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

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Six new terpenoids (two maaliane-type sesquiterpenoids, **1** and **2**, one aromadendrane-type sesquiterpenoid, **3**, one noraromadendrane-type sesquiterpenoid, **4**, and two neodolabellane-type diterpenoids, **5** and **6**) were isolated from the Okinawan soft coral *Clavularia koellikeri*. The structures of these compounds were determined on the basis of the results of spectroscopic analysis, chemical conversion, and X-ray crystallographic analysis. Compound **6** exhibited modest growth-inhibition effect in vitro toward tumor cells.

The Okinawan soft corals of the genus *Clavularia* comprise a number of structurally unique natural products with various bioactivities. For example, *Clavularia viridis* produces antitumor prostanoids, clavulones[1,2] and related compounds,[3−6] and *Clavularia koellikeri* contains cytotoxic diterpenoids, kericembranolides.[7] Recently, we reported the isolation and structural determination of new cembrane-type and dolabellane-type diterpenoids from *C. koellikeri*.[8,9] Further investigation on natural products from *C. koellikeri* resulted in the isolation of six new terpenoids: two maaliane-type sesquiterpenoids, **1** and **2**; one aromadendrane-type sesquiterpenoid, **3**; one noraromadendrane-type sesquiterpenoid, **4**; and two neodolabellane-type diterpenoids, **5** and **6**. Their structures were elucidated on the basis of spectroscopic analysis, chemical conversion, and X-ray crystallographic analysis. This paper describes the isolation, structural determination, and bioactivity of these compounds.

Results and Discussion

The MeOH extract of *C. koellikeri*, collected on a coral reef off Ishigaki Island (Okinawa Prefecture, Japan), was partitioned between EtOAc and H_2O to afford an EtOAc-soluble portion (71.4 g). A part (39.4 g) of the EtOAc-soluble portion was subjected to repeated chromatographic separation and purification to give compounds **1** (2.3 mg), **2** (12.4 mg), **3** (2.3 mg), **4** (2.9 mg), **5** (29 mg), and **6** (20 mg).

The molecular formula of compound **1** was found to be $C_{17}H_{26}O_2$ by HREIMS and ^{13}C NMR data (Table 1). The DEPT spectrum showed five methyls, three sp^3 methylenes, four sp^3 methines, two sp^3 quaternary carbons, one sp^2 methine, and two sp^2 quaternary carbons. The IR absorptions at 1732 and 1245 cm^{-1} indicated the presence of an acetoxyl group. The NMR spectra confirmed the presence of a secondary acetoxyl group: δ_H 2.01 (3H, s, $COCH_3$) and 4.69 (1H, br d, J = 4.1 Hz, H-1); δ_C 21.4 ($CO\mathit{CH}_3$), 75.4 (CH, C-1), and 171.0 ($\mathit{CO}CH_3$). The ^1H NMR spectrum of **1** (Table 1) also disclosed one olefinic proton at 5.25 (1H, br s, H-3), one olefinic methyl at 1.74 (3H, s, H-15), and two cyclopropyl methine protons at 0.53 (1H, dd, J = 7.8, 9.1 Hz, H-6) and 0.60 (1H, dt, J = 2.7, 9.1 Hz, H-7). These spectral data, coupled with the degrees of unsaturation (five), suggested that compound **1** was a tricyclic sesquiterpenoid with a secondary acetoxyl group.

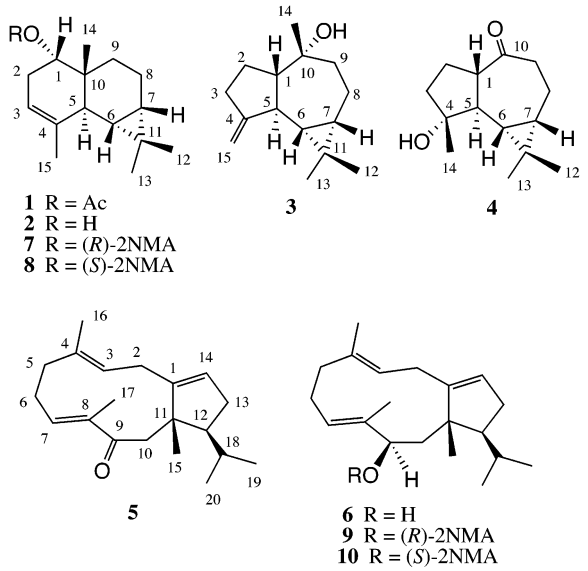

Figure 1. Structures of new terpenoids.

After direct ^1H and ^{13}C correlations were established from the HMQC spectrum, the gross structure of **1** was elucidated on the basis of the analysis of $^1H-^1H$ COSY and HMBC spectra (Figure 2). The $^1H-^1H$ COSY spectrum revealed sequences of the correlations from H-1 [4.69 (1H, br d, J = 4.1 Hz)] to H-3 [5.25 (1H, br s)] and from H-5 [1.84 (1H, m)] to H-9 [1.12 (1H, m), 1.15 (1H, m)] and the long-range correlation between H-3 and H-15 [1.74 (3H, br s)], as shown by the bold lines in Figure 2, indicating two partial structures **a** and **b**. The HMBC correlation from H-15 to C-5 [35.9 (CH)] indicated the connectivity between C-4 and C-5. The presence of a dimethylcyclopropyl group at C-6 and C-7 was exhibited by the HMBC correlations from H-12 [1.07 (3H, s)] to C-11 [18.1 (C)] and C-7 [19.7 (CH)] and from H-13 [0.96 (3H, s)] to C-11 and C-6 [22.1 (CH)]. The correlations from H-1 to the carbonyl carbon [171.0 (C)] demonstrated the presence of the secondary acetoxyl group at C-1. The connections between C-1 and C-10, C-5 and C-10, C-9 and C-10, and C-14 and C-10 were indicated by the correlations from H-14 [0.88 (3H, s)] to C-1 [75.4 (CH)], C-5, C-9 [31.5 (CH_2)], and C-10 [34.8 (C)].

