Tricycloclavulone and Clavubicyclone, Novel Prostanoid-Related Marine Oxylipins, Isolated from the Okinawan Soft Coral *Clavularia viridis*

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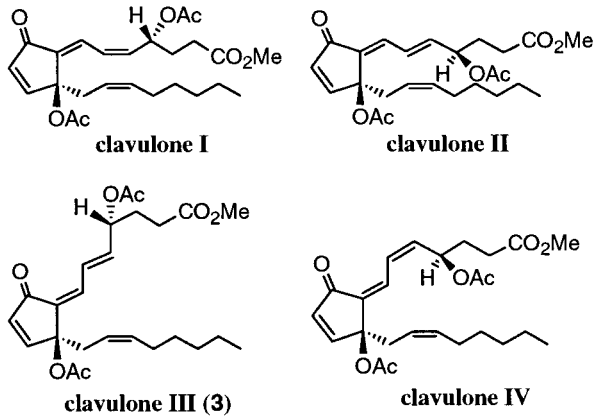
Received November 1, 2001

Two novel prostanoid-related marine oxylipins, tricycloclavulone (**1**) and clavubicyclone (**2**), were isolated from the Okinawan soft coral *Clavularia viridis*. The structures of **1**, having a tricyclo-[5.3.0.0^{1,4}]decane ring system, and **2**, having a bicyclo[3.2.1]octane ring system, were elucidated on the basis of spectroscopic analysis. Clavubicyclone showed a moderate growth inhibition activity against tumor cells in vitro.

Introduction

The Okinawan soft coral *Clavularia viridis* Quoy and Gaimard (class Anthozoa, subclass Octocorallia, order Stolonifera) has been recognized as a rich source for bioactive marine prostanoids such as clavulones[1] (Chart 1) and chlorovulones.[2] These marine prostanoids have attracted much attention because of their unique structural features, biosynthesis,[3] and biological activities.[1,2,4,5] We recently reported new members of prostanoids[6] and related compounds[7] (oxylipins[5]) from *C. viridis*. Our further investigation focused on biologically active prostanoids and oxylipins from Okinawan *C. viridis* has resulted in the discovery of new types of oxylipins, tricycloclavulone (**1**) and clavubicyclone (**2**), as minor components (Chart 2). The structure of **1** possessing a tricyclo[5.3.0.0^{1,4}]decane ring system and that of **2** containing a bicyclo[3.2.1]octane ring system were elucidated on the basis of the spectroscopic analysis. The skeletal carbon frameworks of **1** and **2** are unprecedented in the field of natural products. In the present paper, we describe the results of isolation, structural determination, biological activity, and possible biogenesis for new oxylipins.

Chart 1



Results and Discussion

Isolation of Tricycloclavulone (1) and Clavubicyclone (2). Wet specimens of *C. viridis*, collected on the coral reef of Ishigaki Island (Okinawa, Japan) in December 1995, were immersed in methanol. The methanol extract was partitioned between ethyl acetate (EtOAc) and water (H_2O) to afford an EtOAc-soluble portion, which was chromatographed on a silica gel column by elution with a hexanes−EtOAc gradient and finally methanol to afford nine fractions. The fifth fraction [eluted with hexanes−EtOAc (2:1)] was purified by separations using flash silanized (C_2) silica gel column chromatography, medium-pressure liquid chromatography (MPLC, normal and reversed phase), and recycled HPLC (normal phase) to give tricycloclavulone (**1**, 0.0001%

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(1) (a) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett*. **1982**, *23*, 5171. (b) Kobayashi, M.; Yasuzawa, T.; Yoshihara, M.; Akutsu, H.; Kyogoku, Y.; Kitagawa, I. *Tetrahedron Lett*. **1982**, *23*, 5331. (c) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett*. **1983**, *24*, 1549. (d) Iguchi, K.; Yamada, Y.; Kikuchi, H.; Tsukitani, Y. *Tetrahedron Lett*. **1983**, *24*, 4433. (e) Kitagawa, I.; Kobayashi, M.; Yasuzawa, T.; Son, B. W.; Yoshihara, M.; Kyogoku, Y. *Tetrahedron* **1985**, *41*, 995.

(2) (a) Iguchi, K.; Kaneta, S.; Mori, K.; Yamada, Y.; Honda, A.; Mori, Y. *Tetrahedron Lett*. **1985**, *26*, 5787. (b) Nagaoka, H.; Iguchi, K.; Miyakoshi, T.; Yamada, N.; Yamada, Y. *Tetrahedron Lett*. **1986**, *27*, 223. (c) Iguchi, K.; Kaneta, S.; Mori, K.; Yamada, Y.; Honda, A.; Mori, Y. *J. Chem. Soc., Chem. Commun*. **1986**, 981.

