New Marine Prostanoids from the Okinawan Soft Coral, *Clavularia viridis*

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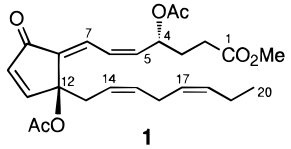
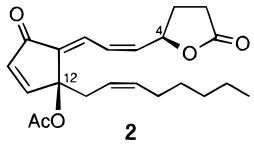
Two new marine prostanoids—17,18-dehydroclavulone I (**1**) and clavulolactone I (**2**)—were isolated from the Okinawan soft coral, *Clavularia viridis*. Their structures, including absolute configurations, were determined based on the results of spectroscopic analysis and chemical conversions.

Marine prostanoids have attracted considerable attention because of their structural features and biological activities.[1] The Okinawan soft coral, *Clavularia viridis* Quoy and Gaimard (Clavularidae), is a rich source of structurally unique antitumor prostanoids such as the clavulones[2−4] and chlorovulones.[5,6] Recently, we reported the isolation and structure determination of the prostanoid γ-lactones, preclavulone lactone I and II,[7] which are possibly precursors of the clavulones, from the MeOH extract of *C. viridis*. During the course of our study on minor chemical congeners from *C. viridis*, two new prostanoids—17,18-dehydroclavulone I (**1**) and clavulolactone I (**2**)—were isolated. 17,18-Dehydroclavulone I (**1**) is the first example in the clavulone family possessing (14*Z*,17*Z*)-double bonds in the ω side chain, which may be biogenetically derived from (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoic acid instead of arachidonic acid. Clavulolactone I (**2**) was not isolated in our previous study[8] on clavulolactones, being present in amounts too small, but subsequently sufficient quantities of this compound have been obtained to permit its characterization. This paper describes the structure elucidation of prostanoids **1** and **2** through spectroscopic analysis and chemical conversions.



1



2

Specimens of *C. viridis*, collected on the coral reef of Ishigaki Island (Okinawa Prefecture, Japan), were immersed in MeOH. The MeOH extract was successively partitioned between EtOAc and H_2O to afford an EtOAc-soluble portion, and then the aqueous layer was extracted with *n*-BuOH to afford *n*-BuOH- and H_2O-soluble portions. The EtOAc-soluble portion was chromatographed on a Si gel column by elution with hexanes, hexane−EtOAc (from 10:1 to 1:1), EtOAc, and MeOH, in turn, to afford nine fractions. Compound **1** (colorless oil, 6.3 mg, $[\alpha]^{25}_D$ −27.1°) from the sixth fraction (eluted with hexane−EtOAc, 3:2) and compound **2** (colorless oil, 9.2 mg, $[\alpha]^{25}_D$ −7.8°) from the seventh fraction (eluted with hexane−EtOAc, 1:1) were isolated by repeated purification using flash column chromatography, MPLC, and HPLC.

The molecular formula of **1** was found to be $C_{25}H_{32}O_7$, by the combination of HREIMS [found *m*/*z* 384.1925 ($M - CH_3CO_2H$)$^+$, $C_{23}H_{28}O_5$ requires 384.1937] and ^{13}C NMR analysis. All 25 carbons appeared in the ^{13}C NMR spectrum of **1** (Table 1). The DEPT spectrum exhibited three methyls, one methoxyl, five sp^3 methylenes, one sp^3 methine, nine sp^2 methines, two sp^3 quaternary carbons, and four sp^2 quaternary carbons. The IR spectrum of **1** showed absorptions due to acetate ester (1738, 1233 cm^{-1}) and α,β-unsaturated cyclopentenone (1706 cm^{-1}) functionalties. The presence of a cross-conjugated system in **1**, corresponding to that of the clavulones, was demonstrated by UV absorptions at 228 (log ϵ 3.98) and 293 (log ϵ 4.01) nm. The ^1H NMR spectrum of **1** (Table 1) disclosed five olefinic protons in the cross-conjugated system at δ 5.86 (1H, ddd, J = 1.1, 8.1, 11.1 Hz, H-5), 6.43 (1H, d, J = 6.1 Hz, H-10), 6.59 (1H, dd, J = 11.1, 12.7 Hz, H-6), 7.27 (1H, d, J = 12.7 Hz, H-7), and 7.48 (1H, d, J = 6.1 Hz, H-11); four olefinic protons on nonconjugated *Z* carbon−carbon double bonds at δ 5.21 (1H, dt, J = 7.6, 10.8 Hz, H-17), 5.23 (1H, ddd, J = 7.4, 8.0, 10.9 Hz, H-14), 5.39 (1H, dt, J = 7.4, 10.8 Hz, H-18), and 5.50 (1H, dt, J = 7.5, 10.9 Hz, H-15); an acetoxyl-bearing methine group at δ 5.78 (1H, dt, J = 5.4, 8.1 Hz, H-4); and a terminal methyl group at δ 0.96 (3H, t, J = 7.5 Hz, H-20). The $^1H-^1H$ COSY spectrum demonstrated $^1H-^1H$ correlations from H-2 to H-7 on the α side chain and from H-13 to H-20 on the ω side chain. These spectroscopic findings showed **1** to have a structure similar to that of clavulone I, except for three carbons on the ω side chain. After assignments between the ^1H and ^{13}C NMR signals were made based on $^{13}C-^1H$ COSY, the gross structure was confirmed by the HMBC correlations shown in Figure 1. The optical rotation of **1** was similar to that of clavulone I ($[\alpha]^{25}_D$ −28.6°), suggesting the absolute stereochemistry of both chiral carbons (C-4 and C-12) in **1** to be the same as for clavulone I (4*R*,12*S*).