The relative configurations of the five successive chiral centers at C-1, C-10, C-5, C-6, and C-7 in **1** were indicated by the following NOE analysis. As shown in Figure 3, NOE

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

	1			**2**	
no.	δ_C	δ_H	no.	δ_C	δ_H
1	75.4 (CH)	4.69 (1H, br d, 4.1)	1	73.5 (CH)	3.42 (1H, br d, 2.7)
2	28.8 (CH_2)	1.99 (1H, m, Hα)	2	31.6 (CH_2)	1.98 (1H, br d, 18.6)
		2.42 (1H, br d, 19.1, Hβ)			2.48 (1H, br d, 18.6)
3	116.5 (CH)	5.25 (1H, br s)	3	116.4 (CH)	5.26 (1H, br s)
4	135.7 (C)		4	136.0 (C)	
5	35.9 (CH)	1.84 (1H, m)	5	35.4 (CH)	1.75 (1H, m)
6	22.1 (CH)	0.53 (1H, dd, 7.8, 9.1)	6	22.1 (CH)	0.51 (1H, dd, 7.7, 9.2)
7	19.7 (CH)	0.60 (1H, dt, 2.7, 9.1)	7	19.8 (CH)	0.61 (1H, dt, 2.5, 9.2)
8	15.4 (CH_2)	1.48 (1H, m, Hα)	8	15.5 (CH_2)	1.50 (1H, m)
		1.87 (1H, qd, 8.8, 15.1, Hβ)			1.90 (1H, m)
9	31.5 (CH_2)	1.12 (1H, m, Hβ)	9	31.5 (CH_2)	1.10 (1H, m)
		1.15 (1H, m, Hα)			1.22 (1H, m)
10	34.8 (C)		10	35.9 (C)	
11	18.1 (C)		11	18.2 (C)	
12	28.5 (CH_3)	1.07 (3H, s)	12	28.5 (CH_3)	1.06 (3H, s)
13	15.5 (CH_3)	0.96 (3H, s)	13	15.6 (CH_3)	0.96 (3H, s)
14	17.7 (CH_3)	0.88 (3H, s)	14	18.1 (CH_3)	0.81 (3H, s)
15	21.0 (CH_3)	1.74 (3H, br s)	15	21.0 (CH_3)	1.72 (3H, br s)
CH$_3$CO	21.4 (CH_3)	2.01 (3H, s)			
CH$_3$*C*O	171.0 (C)				

[a] ^{13}C NMR: 125 MHz for **1**, 100 MHz for **2**. ^{1}H NMR: 500 MHz for **1**, 400 MHz for **2**. *J* in Hz. Assignments of the ^{13}C and ^{1}H signals were made on the basis of HMQC.

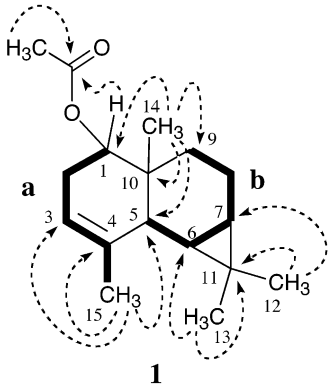

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Figure 2. $^{1}H-^{1}H$ correlations (bold lines) and key HMBC correlations (broken arrows) of compound **1**.

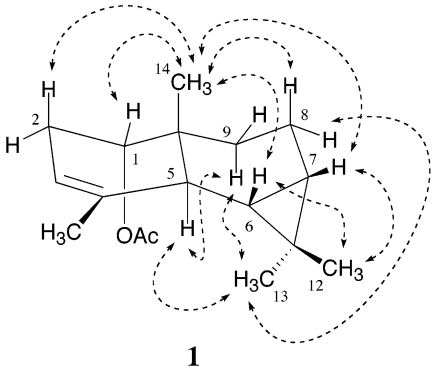

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Figure 3. NOE correlations of compound **1**.

correlations between H-1 and H-14, H-2β [2.42 (br d)] and H-14, H-6 and H-14, H-7 and H-14, H-8β [1.87 (qd)] and H-14, H-6 and H-12, and H-7 and H-12 exhibited that these protons orient to the same side. On the other hand, NOEs between H-5 and H-13, H-13 and H-8α [1.48 (m)], H-5 and H-9α [1.15 (m)], and H-9α and H-13 indicated these protons reside on the opposite side.

The molecular formula of compound **2** was found to be $C_{15}H_{24}O$ by HREIMS and ^{13}C NMR data. The IR spectrum showed an absorption at 3381 cm^{-1} due to a hydroxyl group. The NMR spectra (Table 1) were very similar to those of **1** except for the lack of the acetyl signal as well as

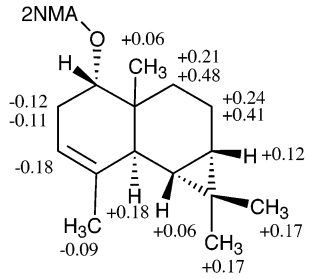


Figure 4. $\delta\Delta$ values (ppm) for 2NMA esters of compound **2**.

the high-field shift of H-1 [3.42 (1H, br d, $J = 2.7$ Hz)] and C-1 [73.5 (CH)], indicating that **2** was a desacetyl congener of **1**. This was confirmed by chemical conversion. Treatment of **2** with acetic anhydride in pyridine afforded the corresponding acetate, the ^{1}H NMR data of which were identical to those of **1**. The optical rotation of the acetate ($[\alpha]_D$ +19°) of **2** was also almost identical to that of **1** ($[\alpha]_D$ +21°). The absolute configuration of **2** was determined on the basis of the modified Mosher's method.[10,11] Esterification of **2** with (*R*)-methoxy(2-naphthyl)acetic acid (2NMA) in CH_2Cl_2 in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethyl-amino)pyridine (DMAP) gave the (*R*)-2NMA ester **7**. Similar esterification of **2** with (*S*)-2NMA gave the (*S*)-2NMA ester **8**. After measuring the ^{1}H NMR spectra of **7** and **8**, the $\delta\Delta$ value ($\delta\Delta = \delta_{R\ \text{ester}} - \delta_{S\ \text{ester}}$) for each proton was calculated and is summarized in Figure 4, indicating the *S* configuration at C-1. These findings concluded the absolute configuration of **2** (and **1**) to be assigned as 1*S*, 5*S*, 6*S*, 7*S*, and 10*R*.