(3) (a) Corey, E. J.; Lansbury, P. T., Jr.; Yamada, Y. *Tetrahedron Lett*. **1985**, *26*, 4171. (b) Corey, E. J.; d'Alarcao, M.; Matsuda, S. P. T.; Lansbury, P. T., Jr. *J. Am. Chem. Soc*. **1987**, *109*, 289. (c) Corey, E. J.; Matsuda, S. P. T. *Tetrahedron Lett*. **1987**, *28*, 4247. (d) Corey, E. J.; Matsuda, S. P. T.; Nagata, R.; Cleaver, M. B. *Tetrahedron Lett*. **1988**, *29*, 2555.

(4) (a) Honda, A.; Yamamoto, Y.; Mori, Y.; Yamada, Y.; Kikuchi, H. *Biochem. Biophys. Res. Commun*. **1985**, *130*, 515. (b) Honda, A.; Mori, Y.; Iguchi, K.; Yamada, Y. *Mol. Pharmacol*. **1987**, *32*, 530. (c) Bader, Th.; Yamada, Y.; Ankel, H. *Antiviral. Res*. **1991**, *16*, 341.

(5) Gerwick, W. H. *Chem. Rev*. **1993**, *93*, 1807. In this review, the term oxylipin was proposed as a general name for prostanoids, eicosanoids, and other related compounds.

(6) (a) Iwashima, M.; Watanabe, K.; Iguchi, K. *Tetrahedron Lett*. **1997**, *48*, 8319. (b) Iwashima, M.; Konno, F.; Iguchi, K. *J. Nat. Prod*. **1999**, *62*, 352. (c) Iwashima, M.; Okamoto, K.; Miyai, Y.; Iguchi, K. *Chem. Pharm. Bull*. **1999**, *47*, 884.

(7) Iwashima, M.; Okamoto, K.; Iguchi, K. *Tetrahedron Lett*. **1999**, *40*, 6455.

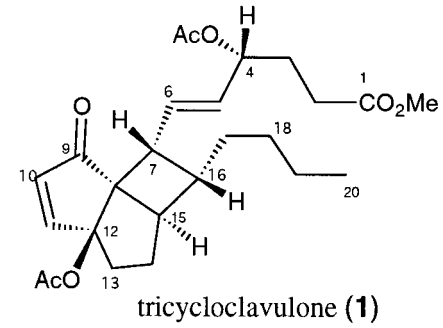
10.1021/jo011043g CCC: $22.00
Published on Web 04/03/2002

Table 1. ^{13}C and ^{1}H NMR Data for Tricycloclavulone (1)[a]

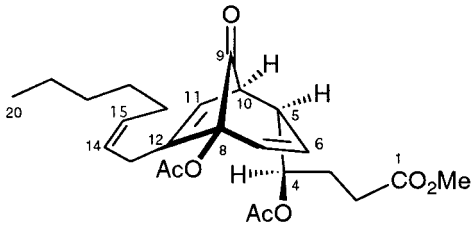
position	δ_C	δ_H	position	δ_C	δ_H
1	173.6 (C)		14	27.9 (CH_2)	1.54 (1H, td, 6.8, 13.8)
2	29.9 (CH_2)	2.34 (2H, t, 7.1)			1.61 (1H, dd, 7.8, 13.8)
3	29.6 (CH_2)	1.96 (2H, m)	15	49.5 (CH)	2.41 (1H, t, 6.8)
4	73.6 (CH)	5.32 (1H, dt, 6.5, 7.1)	16	37.1 (CH)	2.08 (1H, m)
5	129.0 (CH)	5.32 (1H, dd, 7.1, 14.3)	17	30.9 (CH_2)	1.41 (2H, m)
6	131.2 (CH)	6.69 (1H, ddd, 3.9, 10.1, 14.3)	18	29.3 (CH_2)	1.13 (2H, m)
7	40.5 (CH)	3.17 (1H, t, 10.1)	19	22.7 (CH_2)	1.27 (2H, m)
8	60.2 (C)		20	14.1 (CH_3)	0.86 (3H, t, 7.3)
9	205.0 (C)		OCH_3	51.6 (CH_3)	3.66 (3H, s)
10	136.8 (CH)	6.14 (1H, d, 5.8)	CH_3CO	170.0 (C)	
11	156.8 (CH)	7.61 (1H, d, 5.8)	CH_3CO	170.1 (C)	
12	93.9 (C)		CH_3CO	21.2 (CH_3)	2.08 (3H, s)
13	34.2 (CH_2)	2.25 (1H, m)	CH_3CO	21.5 (CH_3)	2.07 (3H, s)
		2.63 (1H, dd, 6.8, 13.9)			

[a] ^{13}C NMR, 125 MHz in $CDCl_3$; ^{1}H NMR, 500 MHz in $CDCl_3$; *J* in hertz. Assignments of the ^{13}C and ^{1}H signals were made based on HMQC.

