Isolation of Three Marine Prostanoids, Possible Biosynthetic Intermediates for Clavulones, from the Okinawan Soft Coral *Clavularia viridis*

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Three marine prostanoids, 1, 2, and 3, were isolated from the extract of the Okinawan soft coral *Clavularia viridis*. The structures of these compounds were assigned based on the results of spectroscopic analysis. Compound 1 was shown to be preclavulone-A methyl ester, and this is the first isolation of the ester of preclavulone-A as a natural product. Preclavulone-A is proposed to be the key intermediate in the biosynthesis of marine prostanoids exemplified by clavulones in *C. viridis*. The new prostanoid 3 was suggested to be a biosynthetic intermediate from preclavulone-A to clavulones, and a possible biogenetic pathway *via* 3 is proposed.

Key words soft coral; *Clavularia viridis*; marine prostanoid; biosynthesis; preclavulone-A methyl ester

Clavulones[1,2] (claviridenones[3,4]) are marine prostanoids isolated from the Okinawan soft coral *Clavularia viridis* in 1982 and have attracted continuing attention owing to their structural features, significant biological activities,[1,5—7] and unique biosynthetic pathway (Fig. 1). Our continuing studies on prostanoids from *C. viridis* have resulted in the isolation of about 50 congeners of clavulones.[8—10] Corey and coworkers proposed a biosynthetic pathway[11,12] from arachidonic acid to clavulones through 8*R*-HPETE, allene oxide, pentadienyl cation, and preclavulone-A as biosynthetic intermediates, as shown in Chart 1. This biosynthesis was based on experimental results showing that preclavulone-A was obtained by treating labeled arachidonic acid as well as labeled 8*R*-HPETE with the cell-free extract or acetone powder prepared from *C. viridis*, although the absolute configuration of preclavulone-A could not be determined due to its small amount and the formation of clavulones was not observed in these biosynthetic experiments. PGA_2-type prostaglandins such as 15*R*-PGA_2 diester found in the Caribbean octocoral *Plexaura homomalla*[13] in 1969 were also proposed to be biosynthesized by the same pathway through preclavulone-A as that for clavulones based on biosynthetic experiments[14,15] using homogenate preparations from *P. homomalla*. Recently, applying a genetic approach, Valmsen *et al.* reported evidence that the PGA_2-type prostaglandins in *P. homomalla* are not biosynthesized through the pathway mediated by lipoxygenase (LOX) shown in Chart 1, but through that mediated by cyclooxygenase (COX), similar to the biosynthetic pathway for mammalian prostaglandins.[16] We assume that the biosynthesis of clavulones in *C. viridis* occurs through the pathway through preclavulone-A rather than through the COX-mediated pathway for PGA_2-type prostaglandins in *P. homomalla* and mammalian prostaglandins, because clavulones have the (*Z*)-2-octenyl goup as the ω side chain instead of the common (*E*)-3-hydroxy-1-octenyl group in the prostaglandins found both in *P. homomalla* and mammals. Thus we attempted to discover biosynthetic intermediates from extracts of *C. viridis*, resulting in the isolation of preclavulone-A methyl ester (**1**) and its stereoisomer **2** as natural products along with the new prostanoid **3**. This paper discusses the structures of **1**, **2**, and **3** based on spectroscopic analysis. A possible biogenetic pathway from preclavulone-A to clavu-