Compounds **1** and **2** are relatively rare maaliane-type sesquiterpenoids exemplified by maaliol[12] isolated from the plant *Canarium samonense*. It is of interest that compounds **1** and **2** have the opposite absolute configurations at the C-6, -7, and -10 positions compared to those of maaliol.

The molecular formula of compound **3** was found to be $C_{15}H_{24}O$ by HREIMS and ^{13}C NMR data. All carbons appeared in the ^{13}C NMR spectrum of **3** (Table 2). The DEPT spectrum showed three methyls, four sp^3 methylenes, four sp^3 methines, two sp^3 quaternary carbons, one sp^2 methylene, and one sp^2 quaternary carbon. The presence of a tertiary hydroxyl group was indicated by the IR

Table 2. ^{13}C and ^{1}H NMR Data of Compounds **3** and **4** in $CDCl_3$[a]

	3			**4**	
no.	δ_C	δ_H	no.	δ_C	δ_H
1	56.5 (CH)	1.86 (1H, td, 6.4, 13.0)	1	57.9 (CH)	2.72 (1H, ddd, 7.9, 8.4, 11.2)
2	26.0 (CH_2)	1.75 (2H, m)	2	21.0 (CH_2)	1.50 (1H, tdd, 7.9. 8.4, 13.2) 2.31 (1H, dddd, 5.9, 7.9, 8.4, 13.2)
3	29.7 (CH_2)	2.25 (1H, m) 2.49 (1H, m)	3	40.9 (CH_2)	1.68 (1H, ddd, 5.9, 7.9, 12.6) 1.76 (1H, td, 7.9, 12.6)
4	157.6 (C)		4	80.1 (C)	
5	42.3 (CH)	2.50 (1H, m)	5	49.6 (CH)	1.39 (1H, t, 11.2)
6	28.3 (CH)	0.34 (1H, t, 9.0)	6	26.6 (CH)	0.68 (1H, dd, 9.4, 11.2)
7	28.4 (CH)	0.63 (1H, ddd, 6.1, 9.0, 11.2)	7	26.3 (CH)	0.89 (1H, ddd, 6.2, 9.4, 12.5)
8	19.2 (CH_2)	1.43 (1H, dt, 2.2, 11.2) 1.67 (1H, m)	8	20.2 (CH_2)	1.10 (1H, dtd, 1.6, 12.5, 14.9) 2.05 (1H, dtd, 2.6, 6.2, 14.9)
9	38.9 (CH_2)	1.62 (1H, br dd, 6.2, 13.7) 1.77 (1H, m)	9	44.0 (CH_2)	2.39 (1H, dt, 2.6, 12.5) 2.51 (1H, ddd, 1.6, 6.2, 12.5)
10	74.7 (C)		10	211.2 (C)	
11	19.1 (C)		11	18.8 (C)	
12	29.2 (CH_3)	1.03 (3H, s)	12	28.7 (CH_3)	1.11 (3H, s)
13	16.1 (CH_3)	1.11 (3H, s)	13	16.1 (CH_3)	1.03 (3H, s)
14	31.4 (CH_3)	1.25 (3H, s)	14	23.7 (CH_3)	1.29 (3H, s)
15	103.2 (CH_2)	4.66 (1H, br s) 4.74 (1H, br s)			

[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.

absorption at 3381 cm^{-1} and ^{13}C signal at δ 74.7 (C, C-10). The ^{1}H NMR spectrum of **3** (Table 2) also disclosed two olefinic protons due to a terminal methylene at δ 4.66 (1H, br s, H-15) and 4.74 (1H, br s, H-15) and two cyclopropyl methine protons at δ 0.34 (1H, t, $J = 9.0$ Hz, H-6) and 0.63 (1H, ddd, $J = 6.1, 9.0, 11.2$ Hz, H-7). These spectral data, coupled with the degrees of unsaturation (four), suggested that compound **3** was a tricyclic sesquiterpenoid with a tertiary hydroxyl group.

After direct ^{1}H and ^{13}C correlations were established from the HMQC spectrum, the gross structure of **3** was elucidated on the basis of the analysis of $^{1}H-^{1}H$ COSY and HMBC spectra (Figure 5). The $^{1}H-^{1}H$ COSY spectrum revealed sequences of the correlations from H-2 [1.75 (2H, m)] to H-3 [2.25 (1H, m), 2.49 (1H, m)] and from H-1 [1.86 (1H, td, $J = 6.4, 13.0$ Hz)] to H-9 [1.62 (1H, br dd, $J = 6.2$, 13.7 Hz), 1.77 (1H, m)], as depicted by the bold lines in Figure 5. The HMBC correlation from H-1 to C-2 [26.0 (CH_2)] indicated the connectivity between C-1 and C-2. The location of the terminal methylene group between C-3 and C-5 was demonstrated by the HMBC correlations from H-15 to C-3 [29.7 (CH_2)] and C-5 [42.3 (CH)]. The presence of a dimethylcyclopropyl group at C-6 and C-7 was exhibited by the HMBC correlations from H-13 [1.11 (3H, s)] to C-6 [28.3 (CH)] and C-11 [19.1 (C)] and from H-12 [1.03 (3H, s)] to C-6 and C-11. Finally, the connections between C-1 and C-10 bearing the tertiary hydroxyl group, C-10 and C-14, and C-10 and C-9 were indicated by the HMBC correlations from H-14 [1.25 (3H, s)] to C-1 [56.5 (CH)], C-10, and C-9 [38.9 (CH_2)].

