10), 5.59 (1H, ddd, *J* = 3.2, 7.8, 10.4 Hz, H-6). The H-5, -9, and -13 proton signals are difficult to discern.

Acetylation of 2. Compound **2** was treated with acetic anhydride (0.1 mL) in pyridine (1.5 mL) at room temperature for 1 h. After concentration under reduced pressure, the residue was purified by HPLC [hexanes−*i*-PrOH (12:1)] to obtain triacetate ester **1** [1.2 mg, 83% yield, $[\alpha]^{25}_D$ −18.0° (*c* 0.15, $CHCl_3$)] as a colorless oil. The spectral data were identical to those of **1**.

Acetylation of 3. Compound **3** was treated with acetic anhydride (0.2 mL) in pyridine (0.5 mL) at room temperature for 1 h. After concentration under reduced pressure, the residue was purified by HPLC [hexanes−*i*-PrOH (15:1)] to obtain the corresponding triacetate ester **1** [1.2 mg, 90% yield, $[\alpha]^{25}_D$ −18.8° (*c* 0.12, $CHCl_3$)] as a colorless oil. The spectral data of this triacetate were identical to those of **1**.

Chemical Conversion of 11 to 12. To a mixture of kericembrenolide A[2] (**11**, 5.5 mg, 0.02 mmol) in MeOH (1 mL) was added potassium carbonate (3 mg) at room temperature. The mixture was vigorously stirred for 1 h at this temperature, and then EtOAc (2 mL) was added. The mixture was filtered by passing over a small plug of Si gel column, the plug was rinsed twice with 2 mL of EtOAc, and the combined filtrates were concentrated under reduced pressure. The oily residue was purified by Si gel column chromatography [hexanes−EtOAc (4:1) as eluent] to obtain **12** (5.2 mg, 100% yield) as a colorless oil.

Compound 12: ^1H NMR ($CDCl_3$, 300 MHz) δ ppm 1.56 (3H, br s, H-20), 1.69 (3H, br s, H-19), 1.80 (3H, br s, H-18), 2.19 (1H, m, H-1), 2.40 (1H, m, H-15), 3.34 (3H, s, OMe), 3.56 (1H, dd, *J* = 4.4, 11.8 Hz, H-16a), 3.61 (1H, dd, *J* = 6.6, 11.8 Hz, H-16b), 4.64 (1H, dd, *J* = 4.7, 8.5 Hz, H-2), 4.68 (1H, ddd, *J* = 4.2, 9.3, 10.8 Hz, H-6), 4.73 (1H, m, H-11), 4.93 (1H, br d, *J* = 8.5 Hz, H-3), 5.17 (1H, br d, *J* = 9.3 Hz, H-7). The H-5, -9, -10, and -13 proton signals are difficult to discern.

Mitsunobu Inversion[6] of 12. To a mixture of **12** (2 mg, 0.006 mmol), acetic acid (2.0 mg, 0.034 mmol), and triphenylphosphine (Ph_3P, 12.1 mg, 0.046 mmol) in THF (0.5 mL) under an argon atmosphere was added diisopropyl azodicarboxylate (0.02 mL, 0.06 mmol) at room temperature, and the mixture was stirred for 1 h. After removal of urea derivatives, Ph_3P, and triphenylphosphine oxide by Si gel column chromatography, the crude product was purified by HPLC [hexanes−*i*-PrOH (12:1)] to afford **13** (0.6 mg, 26% yield) as a colorless oil.

Compound 13: $[\alpha]^{25}_D$ +85.9° (*c* 0.12, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm 1.60 (3H, br s, H-20), 1.70 (3H, br s, H-18), 1.74 (3H, br s, H-19), 2.25 (1H, m, H-1), 2.40 (1H, m,

H-15), 3.36 (3H, s, H-OMe), 3.59 (1H, dd, J = 4.4, 11.1 Hz, H-16a), 3.73 (1H, dd, J = 5.9, 11.1 Hz, H-16b), 4.59 (1H, m, H-11), 4.71 (1H, dd, J = 2.7, 10.8 Hz, H-2), 4.91 (1H, m, H-6), 5.17 (1H, br d, J = 8.8 Hz, H-7), 5.33 (1H, br d, J = 10.8 Hz, H-3), The H-5, -9, -11, and OH proton signals are difficult to discern; EIMS m/z 330 $(M - CH_3CO_2H)^+$.