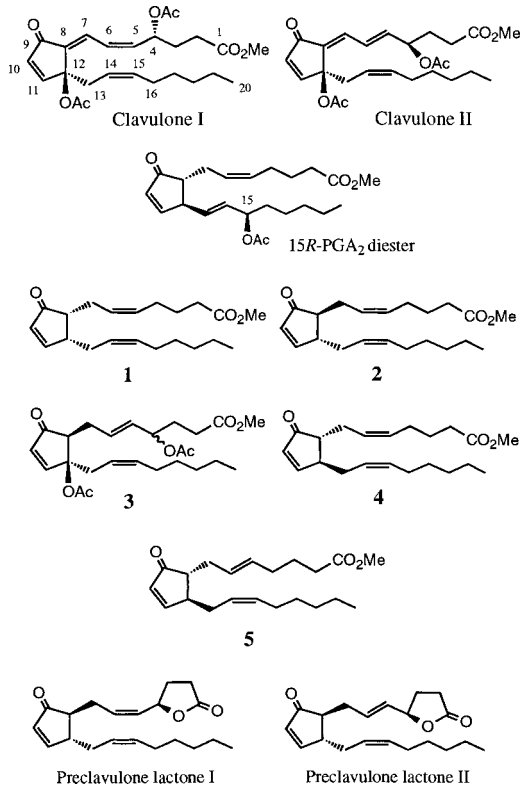

Fig. 1. Structures of Marine Prostanoids

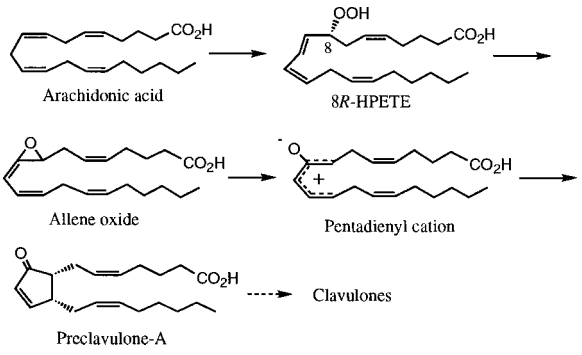

Chart 1. Biosynthetic Pathway for Clavulones Proposed by Corey *et al.*[12]

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lones is also described.

Repeated chromatographic separation and purification of the hexane extract (6.83 g) of freeze-dried *C. viridis* afforded the three compounds **1** (2.8 mg), **2** (0.8 mg), and **3** (1.2 mg) as minor constituents (see Experimental). The molecular formula of compound **1** [a colorless viscous oil, $[\alpha]_D$ −13.9° (THF)] was found to be $C_{21}H_{32}O_3$ from high-resolution electron impact MS (HR-EI-MS) measurement. The degrees of unsaturation (six) were obtained from the molecular formula. All 21 carbons appeared in the ^{13}C-NMR spectrum of **1** (Table 1). The distortionless enhancement by polarization transfer (DEPT) spectrum showed two methyls involving one methoxy group, nine sp^3 methylenes, two sp^3 methines, six sp^2 methines, and two sp^2 quaternary carbons. The presence of a cyclopentenone system was indicated by the UV absorption at 218 nm (ε 7800), ^{1}H-NMR in C_6D_6[17] [δ 5.99 (1H, dd, J=1.7, 5.9 Hz, H-10), 7.12 (1H, dd, J=2.9, 5.9 Hz, H-11)] (Table 2) and ^{13}C-NMR in C_6D_6[17] [δ 132.9 (CH, C-10), 165.7 (CH, C-11), 208.6 (C, C-9)] spectra. ^{1}H- and ^{13}C-NMR spectra also demonstrated the presence of a methoxycarbonyl group [^{1}H; δ 3.36 (3H, s), ^{13}C; δ 51.1 (OCH_3), 173.3 (CO)] and two di-substituted olefins [^{1}H; δ 5.31 (1H, br td, J=7.3, 10.8 Hz, H-5), 5.44 (1H, m, H-6), 5.21 (1H, br ddd, J=6.5, 8.2, 10.8 Hz, H-14), 5.44 (1H, m, H-15) : ^{13}C; δ 130.3 (CH, C-5), 129.5 (CH, C-6), 126.9 (CH, C-14), 132.4 (CH, C-15)]. These spectral data suggest that compound **1** is a prostanoid methyl ester.

After assignment of the direct bonding between ^{1}H and ^{13}C signals by heteronuclear multiple-quantum coherence (HMQC), the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) and heteronuclear multiple-bond correlation (HMBC) spectra were measured and analyzed to give a plane structure for **1**. The stereochemistry of the two di-substituted olefins on the side chains was determined by ^{1}H coupling constants and nuclear Overhauser effect (NOE) correlations. The coupling constant ($J_{5,6}$=10.8 Hz) disclosed the *Z* configuration for the olefin on the α side chain. The coupling constant ($J_{14,15}$=10.8 Hz) also indicated the *Z* configuration for the olefin on the ω side chain. These stereochemistries were supported by NOE correlations between H_2-4 [1.96 (2H, m)] and H-7 [2.65 (1H, br td)] for the double bond at C-5, and between H_2-13 [1.76 (1H, br td), 2.25 (1H, br td)] and H_2-16 [1.90 (2H, br q)] for the double bond at C-14. The stereochemistries of the two chiral centers at C-8 and C-12 are described below.