The relative configurations of the five successive chiral centers at C-10, C-1, C-5, C-6, and C-7 in **3** were determined by the following NOE analysis. As shown in Figure 6, NOE correlations between H-1 and H-14, H-14 and H-6, H-6 and H-12, and H-12 and H-7 exhibited these protons to orient in the same direction. On the other hand, the NOE correlation between H-5 and H-13 indicated these protons to orient in the opposite direction.

Compound **3** is an aromadendrane-type sesquiterpenoid. Although the absolute stereochemistry of **3** was not determined, the absolute configurations at C-6 and C-7 may be the same as those of compounds **1** and **2** present in the same soft coral.

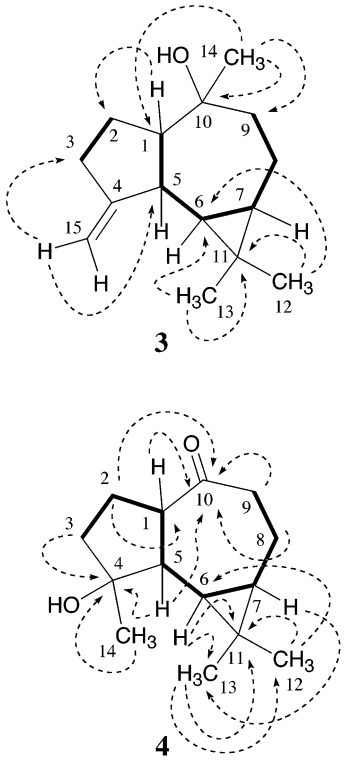


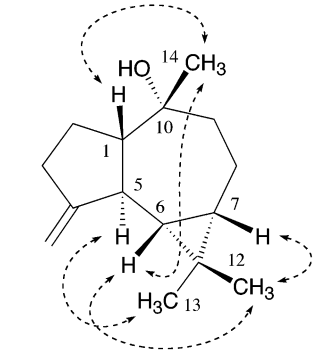
Figure 5. $^{1}H-^{1}H$ correlations (bold lines) and key HMBC correlations (broken arrows) of compounds **3** and **4**.

The molecular formula of compound **4** was found to be $C_{14}H_{22}O$ by HREIMS and ^{13}C NMR data (Table 2). The DEPT spectrum showed three methyls, four sp^3 methylenes, four sp^3 methines, two sp^3 quaternary carbons, and one sp^2 quaternary carbon. The IR and ^{13}C NMR spectra indicated the presence of a tertiary hydroxyl [IR 3440 cm^{-1}, δ_C 80.1 (C, C-4)] and a ketone [IR 1693 cm^{-1}, δ_C 211.2 (C, C-10)] group. The ^{1}H NMR spectrum (Table 2) disclosed signals due to three methyl protons [1.03 (3H, s, H-13), 1.11 (3H, s, H-12), 1.29 (3H, s, H-14)] and two cyclopropyl methine protons [0.68 (1H, dd, $J = 9.4, 11.2$ Hz, H-6), 0.89 (1H, ddd, $J = 6.2, 9.4, 12.5$ Hz, H-7)]. These spectral data, coupled with the degrees of unsaturation (four), suggested

Table 3. ^{13}C and ^{1}H NMR Data of Compounds **5** and **6**[a]

	5 (in C_6D_6)			**6** (in $CDCl_3$)	
no.	δ_C	δ_H	no.	δ_C	δ_H
1	147.5 (C)		1	150.0 (C)	
2	28.2 (CH_2)	2.46 (1H, br dd, 6.1, 13.7) 2.97 (1H, dd, 10.2, 13.7)	2	26.5 (CH_2)	2.46 (1H, dd, 10.2. 14.1) 2.70 (1H, br d, 14.1)
3	125.2 (CH)	5.04 (1H, br dd, 6.1, 10.2)	3	128.4 (CH)	4.77 (1H, br d, 10.2)
4	134.9 (C)		4	131.9 (C)	
5	37.9 (CH_2)	1.89−1.99 (2H, m)	5	38.5 (CH_2)	2.02 (1H, m), 2.12 (1H, m)
6	25.7 (CH_2)	1.88 (1H, m) 2.13−2.25 (1H, m)	6	23.0 (CH_2)	2.01 (1H, m) 2.21 (1H, br dd, 2.2, 11.0)
7	140.3 (CH)	5.50 (1H, br d, 10.4)	7	127.6 (CH)	4.96 (1H, br s)
8	138.2 (C)		8	138.6 (C)	
9	205.6 (C)		9	75.9 (CH)	4.11 (1H, t, 3.8)
10	52.8 (CH_2)	2.22 (1H, d, 11.1) 3.08 (1H, d, 11.1)	10	47.2 (CH_2)	1.69 (2H, m)
11	52.7 (C)		11	51.0 (C)	
12	51.9 (CH)	2.84 (1H, br d, 7.8)	12	49.2 (CH)	2.04 (1H, m)
13	30.9 (CH_2)	1.85 (1H, qd, 2.3, 16.9) 2.35 (1H, tdd, 1.9, 7.8, 16.9)	13	35.6 (CH_2)	1.99 (1H, m) 2.39 (1H, ddd, 2.0, 7.9, 15.4)
14	127.9 (CH)	5.17 (1H, br s)	14	125.6 (CH)	5.38 (1H, br s)
15	22.0 (CH_3)	1.05 (3H, s)	15	21.8 (CH_3)	0.94 (3H, s)
16	15.2 (CH_3)	1.41 (3H, br s)	16	15.4 (CH_3)	1.46 (3H, br s)
17	12.3 (CH_3)	1.72 (3H, br s)	17	11.2 (CH_3)	1.59 (3H, br s)
18	28.5 (CH)	1.91 (1H, m)	18	30.1 (CH)	1.74 (1H, m)
19	18.2 (CH_3)	0.75 (3H, d, 6.6)	19	22.3 (CH_3)	0.92 (3H, d, 6.6)
20	22.8 (CH_3)	1.02 (3H, d, 6.8)	20	22.9 (CH_3)	1.05 (3H, d, 6.6)

[a] ^{13}C NMR: 125 MHz for **5**, 100 MHz for **6**. ^{1}H NMR: 500 MHz for **5**, 400 MHz for **6**. *J* in Hz. Assignments of the ^{13}C and ^{1}H signals were made on the basis of HMQC.