Chart 2



tricycloclavulone (**1**)



clavubicyclone (**2**)

based on the methanol extract). Clavubicyclone (**2**, 0.0036%) was independently isolated from another collection of *C. viridis* in June 1998 by a purification procedure similar to that for **1**. Clavulones were isolated as the major metabolites in each collection.

Structure Elucidation of Tricycloclavulone (1). The molecular formula of tricycloclavulone (**1**) was assigned as $C_{25}H_{34}O_7$ on the basis of HREIMS analysis. This molecular furmula of **1** was the same as that of clavulones; however, the ^{1}H and ^{13}C NMR spectra of **1** (Table 1) were different from those of clavulones. All 25 carbons appeared in the ^{13}C NMR spectrum of **1**. The DEPT spectrum indicated four methyls including one methoxyl, seven sp^3 methylenes, four sp^3 methines including one oxymethine, four sp^2 methines, two sp^3 quaternary carbons, and four carbonyls. The IR, ^{1}H NMR, and ^{13}C NMR spectra of **1** showed the presence of a cyclopentenone [IR 1715 cm^{-1}; δ_H 6.14 (1H, d, J = 5.8 Hz), 7.61 (1H, d, J = 5.8 Hz) ppm; δ_C 136.8 (CH), 156.8 (CH), 205.0 (CO) ppm], a methyl ester [IR 1732 (or 1738) cm^{-1}; δ_H 3.66 (3H, s) ppm; δ_C 51.6 (CH_3), 173.6 (CO) ppm], two acetoxyls [IR 1738 (or 1732), 1238 cm^{-1}; δ_H 2.07 (3H, s), 2.08 (3H, s) ppm; δ_C 21.2 (CH_3), 21.5 (CH_3), 170.0 (CO), 170.1 (CO) ppm], and a trans-disubstituted olefin [δ_H 5.32 (1H, dd, J = 7.1, 14.3 Hz), 6.69 (1H, ddd, J = 3.9, 10.1, 14.3 Hz) ppm; δ_C 129.0 (CH), 131.2 (CH) ppm]. The ^{1}H and ^{13}C signals [δ_H 5.32 (1H, dt, J = 6.5, 7.1 Hz) ppm; δ_C 73.6 (CH) ppm] due to the oxymethine group suggested that one of acetoxyl groups was located on this carbon, and the ^{13}C signal [δ_C 93.9 (C) ppm] due to the quarternary carbon indicated that the other acetoxyl group was on this carbon. HMQC analysis revealed the complete assignment of each C−H bonding in **1**. Sequential $^{1}H-^{1}H$ correlations from H-2 to H-7 on the one side chain, from H-10 to H-11 in the cyclopentenone ring, and from H-13 to H-20 on the other side chain were observed in the $^{1}H-^{1}H$ COSY spectrum. These findings allowed four partial structures, **a**, **b**, **c**, and **d**, to be determined for **1**, as shown in Figure 1.

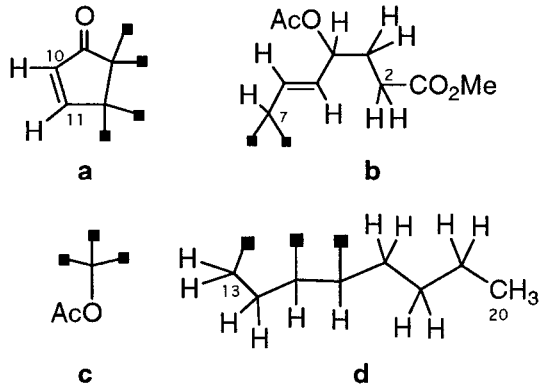


Figure 1. Partial structures for **1**.