The molecular formula of compound **2** [a colorless viscous oil, $[\alpha]_D$ −49.8° (THF)] was found to be the same ($C_{21}H_{32}O_3$) as that of **1** based on the HR-EI-MS measurement. The NMR data of **2** (Tables 1—3) were very similar to those of **1** except for the signals due to the C-7, -8, -11, -12, and -13 positions, defining compound **2** to be a stereoisomer of **1** at the chiral centers. The 5*Z* and 14*Z* configurations of the two di-substituted olefins were indicated by the ^{1}H coupling constants ($J_{5,6}$=10.9 Hz, $J_{14,15}$=10.8 Hz) as well as NOE correlations (between H-4 and H-7, and H-13 and H-16). Therefore **2** should be a diastereomer of **1**.

The relative configuration of the chiral centers at C-8 and C-12 in **1** and **2** was deduced from the ^{1}H coupling constants. The value of $J_{8,12}$=3.7 Hz in **1** is somewhat greater than that of $J_{8,12}$=2.4 Hz in **2**. Although these data do not indicate the relative configurations of **1** and **2**, comparison of these data with those of preclavulone lactones I and II,[18] which were found in *C. viridis* and for which the relative as well as absolute configurations were established by enantioselective synthesis,[18] suggested the relative configurations of **1** and **2**. The coupling constant ($J_{8,12}$=2.3 Hz) of preclavulone lac-

Table 1. ^{13}C-NMR Data[a] for **1**, **2**, and **3**

No.	in $CDCl_3$			in C_6D_6		
	1	**2**	**3**	**1**	**2**	**3**
1	174.0 (C)	174.0 (C)	173.3 (C)	173.3 (C)	173.1 (C)	173.3 (C)
2	33.5 (CH_2)	33.4 (CH_2)	29.8 (CH_2)	33.5 (CH_2)	33.3 (CH_2)	29.91 (CH_2)[d]
3	24.6 (CH_2)	24.8 (CH_2)	29.5 (CH_2)	25.1 (CH_2)	25.1 (CH_2)	29.88 (CH_2)[d]
4	26.8 (CH_2)	27.3 (CH_2)	73.5 (CH)	27.2 (CH_2)	26.8 (CH_2)	73.6 (CH)
5	130.1 (CH)	130.9 (CH)	129.7 (CH)	130.3 (CH)	131.0 (CH)	129.8 (CH)
6	128.6 (CH)	127.0 (CH)	132.0 (CH)	129.5 (CH)	127.5 (CH)	132.6 (CH)
7	24.0 (CH_2)	29.2 (CH_2)	29.7 (CH_2)	24.5 (CH_2)	28.2 (CH_2)	30.0 (CH_2)
8	49.3 (CH)	50.7 (CH)	53.3 (CH)	49.5 (CH)	50.91 (CH)	53.4 (CH)
9	210.6 (C)	211.2 (C)	206.6 (C)	208.6 (C)	208.9 (C)	205.3 (C)
10	132.6 (CH)[b]	133.3 (CH)	134.3 (CH)	132.9 (CH)	133.5 (CH)	134.5 (CH)
11	167.2 (CH)	166.9 (CH)	161.0 (CH)	165.7 (CH)	165.1 (CH)	160.3 (CH)
12	44.4 (CH)	47.2 (CH)	87.5 (C)	44.5 (CH)	47.1 (CH)	87.5 (C)
13	28.6 (CH_2)	31.50 (CH_2)[c]	34.2 (CH_2)	29.0 (CH_2)	31.8 (CH_2)	34.4 (CH_2)
14	125.9 (CH)	125.4 (CH)	121.4 (CH)	126.9 (CH)	126.1 (CH)	122.2 (CH)
15	132.5 (CH)[b]	132.9 (CH)	135.2 (CH)	132.4 (CH)	132.5 (CH)	135.0 (CH)
16	27.4 (CH_2)	27.9 (CH_2)	27.5 (CH_2)	27.8 (CH_2)	27.5 (CH_2)	27.7 (CH_2)
17	29.1 (CH_2)	29.7 (CH_2)	29.1 (CH_2)	29.7 (CH_2)	29.6 (CH_2)	29.5 (CH_2)
18, 19	22.5 (CH_2)	22.5 (CH_2)	22.5 (CH_2)	23.1 (CH_2)	22.9 (CH_2)	22.9 (CH_2)
	31.5 (CH_2)	31.46 (CH_2)[c]	31.5 (CH_2)	32.0 (CH_2)	31.7 (CH_2)	31.8 (CH_2)
20	14.0 (CH_3)	14.0 (CH_3)	14.0 (CH_3)	14.4 (CH_3)	14.2 (CH_3)	14.2 (CH_3)
OCH_3	51.5 (CH_3)	51.5 (CH_3)	51.7 (CH_3)	51.1 (CH_3)	50.86 (CH_3)	51.1 (CH_3)
$CO\underline{CH_3}$			21.1 (CH_3)			20.7 (CH_3)
			21.6 (CH_3)			21.1 (CH_3)
$\underline{CO}CH_3$			169.9 (C)			169.27 (C)
			170.1 (C)			169.33 (C)