Chemical Conversion of 4 to 14. To a mixture of **4** (2.0 mg, 0.006 mmol) in MeOH (1 mL) was added potassium carbonate (5 mg) at room temperature. The mixture was vigorously stirred for 1 h at this temperature, and then EtOAc (2 mL) was added. The mixture was filtered by passing over a small plug of Si gel column, rinsed twice with 2 mL of EtOAc, and the combined filtrates were concentrated under reduced pressure. The oily residue was purified by Si gel column chromatography [hexanes−EtOAc (2:1) as eluent] to obtain **14** (1.9 mg, 100% yield) as a colorless oil.

Compound 14: ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.59 (3H, br s, H-20), 1.72 (3H, br s, H-19), 1.73 (3H, br s, H-18), 2.28 (1H, m, H-1), 2.41 (1H, m, H-15), 3.37 (3H, s, OMe), 3.58 (1H, dd, J = 3.4, 9.4 Hz, H-16a), 3.73 (1H, dd, J = 4.5, 9.4 Hz, H-16b), 4.72 (1H, dd, J = 6.6, 9.4 Hz, H-2), 4.90 (1H, br t, J = 6.3 Hz, H-11), 5.11 (1H, br d, J = 9.2 Hz, H-7), 5.34 (1H, br d, J = 9.4 Hz, H-3), 5.70 (1H, ddd, J = 4.5, 9.2, 10.9 Hz, H-6), The H-5, -9, -10, -13, and -14 proton signals are difficult to discern; EIMS m/z 330 $(M - CH_3CO_2H)^+$.

Acetylation of 14. Compound **14** was treated with acetic anhydride (0.2 mL) in pyridine (0.5 mL) at room temperature for 1 h. After concentration under reduced pressure, the residue was purified by HPLC [hexanes−*i*-PrOH (15:1)] to obtain the corresponding acetate ester [1.3 mg, 61% yield, $[\alpha]^{25}_D$ +93.8° (*c* 0.15, $CHCl_3$)] as a colorless oil. The spectral data of this acetate were identical to those of **13**.

Acetylation of 5. Compound **5** was treated with acetic anhydride (0.3 mL) in pyridine (0.5 mL) at room temperature for 1 h. After concentration under reduced pressure, the residue was purified by HPLC [hexanes−*i*-PrOH (15:1)] to obtain the corresponding diacetate ester **15** [0.8 mg, 68% yield, $[\alpha]^{25}_D$ −67.5° (*c* 0.01, $CHCl_3$)] as a colorless oil. The spectral data of this diacetate were identical to those of kericembrenolide C.[2]

Acknowledgment. The authors express their appreciation to Dr. Y. Shida, Tokyo University of Pharmacy and Life Science, for conducting MS measurements, and Dr. Y. Ichihara, Meiji Institute of Health Science, for conducting the bioassay.

References and Notes

(1) (a) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1982**, *23*, 5171−5174; Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1983**, *24*, 1549−1552. (b) Iwashima, M.; Okamoto, K.; Iguchi, K. *Tetrahedron Lett.* **1999**, *40*, 6455−6459; Iwashima, M.; Nara, K.; Iguchi, K. *Steroids* **2000**, *65*, 130−137.
(2) Kobayashi, M.; Son, B. W.; Kyogoku, Y.; Kitagawa, I. *Chem. Pharm. Bull.* **1986**, *34*, 2306−2309.
(3) (a) Mori, K.; Iguchi, K.; Yamada, N.; Yamada, Y.; Inouye, Y. *Tetrahedron Lett.* **1987**, *28*, 5673−5676. (b) Mori, K.; Iguchi, K.; Yamada, N.; Yamada, Y.; Inouye, Y. *Chem. Pharm. Bull.* **1988**, *36*, 2840−2852.
(4) Bowden, B. F.; Braekman, J. C.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980**, *33*, 927−932.
(5) (a) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096. (b) Kusumi, T.; Takahashi, H.; Xu, P.; Fukushima, T.; Asakawa, Y.; Hashimoto, T.; Kan, Y.; Inouye, Y. *Tetrahedron Lett.* **1994**, *35*, 4397−4400.
(6) Mitsunobu, O. *Synthesis* **1981**, 1−28.

NP000309W