To connect each of the partial structures for constructing the gross structure, HMBC analysis was undertaken; the key correlations observed in the HMBC spectrum are shown in Figure 2. The following HMBC correlations gave the connections for partial structures **a**, **b**, **c**, and **d**, leading to the unique gross structure for **1** possessing the tricyclo[5.3.0.$0^{1,4}$]decane ring system: from H-7 to C-5, 9, 12, 15, and 16; from each proton at H-10 and 11 to C-8, 9 and 12; from one of H-13 to C-8, 11, and 15; from one of H-14 to C-8, 12, and 16; from H-15 to C-9, 12, 13 and 17; and from H-17 to C-7 and 15.

The relative stereochemistry of **1** was deduced on the basis of the NOESY analysis (Figure 3). The NOE correlation between H-6 and H-17 indicated the cis configuration of each side chain. The relative configuration of the core tricyclic ring was also disclosed by the following NOESY analysis: correlations between H-6 and H-15, between H-7 and the acetoxyl at C-12, between

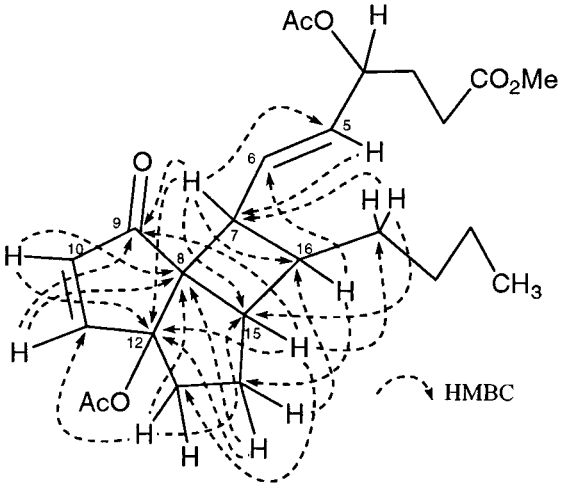


Figure 2. Gross structure and HMBC for **1**.

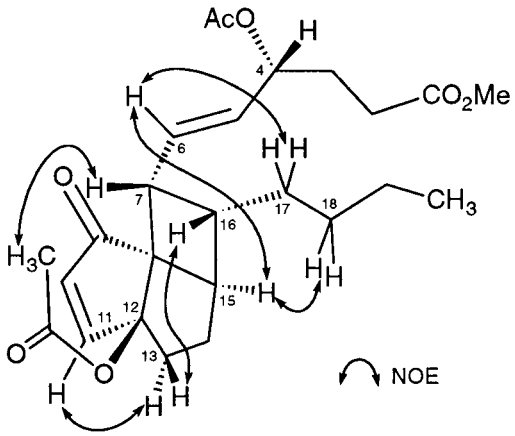


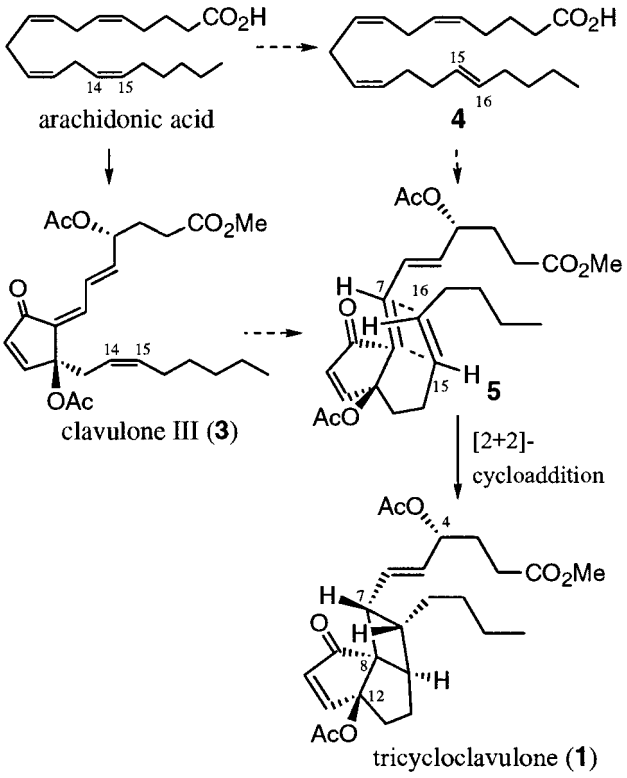
Figure 3. Relative stereochemistry of **1** and key NOE correlations.