a) 125 MHz. *b*, *c*, *d*) Values with the same subscript may be interchanged.

Table 2. ^{1}H-NMR Data[a] for **1**, **2**, and **3** in C_6D_6

No.	1	2	3
1			
2	2.10 (2H, t, 7.4)	2.09 (2H, t, 7.4)	2.24 (2H, m)
3	1.60 (2H, m)	1.58 (2H, quint, 7.4)	1.97 (2H, m)
4	1.96 (2H, quint, 7.3)	1.95 (2H, q, 7.4)	5.42 (1H, q, 6.9)
5	5.31 (1H, br td, 7.3, 10.8)	5.31 (1H, br td, 7.4, 10.9)	5.41 (1H, m)
6	5.44 (1H, m)	5.38 (1H, br td, 7.3, 10.9)	5.90 (1H, m)
7	2.13 (1H, m)	2.30 (1H, br td, 7.3, 14.6)	2.38 (1H, td, 7.9, 14.3)
	2.65 (1H, br td, 6.0, 14.9)	2.51 (1H, br td, 7.3, 14.6)	2.66 (1H, td, 5.7, 14.3)
8	2.18 (1H, ddd, 3.7, 6.0, 10.0)	1.92 (1H, m)	2.32 (1H, dd, 5.1, 8.6)
9			
10	5.99 (1H, dd, 1.7, 5.9)	5.96 (1H, dd, 2.0, 5.7)	5.93 (1H, d, 5.9)
11	7.12 (1H, dd, 2.9, 5.9)	6.95 (1H, dd, 2.4, 5.7)	7.78 (1H, d, 5.9)
12	2.54 (1H, m)	2.36 (1H, qt, 2.4, 7.1)	
13	1.76 (1H, br td, 8.2, 14.0)	2.00 (1H, m)	2.75 (1H, br dd, 6.3, 14.3)
	2.25 (1H, br td, 6.5, 14.0)	2.02 (1H, br td, 7.1, 14.9)	2.88 (1H, br dd, 8.4, 14.3)
14	5.21 (1H, br ddd, 6.5, 8.2, 10.8)	5.25 (1H, br td, 7.1, 10.8)	5.16 (1H, m)
15	5.44 (1H, m)	5.46 (1H, br td, 7.4, 10.8)	5.54 (1H, br td, 7.4, 10.9)
16	1.90 (2H, br q, 7.1)	1.91 (2H, br q, 7.4)	2.03 (2H, quint, 7.0)
17—19	1.20—1.32 (6H, m)	1.21—1.32 (6H, m)	1.26—1.40 (6H, m)
20	0.88 (3H, t, 7.0)	0.89 (3H, t, 7.1)	0.94 (3H, t, 7.2)
OCH_3	3.36 (3H, s)	3.35 (3H, s)	3.37 (3H, s)
$COCH_3$			1.62 (3H, s), 1.70 (3H, s)

a) The spectra (500 MHz) were measured in C_6D_6. *J* in Hz.

tones with the *trans* relative configuration between H-8 and -12 corresponded with that ($J_{8,12}$=2.4 Hz) of **2**, indicating the *trans* configuration for **2** and thus the *cis* configuration for **1**. These findings demonstrate that compound **1** is a methyl ester of preclavulone-A.

