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Clavirins, a new type of marine oxylipins with growth-inhibitory activity from the Okinawan soft coral, *Clavularia viridis*

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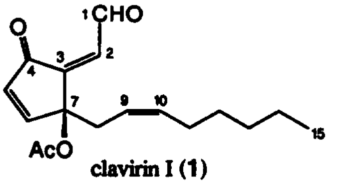
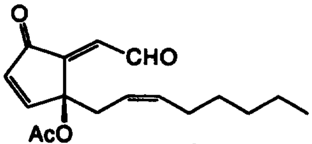
Abstract

Two new marine carbocyclic oxylipins, clavirin I (**1**) and II (**2**), were isolated from the Okinawan soft coral, *Clavularia viridis*. Their structures containing unique α-side chains were determined, based on spectroscopic analysis and stereoselective total synthesis. Clavirins showed growth-inhibitory activity toward HeLa S3 cells.

The Okinawan soft coral, *Clavularia viridis* Quoy and Gaimard (class Anthozoa, subclass Octocorallia, order Stolonifera), contains numerous structurally unique antitumor prostanoids such as clavulones[1] and cytotoxic steroids.[2] During the course of our study on minor chemical congeners of the prostanoids from *C. viridis*,[3] two novel carbocyclic oxylipins,[4] clavirin I (**1**) and clavirin II (**2**), were discovered. These oxylipins showed growth-inhibitory activity toward Hela S3 at 1 μg/ml. This paper describes the isolation and structure determination of clavirins. Their chemical structures, including the absolute configuration, were determined based on spectroscopic analysis and stereoselective total syntheses of **1** and **2** from 4-alkoxy-2-cyclopentenone. Clavirins have proved to be a new type of clavulone-related oxylipins containing short α-side chains with a terminal aldehyde.



clavirin I (**1**)



clavirin II (**2**)

Wet specimens of *C. viridis* (17.1 kg), collected on the coral reef of Ishigaki Island (Okinawa, Japan) in December 1995, were immersed in methanol. The methanol extract (644.0 g) was partitioned between ethyl acetate (EtOAc) and H_2O to afford an EtOAc-soluble portion (123.5 g). The EtOAc-soluble portion (50.0 g) was chromatographed on a silica gel column by elution with hexane, hexane–EtOAc (from 10:1 to 1:1), EtOAc, and MeOH, in turn, to afford nine fractions. Compounds **1** {colorless oil, 2.2 mg, $[\alpha]_D^{25}$

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Table 1
NMR Data for Clavirins I (**1**) and II (**2**)*

	1		**2**	
No	^{13}C	^{1}H	^{13}C	^{1}H
1	191.7 (CH)	10.79 (1H, d, 7.6)	190.6 (CH)	10.33 (1H, d, 8.0)
2	129.9 (CH)	6.21 (1H, d, 7.6)	125.6 (CH)	6.53 (1H, d, 8.0)
3	150.2 (C)		149.4 (C)	
4	192.9 (C)		192.5 (C)	
5	136.1 (CH)#	6.49 (1H, d, 6.2)	136.5 (CH)	6.54 (1H, d, 6.2)
6	158.4 (CH)	7.56 (1H, d, 6.2)	159.3 (CH)	7.65 (1H, d, 6.2)
7	83.5 (C)		84.4 (C)	
8	36.0 (CH_2)	2.77 (1H, dd, 7.4, 14.6) 2.68 (1H, dd, 7.7, 14.6)	37.3 (CH_2)	2.91 (1H, dd, 7.5, 14.5) 2.88 (1H, dd, 7.1, 14.5)
9	120.1 (CH)	5.59 (1H, ddd, 7.4, 7.7, 10.9)	120.1 (CH)	5.62 (1H, ddd, 7.1, 7.5, 10.9)
10	136.0 (CH)#	5.23 (1H, dt, 7.1, 10.9)	134.8 (CH)	5.23 (1H, ddd, 1.6, 7.2, 10.9)
11	27.4 (CH_2)	1.96 (2H, br q, 7.1)	27.5 (CH_2)	1.95 (2H, br q, 7.2)
12	29.0 (CH_2)	1.20~1.36 (2H, m)	28.9 (CH_2)	1.20~1.40 (2H, m)
13	31.4 (CH_2)	1.20~1.36 (2H, m)	31.4 (CH_2)	1.20~1.40 (2H, m)
14	22.5 (CH_2)	1.20-1.36 (2H, m)	22.5 (CH_2)	1.20-1.40 (2H, m)
15	14.0 (CH_3)	0.88 (3H, t, 7.1)	14.0 (CH_3)	0.88 (3H, t, 7.1)
$CH_3\underline{C}O$	169.5 (C)		169.2 (C)	
$\underline{C}H_3CO$	21.3 (CH_3)	2.05 (3H, s)	21.3 (CH_2)	2.09 (3H, s)

* ^{1}H; 500 MHz in $CDCl_3$. *J* in Hz. ^{13}C; 125 MHz in $CDCl_3$.
\# Values in the column are interchangeable. Assignments of the ^{13}C and ^{1}H signals were made based on HMQC.