H-11 and H-13α, between H-13β and H-16, and between H-15 and H-18. The stereochemistry of the chiral center at C-4 bearing the secondary acetoxyl group in the side chain is still unclear, because no NOE correlation was observed from H-4 to the other stereogenic center in **1**. However the same *S* configuration at C-4 in **1** as that at C-4 in clavulones[1c] can be assigned, since clavulone III (**3**) is assumed as a biosynthetic precursor for **1**, as described below.

A possible biogenesis for **1** is proposed in Scheme 1. The characteristic ring system for **1** may be formed from the biogenetic intermediate **5** by the [2 + 2]-cycloaddition between the (*Z*) double bond at C-7 of the α-side chain and the (*E*) double bond at C-15 of the ω-side chain in **5**. The intermediate **5** may be formed through either of the two pathways. One is the pathway from clavulone III (**3**) by a migration of the double bond at C-14 in **3** to C-15. The other is that from the precursor **4** having the (*E*) double bond at C-15, which may be derived from arachidonic acid by the double bond migration at C-14.

Structure Elucidation of Clavubicyclone (2). The molecular formula of clavubicyclone (**2**) was assigned as $C_{25}H_{34}O_7$ on the basis of HREIMS analysis. This molecular formula of **2** was equal to that of **1** and clavulones, but the ^{1}H and ^{13}C NMR spectra of **2** were much different from those of **1** and clavulones[1] (Table 2). ^{13}C NMR and DEPT spectra showed the presence of four methyls including one methoxyl, seven sp^3 methylenes, three sp^3 methines including one oxymethine, five sp^2 methines, one sp^3 quaternary carbon with oxygen functionality, and five sp^2 quaternary carbon including four carbonyls. The ^{1}H NMR spectrum in $CDCl_3$ was slightly complicated by overlaps of signals in the high-field area. Therefore, the ^{1}H NMR spectrum was again recorded in C_6D_6, yielding a crucial signal dispersion, which was helpful for the analysis of the ^{1}H NMR data in $CDCl_3$. The IR, ^{1}H NMR, and ^{13}C NMR data of **2** showed the presence of a cyclopentanone [IR 1744 cm^{-1}; δ_C 203.0 (CO) ppm], a methyl ester [IR 1732 cm^{-1}; δ_H 3.68 (3H, s) ppm; δ_C 51.8 (OCH_3), 173.0 (CO) ppm], a secondary acetoxyl [IR 1732, 1231 cm^{-1}; δ_H 2.05 (3H, s) or 2.16 (3H, s), 4.97 (1H, dt, J = 3.1, 8.6 Hz) ppm; δ_C 20.9 (CH_3) or 20.7 (CH_3), 72.4 (CH), 168.8 (CO) or 170.4 (CO) ppm], a tertiary acetoxyl [IR 1732, 1231 cm^{-1}; δ_H 2.16 (3H, s) or 2.05 (3H, s) ppm; δ_C 20.7 (CH_3) or 20.9 (CH_3), 82.7 (C), 170.4 (CO) or 168.8 (CO) ppm], two cis-disubstituted olefins [δ_H 5.37 (1H, ddd, J = 6.8, 7.8, 10.7 Hz), 5.54 (1H, td, J = 7.3, 10.7 Hz), 5.40 (1H, td, J = 2.2, 10.0 Hz), 6.17 (1H, dd, J = 2.5, 10.0 Hz) ppm; δ_C 123.6 (CH), 133.1 (CH), 125.1 (CH), 135.0 (CH) ppm], and a trisubstituted olefin [δ_H 5.70 (1H, br dd, J = 1.6, 3.1 Hz) ppm; δ_C 118.0 (CH), 154.6 (C) ppm]. These spectroscopic findings together with the ^{1}H−^{1}H COSY and HMQC analyses disclosed four partial structures, **e**, **f**, **g**, and **h**, for **2**, as shown in Figure 4.

Scheme 1. Proposed Biogenesis for Tricycloclavulone (1)



To verify the gross structure of **2**, HMBC experiments were conducted in both $CDCl_3$ and C_6D_6. Combined key HMBC correlations are shown in Figure 5. Important HMBC correlations were detected from H-6 to C-8 (acetoxyl quaternary carbon); from H-7 to C-9 (carbonyl carbon); from H-10 to C-9 and 12 (olefinic quaternary carbon); from H-11 to C-8, 9, and 13; and from H-13 (two protons) to C-8 and 11. These observation provided the connectivities among the partial structures **e**, **f**, **g**, and

Table 2. ^{13}C and ^{1}H NMR Data of Clavubicyclone (2)[a]