(8*R*,12*R*)-Preclavulone-A methyl ester was previously synthesized[19] in enantiomerically pure form and had $[\alpha]_D$ −131.8° (THF). The ^{1}H-NMR data measured in $CDCl_3$ for the present compound **1** were identical with those[20] of the synthetic methyl ester. The $[\alpha]_D$ of preclavulone-A derived from biosynthetic experiments using the acetone powder of *C. viridis* was not measured due to its small amount,[11] and the preclavulone-A methyl ester obtained from biosynthetic experiments using the acetone powder of *P. homomalla* was found to be racemic.[15] Interestingly, compound **1** produced in the current study showed an $[\alpha]_D$ value of −13.9° (THF). This demonstrated compound **1** to be a mixture of 55% of the (8*R*,12*R*) isomer of preclavulone-A methyl ester and 45% of the enantiomeric (8*S*,12*S*) isomer, which was confirmed by HPLC analysis of **1** using a chiral column: two peaks appeared in a ratio of 54 : 46 after elution with hexane–2-propanol=99 : 1.

Compound **4** (Fig. 1) is the same plane structure as **1** and **2**, and has the *trans* configuration between C-8 and C-12; it was recently isolated from the Caribbean octocoral *Plexaura nina*.[21] The structure of **4** was previously reported to be the epimeric product obtained by treatment of preclavulone-A methyl ester with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU).[12] Compound **4** also formed along with preclavulone-A in biosynthetic experiments using the acetone powder of *P. homomalla*.[15] However, the NMR data of **2** were found to be different from those[22] of **4** isolated from *P. nina*. We examined the reported NMR data of **4** and believe that **4** has the *trans* olefin on the α side chain (structure **5**) instead of the *cis* olefin based on the fact that the ^{13}C-NMR chemical shift [δ 39.7 (t) ppm] of C-7 or -4 for **4** is too low to assign to the allylic methylene carbon of the *cis* olefin. The structure, as well as the optical purity of **2**, will be confirmed by our ongoing synthetic studies.

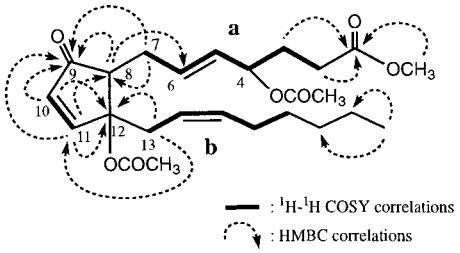

Fig. 2. ^{1}H–^{1}H COSY and Key HMBC Correlations of **3**

The molecular formula of compound **3** [a colorless viscous oil, $[\alpha]_D$ +22.8° ($CHCl_3$)] was found to be $C_{25}H_{36}O_7$ from high-resolution electron-spray ionization MS (HR-ESI-MS) measurement. The degrees of unsaturation (eight) were obtained from the molecular formula. All 25 carbons appeared in the ^{13}C-NMR spectrum of **1** (Table 1). The DEPT spectrum showed four methyls involving one methoxy group, eight sp^3 methylenes, two sp^3 methines, one sp^3 quaternary carbon, six sp^2 methines, and four sp^2 quaternary carbons. The presence of a conjugated cyclopentenone system was indicated by the UV absorption at 208 nm (ε 8900), ^{1}H-NMR in $CDCl_3$ (Table 3) [δ 6.21 (1H, d, *J*=5.9 Hz, H-10), 7.98 (1H, d, *J*=5.9 Hz, H-11)], and ^{13}C-NMR in $CDCl_3$ (Table 1) [δ 134.3 (CH, C-10), 161.0 (CH, C-11), 206.6 (C, C-9)] spectra. The ^{1}H- and ^{13}C-NMR spectra also demonstrated the presence of a methoxycarbonyl group [^{1}H; δ 3.67 (3H, s), ^{13}C; δ 51.7 (OCH_3), 173.3 (CO, C-1)], two acetoxyl groups [^{1}H; δ 2.02 (3H, s), 2.03 (3H, s), ^{13}C; δ 21.1 (CH_3), 21.6 (CH_3), 169.9 (CO), 170.1 (CO)], and two di-substituted olefins [^{1}H; δ 5.48 (1H, dd, *J*=7.1, 15.5 Hz, H-5), 5.82 (1H, td, *J*=6.3, 15.5 Hz, H-6), 5.19 (1H, m, H-14), 5.57 (1H, br td, *J*=7.3, 10.9 Hz, H-15), ^{13}C; δ 129.7 (CH, C-5), 132.0 (CH, C-6), 121.4 (CH, C-14), 135.2 (CH, C-15)]. The NMR data of **3** were similar to those of clavulone II[1] except for the sig-

nals due to the C-7 and -8 positions, suggesting **3** to be a 7,8-dihydro congener of clavulone II.