−17.1° (*c* 0.48, $CHCl_3$)} and **2** {colorless oil, 2.5 mg, $[\alpha]_D^{25}$ −33.7° (*c* 0.43, $CHCl_3$)} from the fifth fraction [eluted with hexane–EtOAc (2:1)] were isolated in addition to clavulones by separation using flash column chromatography, medium-pressure liquid chromatography (MPLC), and HPLC.

The molecular formula of clavirin I (**1**) was assigned as $C_{17}H_{22}O_4$ by the combination of HREIMS [found: 230.1298; calcd: 230.1307 $(M-CH_3CO_2H)^+$] and ^{13}C NMR analysis. All 17 carbons appeared in the ^{13}C NMR spectrum of **1** (Table 1). DEPT indicated two methyls, five sp^3 methylenes, six sp^2 methines including one aldehyde, and four quaternary carbons containing two carbonyls. The IR, ^{1}H NMR (Table 1) and ^{13}C NMR spectra of **1** showed the presence of a conjugated cyclopentenone [IR 1714, 1682 cm^{-1}, δ_H 6.49 (1H, d), 7.56 (1H, d) ppm, δ_C 136.1 (CH), 158.4 (CH), 192.9 (C=O) ppm], a conjugated aldehyde [IR 1682 cm^{-1}, δ_H 6.21 (1H, d), 10.79 (1H, d) ppm, δ_C 129.9 (CH), 150.2 (C), 191.7 (CHO) ppm], an acetoxyl [IR 1735, 1227 cm^{-1}, δ_H 2.05 (3H, s) ppm, δ_C 21.3 (CH_3), 169.5 (C=O) ppm], and a disubstituted olefin [δ_H 5.23 (1H, dt), 5.59 (1H, ddd) ppm, δ_C 120.1 (CH), 136.0 (CH) ppm]. Sequential ^{1}H–^{1}H correlations between H-1 and H-2, H-5 and H-6, and from H-8 to H-11 were observed in ^{1}H–^{1}H COSY spectra. HMBC between H-2 and C-4, H-5 and C-3, H-6 and C-7, and H-8 and C-7 revealed carbon–carbon connections around three quaternary carbons (C-3, C-4 and C-7). These spectroscopic findings led to the gross structure of clavirin I (**1**). The *Z* stereochemistry of the disubstituted olefin in the ω-side chain was indicated by the coupling constant of the olefinic protons ($J_{9,10}$=10.9 Hz). The *Z* stereochemistry of the trisubstituted olefin in the short α-side chain was determined by the following evidence: the chemical shift value of H-1, the terminal aldehyde proton at δ 10.79, shifts much downfield due to the deshielding effect by C-4 carbonyl, however, the H-2 proton at δ 6.21 receives no such neighboring effect. The nuclear Overhauser effect between H-2 and H-8 observed in NOESY of **1** supported this stereochemistry. The absolute configuration at C-7 could not be confirmed by spectroscopic analyses.

Clavirin II (**2**) was found to have the molecular formula, $C_{17}H_{22}O_4$, the same as that for **1** by both HREIMS and ^{13}C NMR analyses. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were quite similar to those of **1**, except for the signals assigned to the α-side chain. The chemical shift value in the ^{1}H NMR spectrum of H-2 at δ 6.53 (1H, d) slides downfield due to the C-4 carbonyl deshielding, and H-1 at δ 10.33 (1H, d) shifts upfield compared to that of **1**, thus confirming the *E* stereochemistry of the trisubstituted olefin.

An answer to the remaining question in **1** and **2** on the absolute configuration at the C-7 chiral center bearing an oxygen function, corresponding to C-12 in clavulones, was important for not only determining the stereostructure but also understanding the biosynthesis of these oxylipins in *C. viridis*. In spite of the opposite absolute configuration, clavulones with 12*S* tertiary acetate and chlorovulones,[5] chlorinated antitumor marine prostanoids with 12*R* tertiary alcohol, were both found from the same organism, *C. viridis*.