	$CDCl_3$		C_6D_6	
position	δ_C	δ_H	δ_C	δ_H
1	173.0 (C)		172.4	
2	29.5 (CH_2)	2.32 (2H, m)	29.4	2.07 (2H, t, 7.5)
3	26.9 (CH_2)	1.82 (1H, dt, 8.6, 14.7)	26.9	1.45 (1H, dtd, 1.9, 7.5, 8.8)
		2.04 (1H, m)		1.80 (1H, m)
4	72.4 (CH)	4.97 (1H, dt, 3.1, 8.6)	72.2	5.03 (1H, dt, 3.0, 8.8)
5	45.2 (CH)	2.83 (1H, m)	45.6	2.57 (1H, m)
6	125.1 (CH)	5.40 (1H, td, 2.2, 10.0)	125.5	5.23 (1H, td, 2.2, 10.0)
7	135.0 (CH)	6.17 (1H, dd, 2.5, 10.0)	135.3	6.20 (1H, dd, 2.4, 10.0)
8	82.7 (C)		83.0	
9	203.0 (C)		202.2	
10	49.5 (CH)	3.19 (1H, br s)	49.7	2.99 (1H, br s)
11	118.0 (CH)	5.70 (1H, br dd, 1.6, 3.1)	118.4	5.68 (1H, br dd, 1.5, 3.1)
12	154.6 (C)		154.8	
13	26.5 (CH_2)	2.80 (1H, br dd, 6.8, 17.0)	27.1	2.93 (1H, br dd, 6.2, 17.0)
		2.91 (1H, br dd, 7.8, 17.0)		3.02 (1H, br dd, 7.4, 17.0)
14	123.6 (CH)	5.37 (1H, ddd, 6.8, 7.8, 10.7)	124.4	5.53 (1H, m)
15	133.1 (CH)	5.54 (1H, td, 7.3, 10.7)	133.2	5.58 (1H, td, 7.1, 10.8)
16	27.1 (CH_2)	2.01 (2H, q, 7.3)	27.5	2.01−2.19 (2H, m)
17	29.1 (CH_2)	1.30 (2H, m)	29.6	1.34 (2H, m)
18	31.5 (CH_2)	1.30 (2H, m)	31.8	1.34 (2H, m)
19	22.5 (CH_2)	1.30 (2H, m)	22.9	1.34 (2H, m)
20	14.0 (CH_3)	0.88 (3H, t, 6.9)	14.2	0.93 (3H, t, 6.9)
OCH_3	51.8 (CH_3)	3.68 (3H, s)	51.2	3.39 (3H, s)
CH_3CO	20.9 (CH_3)	2.05 (3H, s)	20.3	1.61 (3H, s)
CH_3CO	20.7 (CH_3)	2.16 (3H, s)	20.2	1.81 (3H, s)
CH_3CO	170.4 (C)		169.6	
CH_3CO	168.8 (C)		168.3	

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

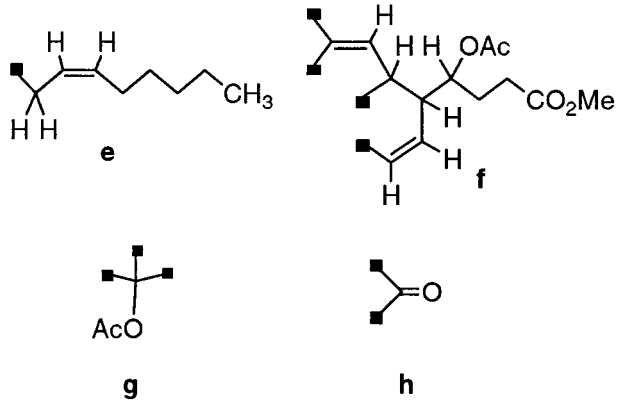


Figure 4. Partial structures for **2**.

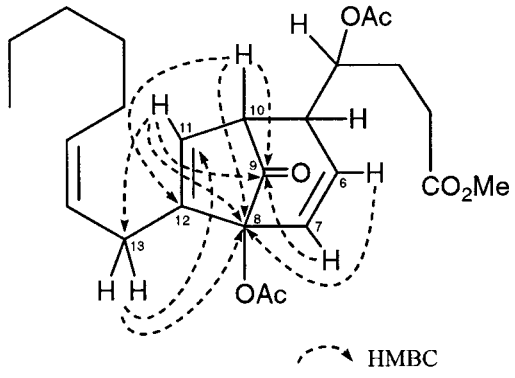


Figure 5. Gross structure and HMBC for **2**.

h, leading to the gross structure of **2** possessing the bicyclo[3.2.1]octane ring system.