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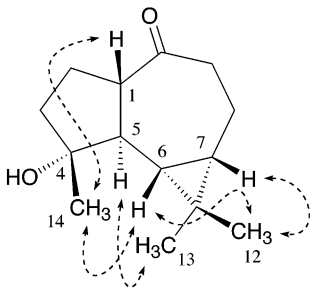

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Figure 6. NOE correlations of compounds **3** and **4**.

that compound **4** was a tricyclic norsesquiterpenoid ketone with a tertiary hydroxyl group.

After assignments of all the direct $^{1}H-^{13}C$ bondings were made based on HMQC analysis, the gross structure of **4** was determined by $^{1}H-^{1}H$ COSY and HMBC analysis (Figure 5). The $^{1}H-^{1}H$ COSY spectrum revealed a sequence of correlations from H-3 [1.68 (1H, ddd, J = 5.9, 7.9, 12.6 Hz), 1.76 (1H, td, J = 7.9, 12.6, Hz)] to H-9 [2.39 (1H, dt, J = 2.6, 12.5 Hz), 2.51 (1H, ddd, J = 1.6, 6.2, 12.5 Hz)], as depicted by the bold lines in Figure 5. The HMBC correlations from H-3 to C-4 bearing the tertiary hydroxyl group, from H-5 [1.39 (1H, t, J = 11.2 Hz)] to C-4, and from H-14 to C-4 indicated the location of the quaternary carbon (C-4) bearing hydroxyl and methyl groups between C-3 and C-5. The presence of a dimethylcyclopropyl group at C-6 and C-7 was exhibited by the HMBC correlations from H-6 to C-11 [18.8 (C)] and C-13 [16.1 (CH_3)], from H-7 to C-13, from H-13 to C-11 and C-12 [28.7 (CH_3)], and from H-12 to C-11 and C-6 [26.6 (CH)]. The location of the ketone group (C-10) between C-1 and C-9 was indicated by the correlations from H-1, H-2, H-5, H-8, and H-9 to C-10.

The relative configurations of the chiral centers at C-1, C-4, C-5, C-6, and C-7 in **4** were determined by the following NOE analysis. As depicted in Figure 6, the NOE correlation between H-1 and H-14, H-14 and H-6, H-6 and H-12, and H-12 and H-7 exhibited these protons to orient in the same direction. On the other hand, the NOE correlation between H-5 and H-13 indicated these protons to orient in the opposite direction.

Compound **4** is the first natural sesquiterpenoid having a noraromadendrane skeleton. Both enantiomers of **4** were previously reported as synthetic intermediates for the synthesis of sesquiterpenoids.[13−15] The ^{1}H and ^{13}C NMR data of **4** were identical with those of the synthetic intermediate[15] prepared from (+)-aromadendrene. However, the sign of the optical rotation ($[\alpha]_D$ −21.3°) for **4** was shown to be opposite of that for the synthetic intermediate ($[\alpha]_D$ +21.3°). Thus, the absolute configuration of **4** was assigned as 1*S*, 4*R*, 5*R*, 6*S*, and 7*S*.

The molecular formula of compound **5** was found to be $C_{20}H_{30}O$ by HREIMS and ^{13}C NMR data (Table 3).[16] The DEPT spectrum showed five methyls, five sp^3 methylenes, two sp^3 methines, one sp^3 quaternary carbon, three sp^2 methines, and four sp^2 quaternary carbons. The presence of a conjugated enone group was indicated by the UV [234 nm (ϵ 5600)] and IR (1656 cm^{-1}) absorptions and by the ^{13}C signal at δ 205.5 (C, C-9). The ^{1}H NMR spectrum disclosed three olefinic protons due to trisubstituted olefins at δ 5.04 (1H, br dd, J = 6.1, 10.2 Hz, H-3), 5.50 (1H, br d, J = 10.4 Hz, H-7), and 5.17 (1H, br s, H-14). These spectral data, coupled with the degrees of unsaturation (six),

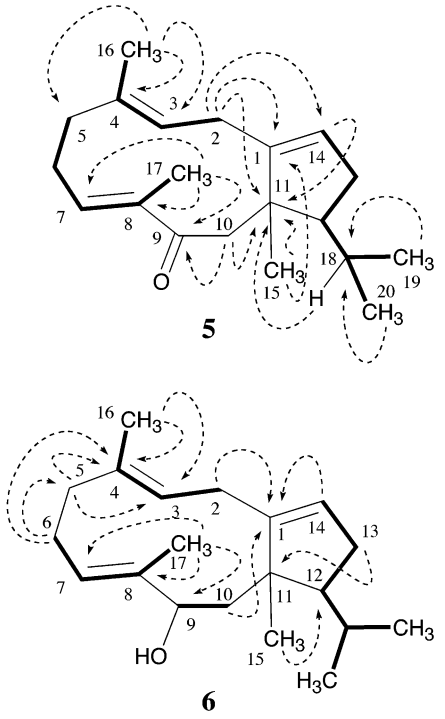

Figure 7. $^1H-^1H$ correlations (bold lines) and key HMBC correlations (broken arrows) of compounds **5** and **6**.

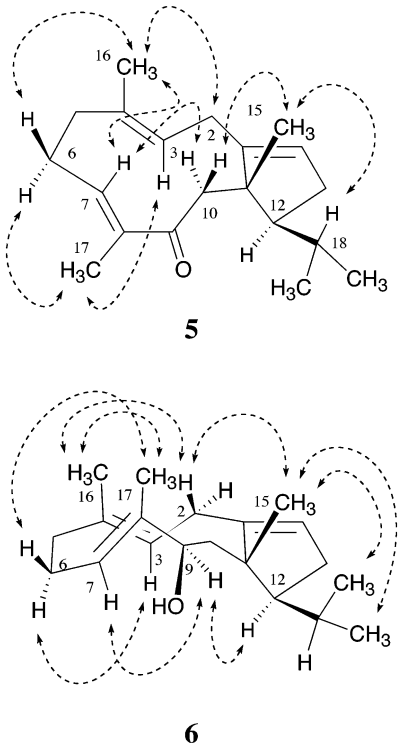

Figure 8. NOE correlations of compounds **5** and **6**.