The absolute configuration of **1** was determined from the results of the following total synthesis. Because of the presence of a tertiary acetate at C-7 which suggested **1** to be biosynthesized from clavulone itself or clavulone-type oxylipin, (*S*)-**1** was first chosen for the total synthesis. The synthetic outline for (*S*)-**1** consists of the preparation of the optically pure 4-alkoxy-2-cyclopentenone derivative with the ω-side chain, such as **7** in Scheme 1, followed by construction of the α-side chain using aldol condensation with (+)-glyceraldehyde acetonide. The compound **7** has already been reported for the stereoselective total synthesis of clavulones from (*S*)-4-hydroxy-2-cyclopentenone **a** through an intermediate **b**.[1c] During the crucial Claisen condensation, the proton at chiral C-4 bearing a secondary alcohol was found to be partially deprotonated with the generating lithium enolate at −78°C (Fig. 1).[6] The report indicates **7** could not be obtained in 100% ee by using this method. In order to unambiguously determine the absolute configuration of **1**, we decided to execute the new diastereomer-separation strategy, which involves the 1,2-addition to (±)-4-alkoxy-2-cyclopentenone with lithium enolate of (−)-acetoxymenthane to obtain the menthyl esters as a mixture of diastereomers, followed by HPLC separation to afford a reliable compound for the optical purity. This method provides an advantage to prepare the antipodal (*R*)-**1** such as chlorovulone-type oxylipins.

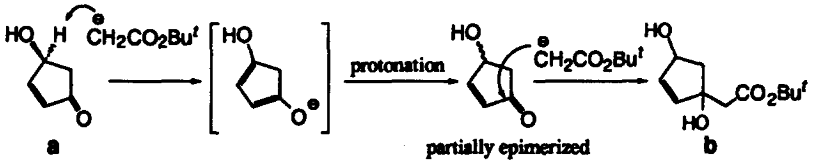
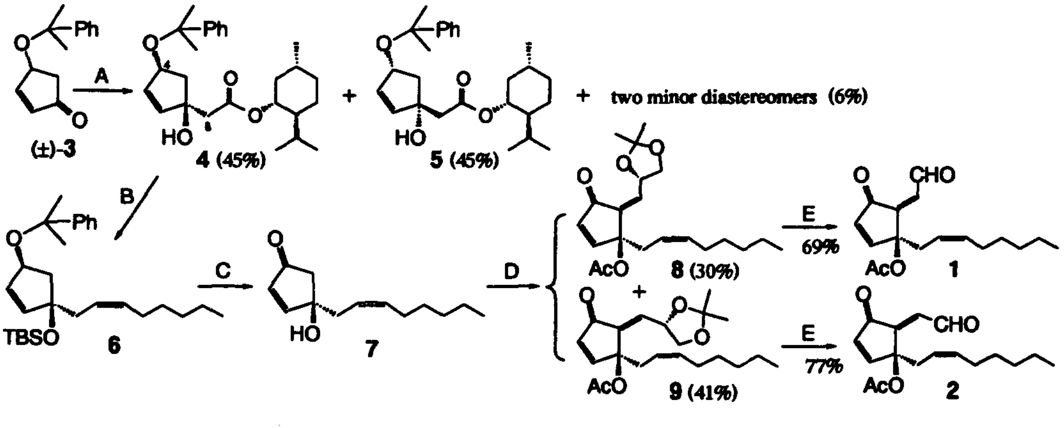


Figure 1.



Scheme 1. Reagents: (A) (−)-Menthyl-OAc, LDA, −78°C. The mixture of **4** and **5** was separated by recycling HPLC. (B) (1) TBSOTf, 2,6-lutidine, 92%; (2) DIBAL, −78°C, 81%; (3) hexyltriphenylphosphonium bromide, BuLi, HMPA, −50°C, 98%. (C) (1) Li, liq. NH_3, EtOH, −33°C, 77%; (2) CrO_3, H_2SO_4, 98%; (3) TBAF, 83%. (D) (1) TMSCl, iPr_2NEt, DMAP, 92%; (2) LiHMDS, −78°C, then (+)-glyceraldehyde acetonide, 76% (four diastereomers); (3) $AcOH–H_2O$; (4) Ac_2O, pyridine, DMAP. (E) (1) $AcOH–H_2O$, 40°C; (2) $NaIO_4$, 0°C