After direct ^{1}H–^{13}C correlations were established from the HMBC spectrum, the plane structure of **3** was elucidated on the basis of the analysis of the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 2). The partial structures of **a** and **b** on the side chains as depicted by the bold lines in Fig. 2 were revealed by the sequential correlations obtained from the ^{1}H–^{1}H COSY spectrum. The connectivity between H-10 and H-11 on the cyclopentenone was also confirmed by ^{1}H–^{1}H COSY analysis. The location of two acetoxyl groups at C-4 and C-12 was indicated by the NMR data (in $CDCl_3$) [for C-4, ^{1}H; δ 5.24 (1H, q, J=7.1 Hz), ^{13}C; δ 73.5 (CH) and for C-12, ^{13}C; δ 87.5 (C)]. The presence of the cyclopentenone moiety bearing a tertiary acetoxyl group was indicated by the HMBC correlations from the methine proton (H-8) to the carbonyl carbon (C-9), and from each olefinic proton (H-10 and -11) to the carbonyl (C-9) and the quaternary (C-12) carbons. Further HMBC correlations from the methine proton (H-8) to the olefinic carbon (C-6) on the α-side chain, and from the methylene protons (H_2-7) to the methine (C-8) and carbonyl (C-9) carbons, demonstrated the connectivity of the partial structure **a** to the methine carbon at C-8 of the cyclopentenone. Similarly, the connectivity of the partial structure **b** to the quaternary carbon at C-12 was indicated by the HMBC correlations from the methylene protons (H_2-13) on the ω-side chain to the olefinic (C-11) and quaternary (C-12) carbons. The HMBC correlations from the methylene protons (H_2-2 and H_2-3) to the carbonyl carbon (C-1) indicated the connectivity of the methoxycarbonyl group to the partial structure **a**. Finally, a terminal propyl group should be connected to the partial structure **b** to complete the prostanoid structure of **3**.

The (5*E*,14*Z*) configurations of two di-substituted double bonds were determined on the basis of the ^{1}H coupling constants and the NOE correlation. The ^{1}H coupling constant between the olefinic protons, H-5 and H-6 ($J_{5,6}$=15.5 Hz, Table 3), indicated an *E*-configuration, while that between H-14 and H-15 ($J_{14,15}$=10.9 Hz, Table 3) indicated a *Z*-configuration. The NOE correlations between allylic protons (H_2-13

Table 3. ^{1}H-NMR Data[a] for **1**, **2**, and **3** in $CDCl_3$

No.	**1**	**2**	**3**
1			
2	2.32 (2H, t, 7.6)	2.31 (2H, t, 7.4)	2.36 (2H, t, 7.1)
3	1.71 (2H, m)	1.69 (2H, quint, 7.4)	1.96 (2H, q, 7.1)
4	2.10 (2H, quint, 8.0)	2.09 (2H, q, 7.4)	5.24 (1H, q, 7.1)
5	5.43 (1H, m)	5.43 (1H, br td, 7.4, 10.8)	5.48 (1H, dd, 7.1, 15.5)
6	5.47—5.54 (1H, m)[b]	5.31—5.39 (1H, m)[c]	5.82 (1H, td, 6.3, 15.5)
7	2.16 (1H, br td, 7.9, 15.1)	2.27 (1H, m)[d]	2.35 (1H, m)
	2.53 (1H, br td, 6.2, 15.1)	2.47 (1H, br td, 5.6, 14.5)	2.49 (1H, br td, 6.3, 13.0)
8	2.46 (1H, ddd, 5.0, 6.2, 7.9)	2.03 (1H, ddd, 2.3, 5.6, 7.9)	2.38 (1H, m)
9			
10	6.18 (1H, dd, 1.7, 5.8)	6.15 (1H, dd, 1.9, 5.8)	6.21 (1H, d, 5.9)
11	7.68 (1H, dd, 2.8, 5.8)	7.58 (1H, dd, 2.4, 5.8)	7.98 (1H, d, 5.9)
12	3.03 (1H, m)	2.64 (1H, qt, 2.3, 7.0)	
13	1.91 (1H, m)	2.27 (2H, m)[d]	2.78 (1H, br dd, 6.9, 14.6)
	2.44 (1H, m)		2.83 (1H, br dd, 8.4, 14.6)
14	5.38 (1H, m)	5.31—5.39 (1H, m)[c]	5.19 (1H, m)
15	5.47—5.54 (1H, m)[b]	5.51 (1H, br td, 7.4, 10.9)	5.57 (1H, br td, 7.3, 10.9)
16	1.97 (2H, q, 7.2)	2.00 (2H, q, 7.4)	2.01 (2H, q, 7.3)
17	1.32 (2H, quint, 7.2)	1.34 (2H, quint, 7.4)	1.33 (2H, m)
18, 19	1.24—1.31 (4H, m)	1.24—1.32 (4H, m)	1.24—1.31 (4H, m)
20	0.88 (3H, t, 6.8)	0.89 (3H, t, 6.8)	0.89 (3H, t, 7.2)
OCH_3	3.66 (3H, s)	3.67 (3H, s)	3.67 (3H, s)
$COCH_3$			2.02 (3H, s), 2.03 (3H, s)