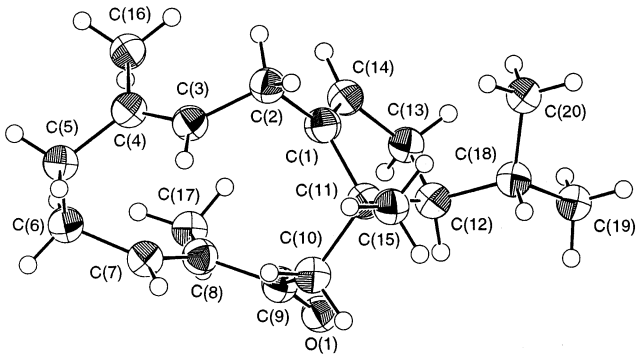

Figure 9. Perspective view (ORTEP) of the molecule of compound **5**.

suggested that compound **5** was a bicyclic diterpenoid with a conjugated enone group.

After assignments of all the direct $^1H-^{13}C$ bondings were made based on the HMQC analysis, the gross structure of **5** was determined by $^1H-^1H$ COSY and HMBC analysis (Figure 7). The $^1H-^1H$ COSY spectrum revealed sequences of the correlations depicted by the bold lines in Figure 7. The HMBC correlations from H-2 [2.97 (1H, dd, J = 10.2, 13.7 Hz)] to C-1 [147.5 (C)] and C-14 [127.9 (CH)] indicated the connectivity between C-1 and C-2. The connection between C-4 and C-5 was indicated by the HMBC correlation from H-16 [1.41 (3H, br s)] to C-5 [37.9 (CH_2)]. The presence of a methyl group (H-17) on the α position of the conjugated enone was demonstrated by the correlations from H-17 [1.72 (3H, br s)] to C-7 [140.3 (CH)], C-8 [138.2 (C)], and C-9. The HMBC correlation from H-10 [3.08 (1H, d, J = 11.1 Hz)] to C-9 [205.6 (C)] indicated the connectivity between C-10 and C-9. Finally, the HMBC correlations from H-10 to C-11 [52.7 (C)], from H-15 [1.05 (3H, s)] to C-11 and C-1, from H-2 to C-11, from H-18 [1.91 (1H, m)] to C-11, and from H-14 [5.17 (1H, br s)] to C-11 revealed connectivities around the angular quaternary carbon at C-11.

The stereochemistry of the two trisubstituted olefins in **5** was determined by the NOE analysis. As shown in Figure 8, the NOE correlation between H-2 and H-16 indicated a 3*E* configuration, and that between H-6 and H-17 a 7*E* configuration. The relative configurations of the two chiral centers at C-11 and C-12 were also determined by NOE analysis. The NOE correlation between the angular methyl proton (H-15) and the methine proton (H-18) demonstrated a cis configuration between the methyl at C-11 and the isopropyl at C-12. The structure of **5** except for the absolute stereochemistry was confirmed by X-ray crystallographic analysis on a single crystal of **5**. The result of the X-ray analysis is shown in Figure 9.

The molecular formula of compound **6** was found to be $C_{20}H_{32}O$ by HREIMS and ^{13}C NMR data. The IR spectrum showed an absorption at 3417 cm^{-1} due to a hydroxyl group. The NMR spectrum (Table 3) indicated the presence of a secondary hydroxyl group: δ_H 4.11 (1H, t, J = 3.8 Hz, H-9), δ_C 75.9 (CH, C-9). The ^1H NMR spectrum disclosed three olefinic protons due to trisubstituted olefins at 4.77 (1H, br d, J = 10.2 Hz, H-3), 4.96 (1H, br s, H-7), and 5.38 (1H, br s, H-14). The NMR spectra of **6** were very similar to those of **5** except for the lack of the carbonyl signal and appearance of the signal due to the secondary hydroxyl group, indicating that **6** was a corresponding alcohol of the ketone **5**. This was confirmed by chemical conversion. Oxidation of **6** with Dess−Martin periodinane afforded a conjugated enone, the NMR as well as optical rotation data of which were identical with those of compound **5**.

The relative configuration at C-9 bearing a secondary hydroxyl group was deduced on the basis of the NOE correlations and analysis of conformation of **6**. The NOE correlations between H-2β and H-16, H-16 and H-17, H-17 and H-6β, H-6α and H-3, and H-2β and H-15 demonstrated the conformation from C-2 to C-9 as depicted in Figure 8. The NOE correlations between H-9 and H-12, and H-9 and H-7, thus indicated the relative configuration at C-9 (9*R**).

The absolute configuration of **6** was determined on the basis of the modified Mosher's method. (*R*)- and (*S*)-2NMA esters **9** and **10** were prepared from **6** by a method similar

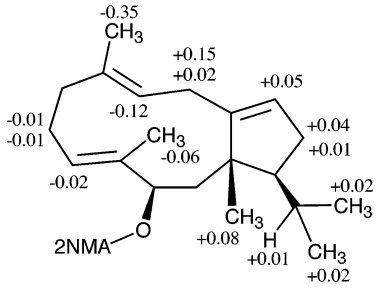

Figure 10. $\delta\Delta$ values (ppm) for 2NMA esters of compound **6**.

to that used in the case of **2**. The $\delta\Delta$ values summarized in Figure 10 indicated the *R* configuration at C-9. These findings concluded the absolute configuration of **6** and **5** to be assigned as 9*R*, 11*S*, 12*S* for **6** and 11*S* and 12*S* for **5**.