The synthesis was performed starting from the known cyclopentenone (±)-**3** prepared from cumene hydroperoxide in three steps.[7] Stereoselective Claisen condensation of (±)-**3** gave diastereomeric menthyl esters. After separation of two minor diastereomers (6% yield) by column chromatography, the desired two major esters were purified by recycled HPLC to obtain **4** ($[\alpha]_D^{25}$ −54.7°, 45% yield) and **5** ($[\alpha]_D^{25}$ −20.4°, 45% yield), respectively. The compound **4** showed a slightly shorter retention time in HPLC. The relative stereochemistry of each compound was confirmed by NOEs between protons at H-4 and H-8, corresponding to clavirin's numbering, but the absolute configurations were not determined at this stage because the known compound **7** was present in the next several steps. Further transformations were carried out for **4**; TBS protection, DIBAL reduction, and Wittig olefination provided **6** (three steps, 73% yield). The key intermediate **7**[8] was prepared in three standard steps (63% yield). The optical purity of **7** showed more than 99% ee by chiral HPLC analysis. All the spectral data of **7** were essentially identical to those of the known clavulone intermediate.[1c,8] According to these results, the stereo-structures of **4** and **5** as well as the other synthetic intermediates from **4** to **7** were automatically determined as shown in Scheme 1. The construction of the α-side chain was carried out by the following reaction sequence. After TMS protection of the tertiary alcohol (92% yield), the aldol coupling with the (+)-glyceraldehyde acetonide gave four diastereomeric aldols (76% yield). The mixture of the diastereomers was treated with acetic acid–H_2O to remove TMS, and then acetylation of the diol with acetic anhydride and catalytic amount of 4-dimethylaminopyridine in pyridine, in which β-elimination occurred simultaneously, gave cross-conjugated enones **8** (30% yield) and **9** (41% yield). These stereoisomes were separated by MPLC. The stereochemistry of each trisubstituted double bond was confirmed by the chemical shift value in ^{1}H NMR, already mentioned in the structural elucidation of **1** and **2**. Finally, deprotection of acetonide on the α-side chain in **8** followed by oxidative cleavage with $NaIO_4$ to remove the extra carbon provided **1** in 69% yield for two steps. The spectral data of synthetic **1** including the optical rotation {$[\alpha]_D^{25}$ −21.3° (*c* 0.04, $CHCl_3$)} were identical to those of natural **1**, thus indicating that the absolute stereochemistry at C-7 was confirmed as *S* configuration, the same as that of clavulones. Stereoisomer **2** was also synthesized similarly from **9** in 77% yield for two steps {$[\alpha]_D^{25}$ −49.3° (*c* 0.28, $CHCl_3$)}, concluding the *S* configuration.

The structural similarity of clavirins to clavulones, including the absolute stereochemistry, suggested the biosynthesis of clavirins derived from clavulones. A possible biogenetic pathway is shown in Fig. 2. Oxidative fragmentation between C-5 and C-6 for clavulone III and/or IV provides clavirin I (**1**), and clavulone I and/or II affords clavirin II (**2**). We are now seeking the related prostanoids from *C. viridis* to reinforce this biogenesis.

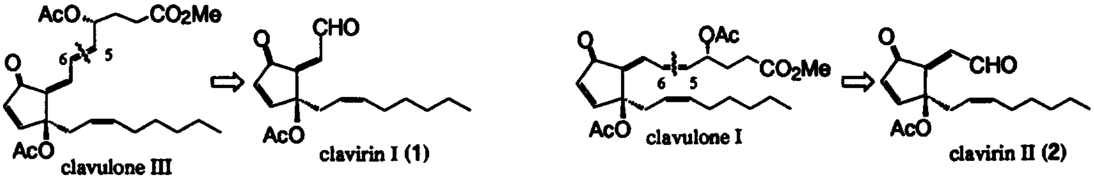


Figure 2.

Acknowledgements

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7. Stork, G.; Isobe, M. *J. Am. Chem. Soc.* **1975**, *97*, 6260.
8. Compound **7**: $[\alpha]_D$ −89.1° (*c* 0.44, $CHCl_3$); IR (film) 3417, 1713, 1674 cm^{-1}; ^1H NMR (400 MHz, δ ppm, *J* in hertz) 7.42 (1H, d, 5.7), 6.14 (1H, d, 5.7), 5.67 (1H, ddd, 10.7, 7.5, 7.3), 5.37 (1H, dt, 10.7, 7.6), 2.56 (1H, dd, 14.1, 7.5), 2.55 (1H, d, 18.2), 2.47 (1H, dd, 14.1, 7.3), 2.46 (1H, d, 18.2), 2.12 (1H, br s), 2.05 (2H, dt, 7.6, 7.1), 1.23–1.38 (6H, m), 0.88 (3H, t, 7.1); ^{13}C NMR (100 MHz, δ ppm) 206.5 (C), 165.2 (CH), 135.9 (CH), 133.8 (CH), 121.9 (CH), 78.7 (C), 48.6 (CH_2), 38.0 (CH_2), 31.5 (CH_2), 29.1 (CH_2), 27.4 (CH_2), 22.5 (CH_2), 14.0 (CH_3); FABMS *m/z* 209 $(M+H)^+$; Anal. calcd for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68; found: C, 74.84; H, 9.68; previously reported **7**: $[\alpha]_D$ −54.1° (*c* 1.52, $CHCl_3$).[1c]