The NOESY analysis revealed the stereochemistry of the double bond at C-14 in the side chain and the relative configuration at C-5 bearing the other side chain, as shown in Figure 6. The NOE correlation between H-13 and H-16 confirmed the cis stereochemistry of the double bond at C-14. The NOE correlation between H-4 and H-11 indicated the anti orientation of the side chain connected at C-5 against the carbonyl bridge in the bicyclo[3.2.1]octane. The alternative stereochemistry at C-5 was inconsistent with the NOE correlation observed. The stereochemistry at C-4 on the side chain was not determined by NOE analysis, but the relative as well as absolute stereochemistry at C-4 in **2** must be the same as those of clavulones by considering the biogenesis for **2** as described below.

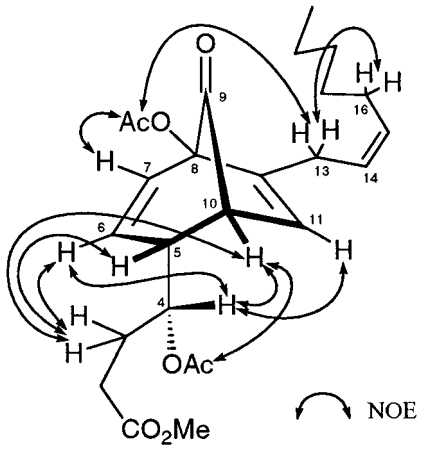
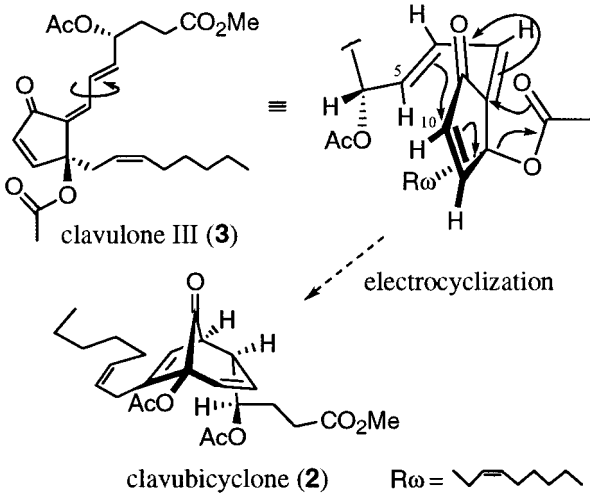


Figure 6. Relative stereochemistry of **2** and key NOE correlations.

Although it is somewhat difficult to propose a plausible biogenesis for **2** possessing an unprecedented structure, we dare to describe a biogenesis in Scheme 2: an electrocyclization reaction may take place in clavulone III (**3**) to give **2**.

Biological Activity. Clavubicyclone (**2**) showed a moderate growth inhibition activity toward cultured

Scheme 2. Proposed Biogenesis for Clavubicyclone (2)



tumor cells, breat carcinoma MCF-7 (IC_{50} 2.7 μg/mL), and ovarian carcinoma OVCAR-3 (IC_{50} 4.5 μg/mL). Further investigations for the bioactivity of **2** as well as tricycloclavulone (**1**) are currently being conducted.

Experimental Section

General Methods. IR spectra were recorded with a FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC experiments were measured with a 400 or 500 MHz FT-NMR spectrometer. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.0 ppm) or C_6H_6 (^{1}H, 7.20 ppm; ^{13}C, 128.0 ppm) as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Column chromatography was carried out on silica gel 60 (70−230 mesh), and flash column chromatography was performed on silica gel 60 (230−400 mesh) and on silanized silica gel 60 (C_2 silica gel, 70−230 mesh).