Compounds **5** and **6** are the rare neodolabellane-type diterpenoids such as neodolabellin[17] from *Clavularia koellikeri* and neodolabellenol[18] from *Clavularia inflata*. Compound **6** exhibited modest growth-inhibitory activity in vitro against lung cancer (NCI-H522, GI_{50} 5.2 μg/mL), melanoma (LOX-IMVI, GI_{50} 4.9 μg/mL), stomach cancer (MKN74, GI_{50} 5.2 μg/mL), and central nervous system cancer (SF-539 and SNB75, GI_{50} each 4.9 μg/mL) cells, evaluated in the Japanese Foundation for Cancer Research 39 cell line assay.[19]

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) or DPX-400 (^{1}H, 400 MHz; ^{13}C, 100 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) or C_6H_6 (^{1}H, 7.20 ppm) and C_6D_6 (^{13}C, 128.0 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, for normal-phase) and a YMC-Pack ODS-AM column (ODS silica gel, SH-343−5AM, for reversed-phase).

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

Extraction and Isolation. Wet specimens (5.4 kg) were extracted with MeOH. The MeOH extract (237 g) was partitioned between EtOAc and H_2O to obtain an EtOAc-soluble portion (71.4 g). An aliquot of the EtOAc-soluble portion (39.4 g) was chromatographed on a silica gel column. Stepwise elution with hexane (2000 mL), hexane−EtOAc (2:1, 2000 mL), EtOAc (2000 mL), and MeOH (2000 mL) afforded four fractions. The second fraction [22.3 g, eluted with hexane−EtOAc (2:1)] was further chromatographed on a silica gel column by stepwise elution with hexane, hexane−EtOAc (10:1 and 4:1), and EtOAc to afford four fractions (fractions I−IV). Silica gel column chromatography of fraction II [11.7 g, eluted with hexane−EtOAc (10:1)] afforded nine fractions (fractions A−I) by stepwise elution with hexane−EtOAc (15:1 and 25:1).

Separation and purification of fraction G (2.12 g) using flash silica gel column chromatography [eluted with hexane−EtOAc (30:1)] and MPLC (reversed-phase, eluted with acetonitrile) afforded compounds **1** (2.3 mg) and **5** (29 mg). From fraction I (2.59 g), compound **2** (12.4 mg) was isolated along with the known diterpenoids (−)-*trans*-cembranolide (75 mg)[9] and neodolabellenol (144 mg)[18] by silica gel column chromatography [hexane−EtOAc (7:1) as an eluent], MPLC [normal phase, hexane−EtOAc (10:1) as an eluent], and HPLC [normal phase, hexane−EtOAc (10:1) as an eluent, and then reversed-phase, acetonitrile−H_2O (95:5) as an eluent]. Similar separation and purification of fraction H (0.47 g) using flash silica gel column chromatography [hexane−EtOAc (15:1) as an eluent], MPLC [(normal phase, hexane−EtOAc (15:1) as an eluent], and HPLC (reversed-phase, acetonitrile as an eluent) afforded compounds **3** (2.3 mg) and **6** (20 mg).

From a portion (2.58 g) of fraction III [3.36 g, eluted with hexane−EtOAc (4:1)], silica gel column chromatography (normal-phase) was conducted three times by elution with a hexane−EtOAc mixture to afford crude compound **4**, which was purified by reversed-phase column chromatography by elution with MeOH−H_2O (75:25) to afford compound **4** (2.9 mg).

Compound 1: colorless oil; $[\alpha]^{25}_D$ +21.9° (*c* 0.08, $CHCl_3$); IR ν_{max} (film) 1732, 1245 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 1; HREIMS *m*/*z* 262.1958 [calcd for $C_{17}H_{26}O_2$, 262.1933].

Compound 2: colorless oil; $[\alpha]^{25}_D$ −3.8° (*c* 0.15, $CHCl_3$); IR ν_{max} (film) 3380 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 1; HREIMS *m*/*z* 220.1824 [calcd for $C_{15}H_{24}O$, 220.1827].

Compound 3: colorless oil; $[\alpha]^{25}_D$ +7.1° (*c* 0.21, $CHCl_3$); IR ν_{max} (film) 3381 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 2; HREIMS *m*/*z* 220.1835 [calcd for $C_{15}H_{24}O$, 220.1827].

Compound 4: colorless oil; $[\alpha]^{25}_D$ −21.3° (*c* 0.13, $CHCl_3$); IR ν_{max} (film) 3440, 1693 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 2; HREIMS *m*/*z* 222.1619 [calcd for $C_{14}H_{22}O_2$, 220.1620].

Compound 5: colorless needles; $[\alpha]^{25}_D$ +153° (*c* 0.18, $CHCl_3$); UV λ_{max} (EtOH) 234 nm (ϵ 5600); IR ν_{max} (film) 1656 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 3; HREIMS *m*/*z* 286.2295 [calcd for $C_{20}H_{30}O$, 286.2297].

Compound 6: colorless plates; $[\alpha]^{25}_D$ +131° (*c* 0.43, $CHCl_3$); IR ν_{max} (film) 3417 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 3; HREIMS *m*/*z* 288.2449 [calcd for $C_{20}H_{32}O$, 288.2453].

Esterification of 2 with 2NMA. To a solution of **2** (2.2 mg) in CH_2Cl_2 (1.5 mL) were added successively (*R*)-2NMA (2.2 mg), EDC hydrochloride (5.0 mg), and DMAP (5.0 mg). The mixture was stirred for 2.5 h at room temperature under an argon atmosphere and was concentrated under reduced pressure. The residue was partitioned between ether and H_2O. The ethereal layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography [hexane−EtOAc (4:1) as an eluant] to give (*R*)-2NMA ester **7** (3.3 mg). Similar esterification of **2** with (*S*)-2NMA afforded (*S*)-2NMA ester **8**.