a) The spectra (500 MHz) were measured in $CDCl_3$. *J* in Hz. *b*, *c*, *d*) The signals were overlapped and unresolved.

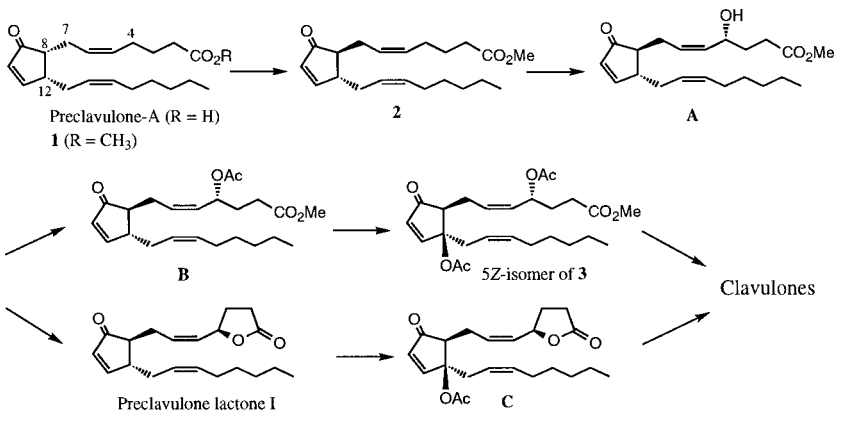

Chart 2. Possible Biogenetic Pathway from Preclavulone-A to Clavulones

and H_2-16) observed in the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum confirmed the *Z*-configuration for the double bond at C-14.

The *trans* orientation of the α- and ω-side chains on the cyclopentenone ring was concluded from the NOE correlations between H-8 and H_2-13. The relative stereochemistry at C-4 as well as the absolute stereochemistries of the three chiral centers at C-4, -8, and -12 will be determined by enantioselective synthesis.

Isolation of the prostanoids **1**, **2**, and **3** coupled with the previous isolation of preclavulone lactones[18] suggests a possible biogenetic pathway from preclavulone-A to clavulones. Clavulones must be converted from preclavulone-A through a three-step oxidation (oxygenation at C-4, dehydration between C-7 and C-8, and oxygenation at C-12). The previous isolation of preclavulone lactones led us to propose the oxygenation at C-4 as the first oxidation step.[18] The isolation of **3** in the present study indicates that the second oxidation step is oxygenation at C-12. The possible biogenesis is summarized in Chart 2. Isomerization at C-8 of preclavulone-A or its methyl ester **1** may provide the *trans* isomer **2**. Oxygenation at C-4 in **2** gives the hydroxy intermediate **A**, which may be esterified to provide the ester intermediate **B** and/or preclavulone lactone I. Further oxygenation (or acetoxylation) at C-12 gives a diester **3** and/or a lactonic acetate intermediate **C**, which may be converted to clavulones.