Animal and Material. The soft coral *Clavularia viridis* Quoy and Gaimard was collected twice on the coral reef of Ishigaki Island, Okinawa Prefecture, Japan, at a depth of 1−2 m in December 1995, for the isolation of tricycloclavulone (**1**), and in June 1998 for that of clavubicyclone (**2**). Voucher specimens (Nos. SC-95-1 and SC-98-6, respectively) are on deposit at Tokyo University of Pharmacy and Life Science, Tokyo, Japan. The wet specimens collected in 1995 (17.1 kg) were immersed in methanol (3 × 19 L) and then EtOAc (3 × 6 L). Following filtration, the combined extracts were concentrated under reduced pressure. The methanol extract (644 g) was partitioned between EtOAc and H_2O, and the aqueous layer was extracted with *n*-butanol. Each layer was concentrated under reduced pressure to give, in turn, EtOAc- (124 g), *n*-butanol- (40 g), and H_2O-soluble (379 g) portions. An aliquot of the EtOAc-soluble portion (50 g) was chromatographed on a silica gel column (600 g). Stepwise elution with hexane (400 mL), hexane/EtOAc (10:1, 6:1, 4:1, 3:2, and 1:1; 400 mL of each), and methanol (400 mL) gave nine fractions. The ^{1}H NMR spectrum of the fifth fraction (8.4 g) [eluted with hexane/EtOAc (3:2)] showed the fraction to contain prostanoids along with fatty acids and steroids. A part (5.6 g) of the fifth fraction was subjected to silica gel column chromatography [silanized (C_2) silica gel; H_2O/methanol (3:1 and 1:2) and then 1,4-dioxane as eluents] to obtain a mixture of prostanoids eluted with H_2O/methanol (3:1). Separation and purification of this fraction by normal and reversed-phase MPLC and recycled HPLC [hexane/EtOAc (7:3); flow rate, 2.0 mL/min; UV detection at 254 nm] gave tricycloclavulone (**1**, 1.5 mg, 0.0001% based on the methanol extract) as a minor oxylipin. Clavulones II (1.09 g) and III (**3**, 242 mg) were also obtained in this separation.

The wet specimens of *C. viridis* collected in 1998 (2.28 kg) were also immersed in methanol (3 × 3L). Following filtration, the combined extracts were concentrated under reduced pressure. The methanol extract (84 g) was partitioned between EtOAc and H_2O, and the aqueous layer was extracted with *n*-butanol. Each layer was concentrated under reduced pressure. The EtOAc-soluble portion (16.1 g) was chromatographed on a silica gel column (300 g). Stepwise elution with hexane (300 mL), hexane/EtOAc (5:1, 3:1. 2:1, and 1:1; 300 mL of each), EtOAc (300 mL), and methanol (300 mL) gave seven fractions. The prostanoids and related compounds were shown to be mainly contained in the third and fourth fractions [5.46 and 1.60 g eluted with hexane/EtOAc (3:1 and 2:1), respectively] by ^{1}H NMR analysis. Combined fractions were separated by silica gel flash column chromatography [silanized (C_2) silica gel; H_2O/methanol (5:1) and methanol] to obtain four fractions. The ^{1}H NMR spectrum suggested the desired fraction to be the first one. This fraction (3.04 g) was separated using MPLC [normal phase, hexane/EtOAc (3:1)] to give almost pure clavulones I (241 mg), II (1.14 g), and III (**3**, 751 mg) and the oxylipin-containing fraction (345 mg). The oxylipin-containing fraction was purified by normal phase HPLC [hexane/EtOAc (7:2); flow rate, 2.0 mL/min; UV detection at 254 nm], and then reverse-phase HPLC [acetonitrile/H_2O (5:1); flow rate, 2.0 mL/min; UV detection at 210 nm] to afford clavubicyclone (**2**, 8.2 mg, 0.0036%).

Tricycloclavulone (1): a colorless oil; $[\alpha]^{25}_D$ +9.7° (*c* 0.12, $CHCl_3$); UV (ethanol) λ_{max} 205 nm (ϵ 12800); IR (dry film) ν_{max} 1738, 1732, 1715, 1238 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HREIMS *m/z* 446.2329 [calcd for $C_{25}H_{34}O_7$ (M)$^+$, 446.2305].

Clavubicyclone (2): a colorless oil; $[\alpha]^{25}_D$ −59.4° (*c* 0.53, $CHCl_3$); UV (ethanol) λ_{max} 203 nm (ϵ 7600); IR (dry film) ν_{max} 1744, 1732, 1231 cm^{-1}; ^{1}H and ^{13}C NMR (Table 2); HREIMS *m/z* 404.2226 [calcd for $C_{23}H_{32}O_6$ (M − C_2H_2O)$^+$, 404.2199].

Acknowledgment. The authors thank Dr. Takao Yamori, of the Screen Committee of New Anticancer Agents supported by Grant-in-Aid for Scientific Research on Priority Area "Cancer" from The Ministry of Education, Science, Sports and Culture of Japan, for performing bioassay. The authors also thank Dr. Yasuo Shida, of the Tokyo University of Pharmacy and Life Science, for measurement of mass spectra. This work was partly supported by Grant-in-Aid of Scientific Research (K.I., 1268061; M.I., 13771340) from The Ministry of Education, Science, Sports and Culture of Japan.

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and NEOSY spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO011043G