(*R*)-2NMA ester 7: colorless viscous oil; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm) 0.41 (1H, t, *J* = 8.3 Hz, H-6), 0.43 (1H, dt, *J* = 2.7, 8.3 Hz, H-7), 0.77 (3H, s, H-12), 0.91 (3H, s, H-13), 1.00 (1H, ddd, *J* = 3.3, 9.4, 12.9 Hz, H-9), 1.01 (3H, s, H-14), 1.18 (1H, dd, *J* = 8.7, 12.9 Hz, H-9), 1.30 (1H, br dd, *J* = 9.1, 15.1 Hz, H-8), 1.66 (3H, d, *J* = 0.8 Hz, H-15), 1.94 (1H, br s, H-5), 2.00 (1H, br d, *J* = 18.5 Hz, H-2), 2.23 (1H, br dd, *J* = 2.5, 18.5 Hz, H-2), 4.90 (1H, m, H-1), 5.06 (1H, br s, H-3).

(*S*)-2NMA ester 8: colorless viscous oil; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm) 0.31 (1H, dt, *J* = 3.6, 9.1 Hz, H-7), 0.35 (1H, t, *J* = 9.1 Hz, H-6), 0.60 (3H, s, H-12), 0.70 (1H, dd, *J* = 8.6, 13.6 Hz, H-9), 0.74 (3H, s, H-13), 0.79 (1H, ddd, *J* = 3.6, 9.1, 13.6 Hz, H-9), 0.89 (1H, dd, *J* = 8.6, 15.1 Hz, H-8), 0.95 (3H, s, H-14), 1.45 (1H, br dd, *J* = 9.1, 15.1 Hz, H-8), 1.75 (3H, d, *J* = 0.9 Hz, H-15), 1.76 (1H, m, H-5), 2.12 (1H, br d, *J* = 18.9 Hz, H-2), 2.34 (1H, br dd, *J* = 3.9, 18.9 Hz, H-2), 4.96 (1H, br dd, *J* = 8.6, 13.6 Hz, H-1), 5.24 (1H, br s, H-3).

Oxidation of 6. To a solution of **6** (2.2 mg) in $CHCl_3$ (1 mL) was added pyridine (60 μL) and Dess−Martin periodinane (5 mg), and the mixture was stirred for 20 min at room temper-

ature. The mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane−EtOAc, 10:1, as an eluent) to afford a ketone (1.3 mg): $[\alpha]^{25}_{D}$ +151° (*c* 0.05, $CHCl_3$). The ^1H NMR data of the ketone were identical with those of **5**.

Esterification of 6 with 2NMA. Compound **6** was converted to 2NMA esters **9** and **10**, respectively, by using a method similar to that in the case of compound **2**.

(*R*)-2NMA ester 9: colorless viscous oil; ^1H NMR (500 MHz, $CDCl_3$, δ ppm) 0.80 (3H, d, J = 6.4 Hz, H-19), 0.92 (3H, s, H-15), 0.93 (3H, d, J = 6.4 Hz, H-20), 1.13 (3H, br s, H-17), 1.38 (3H, s, H-16), 1.61 (1H, m, H-10a), 1.65 (1H, m, H-18), 1.76 (1H, q, J = 8.6 Hz, H-2a), 1.86 (1H, dd, J = 4.6, 15.5 Hz, H-10b), 1.93 (1H, dd, J = 9.7, 14.2 Hz, H-12), 1.98 (1H, m, H-5), 2.38 (1H, br d, J = 10.7 Hz, H-2b), 2.41 (1H, dd, J = 10.5, 14.1 Hz, H-6a), 2.68 (1H, br d, J = 13.0 Hz, H-6b), 4.75 (1H, dd, J = 4.2, 9.8 Hz, H-7), 4.99 (1H, br s, H-3), 5.20 (1H, t, J = 3.9 Hz, H-9), 5.38 (1H, s, H-14); EIMS *m*/*z* 486 $(M)^+$.

(*S*)-2NMA ester 10: colorless viscous oil; ^1H NMR (500 MHz, $CDCl_3$, δ ppm) 0.78 (1H, d, J = 6.4 Hz, H-19), 0.84 (3H, s, H-15), 0.91 (3H, d, J = 6.4 Hz, H-20), 1.44 (3H, br s, H-16), 1.48 (3H, s, H-17), 1.61 (1H, m, H-10a), 1.74 (1H, q, J = 8.6 Hz, H-2a), 1.76 (1H, m, H-18), 1.87 (1H, dd, J = 4.3, 15.0 Hz, H-10b), 1.93 (1H, dd, J = 9.7, 14.2 Hz, H-12), 1.98 (1H, m, H-5), 2.02 (1H, m, H-5a), 2.09 (1H, m, H-5b), 2.24 (1H, br d, J = 10.7 Hz, H-2b), 2.42 (1H, dd, J = 10.5, 14.1 Hz, H-6a), 2.69 (1H, br d, J = 13.0 Hz, H-6b), 4.77 (1H, dd, J = 4.2, 9.8 Hz, H-7), 5.11 (1H, br s, H-3), 5.17 (1H, t, J = 3.9 Hz, H-9), 5.33 (1H, s, H-14); EIMS *m*/*z* 486 $(M)^+$.

X-ray Crystal Structure Determination of 5. A colorless needle crystal of **6** was obtained by recrystallization from MeOH−H_2O. A single crystal with dimensions of 0.4 × 0.2 × 0.2 mm was used for X-ray diffraction studies on a Mac Science MXC18 diffractometer employing graphite-monochromated Cu Kα radiation (l.54178 Å). The structure was solved by a direct method using SIR92[20] in the CRYSTAN GM program system and refined by a full-matrix least-squares method using 1723 reflections [$I > 3.00\sigma(I)$] for 220 parameters. The final R value is 0.058.

Crystal Data: $C_{20}H_{30}O$, orthorhombic with space group $P2_12_12_1$, with a = 12.549(4) Å, b = 12.467(5) Å, c = 10.899(4) Å, V = 1730(1) Å, and Z = 4.

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Supporting Information Available: X-ray crystallographic data of compound **5** (Tables 4, 5, and 6). This material is available free of charge via the Internet at http://pubs.acs.org.

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