Experimental

Optical rotations were measured on a JASCO DIP-370 automatic polarimeter. UV spectra were recorded with a JASCO V-520 spectrophotometer. Mass (MS) spectra were recorded with a Micromass Auto Spec spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, with a Bruker DRX-500 in C_6D_6 or $CDCl_3$. Proton chemical shifts were referenced to the residual C_6H_6 signal at δ 7.20 ppm or $CHCl_3$ signal at δ 7.26 ppm (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br, broad). Carbon chemical shifts were referenced to the central peak of C_6D_6 at δ 128.0 ppm or $CDCl_3$ at δ 77.0 ppm. Two-dimensional (2D) NMR spectra (^{1}H–^{1}H COSY, HMQC, HMBC, NOESY) were measured with a Bruker DRX-500 using standard Bruker pulse sequences. Liquid column chromatography (LCC) was carried out on a Merck Si gel 60 (particle size 0.063—0.200 mm). HPLC was performed on 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-343-5, reverse phase). The HPLC system was equipped with a recycle loop, which was used depending on need. The enantiomeric ratio was analyzed with HPLC using a CHIRALCELL OD-H column (DAICEL).

Extraction and Isolation The soft coral *C. viridis* Quoy and Gaimard (order Stolonifera, family Clavulariidae) was collected from a coral reef off Ishigaki Island, Okinawa Prefecture, Japan, in March 1988, at a depth of 1—2 m. The freeze-dried specimens were stored at −50 °C. A voucher specimen has been deposited at Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Freeze-dried specimens (470 g) were extracted successively with hexane (2 l×2), EtOAc (2 l×2), and MeOH (2 l×2). After filtration, each extract was concentrated under reduced pressure to give hexane (14.5 g), EtOAc (3.7 g), and MeOH (33.4 g) extracts. Part of the hexane extract (6.83 g) was chromatographed on a silica gel column eluted with hexane (1 l), hexane–EtOAc (3 : 1, 850 ml and 1 : 1, 700 ml), EtOAc (700 ml), and MeOH (700 ml), in succession.

The third fraction [0.77 g, eluted with hexane–EtOAc (1 : 1)] containing mainly clavulones I, II, and III was separated by reverse-phase HPLC [eluted with CH_3CN–H_2O (8 : 2)] to give clavulone II (281 mg) and a mixture of clavulones I and III (325 mg).

The second fraction [4.54 g, eluted with hexane–EtOAc (3 : 1)] was separated by normal-phase LCC eluted with hexane–EtOAc (9 : 1, 8 : 2, 7 : 3) to give seven fractions (fractions A—G). Repeated separation and purification of fraction C (306 mg) by normal-phase [eluent; hexane–ether (6 : 4)] and reverse-phase [eluent CH_3CN–H_2O (8 : 2)] recycling HPLC afforded compounds **1** (2.8 mg) and **2** (0.8 mg). Further separation and purification of fraction F (636 mg) afforded compound **3** (1.2 mg), along with halogenated prostanoids previously reported.[23]

Compound **1**: Colorless viscous oil; $[\alpha]_D^{25}$ −13.9° (*c*=0.05, THF), −17.9° (*c*=0.05, $CHCl_3$); UV (MeOH) λ_{max} 218 (ε 7800) nm; ^{13}C- and ^{1}H-NMR, see Tables 1, 2, and 3; HR-EI-MS (*m*/*z*) 332.2354 $[M]^+$ (Calcd for $C_{21}H_{32}O_3$, 332.2351).

Compound **2**: Colorless viscous oil; $[\alpha]_D^{25}$ −49.8° (*c*=0.08, THF), −51.0° (*c*=0.08, $CHCl_3$); UV (MeOH) λ_{max} 216 (ε 9900) nm; ^{13}C- and ^{1}H-NMR, see Tables 1, 2, and 3; HR-EI-MS (*m*/*z*) 332.2354 $[M]^+$ (Calcd for $C_{21}H_{32}O_3$, 332.2351).

Compound **3**: Colorless viscous oil; $[\alpha]_D^{25}$ +22.8° (*c*=0.08, $CHCl_3$); UV (MeOH) λ_{max} 208 (ε 8900) nm; ^{13}C- and ^{1}H-NMR, see Tables 1, 2, and 3; HR-ESI-MS (*m*/*z*) 471.2359 $[M+Na]^+$ (Calcd for $C_{25}H_{36}O_7Na$, 471.2388).

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22) The ^{1}H- and ^{13}C-NMR spectra of **4** were measured in $CDCl_3$. The synthesis of the compound with structure **4** was reported in ref. 11, but NMR data were not given.
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