Compound **2** had the molecular formula $C_{22}H_{28}O_5$ by HREIMS (found *m*/*z* 372.1916, M^+, $C_{22}H_{28}O_5$ requires 372.1937). The IR spectrum of **2** indicated absorptions due to γ-lactone (1770 cm^{-1}), ester (1732 cm^{-1}), and α,β-unsaturated cyclopentenone (1704 cm^{-1}) moieties. The ^{13}C and ^1H NMR spectra of **2** (Table 1) were quite similar to those of clavulone I, except for missing signals for one methyl ester group and one acetate functionality, thus showing **2** to be a member of the clavulolactone series.[8] The structure, including absolute configurations at C-4 and C-12, was determined by chemical conversion from clavulone I to **2** in two steps (Scheme 1).[8] Enzymatic hydrolysis of the C-4 acetate, followed by γ-lactonization gave synthetic **2**. The physical properties, including the optical rotation ($[\alpha]^{25}_D$ −7.9°) of synthesized **2**, were identical to those of natural **2**. The structure of **2** was thus determined to be clavulolactone I (4*R*,12*S*). Compound **2**, possessing (5*Z*,7*E*) stereochemistries of the carbon−carbon double

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Table 1. ^{1}H and ^{13}C NMR Spectral Data of **1** and **2** ($CDCl_3$, δ ppm)

	^{1}H (500 MHz; *J* in Hz)		^{13}C (125 MHz)	
position	**1**	**2**	**1**	**2**
1			172.9	176.3
2	2.38 (2H, q; 7.3)	2.62 (1H, dd; 2.5, 9.5) 2.60 (1H, d; 9.5)	29.8	28.7[a]
3	2.00−2.10 (2H, m)[b]	2.48−2.58 (1H, m) 1.97−2.04 (1H, m)	29.8	29.0[a]
4	5.78 (1H, dt; 5.4, 8.1)	5.53 (1H, q; 7.3)	69.4	75.5
5	5.86 (1H, ddd; 1.1, 8.1, 11.1)	6.01 (1H, ddd; 0.6, 8.7, 10.9)	138.9	138.4
6	6.59 (1H, dd; 11.1, 12.7)	6.67 (1H, ddd; 1.2, 10.9, 12.8)	124.2	123.3
7	7.27 (1H, d; 12.7)	7.01 (1H, d; 12.8)	124.6	125.0
8			137.4	138.2
9			193.1	193.3
10	6.43 (1H, d; 6.1)	6.45 (1H, d; 6.1)	135.2	135.4
11	7.48 (1H, d; 6.1)	7.48 (1H, d; 6.1)	157.8	158.2
12			85.1	85.1
13	2.98 (1H, dd; 7.4, 14.2) 2.69 (1H, dd; 8.0, 14.2)	2.93 (1H, dd; 6.9, 14.6) 2.70 (1H, dd; 8.3, 14.6)	35.8	36.0
14	5.23 (1H, ddd; 7.4, 8.0, 10.9)	5.18 (1H, ddd; 6.9, 8.3, 10.8)	121.4	120.7
15	5.50 (1H, dt; 7.5, 10.9)	5.53−5.59 (1H, m)	133.1	135.1
16	2.67−2.76 (2H, m)	1.94 (2H, q; 7.0)	25.7	27.4
17	5.21 (1H, dt; 7.6, 10.8)		126.2	29.3[a]
18	5.39 (1H, dt; 7.4, 10.8)	1.20−1.35 (6H, m)	132.5	31.5
19	1.90−2.02 (2H, m)[b]		20.6	22.5
20	0.96 (3H, t; 7.5)	0.88 (3H, t; 6.9)	14.2	14.0
OCH_3	3.69 (3H, s)		51.8	
CH_3CO	2.03 (3H, s)	2.04 (3H, s)	21.0	21.3
CH_3CO	2.04 (3H, s)		21.2	
CH_3CO			169.9	169.2
CH_3CO			169.1	

[a,b] Values with the same superscript in each column are interchangeable.

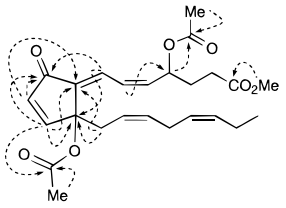
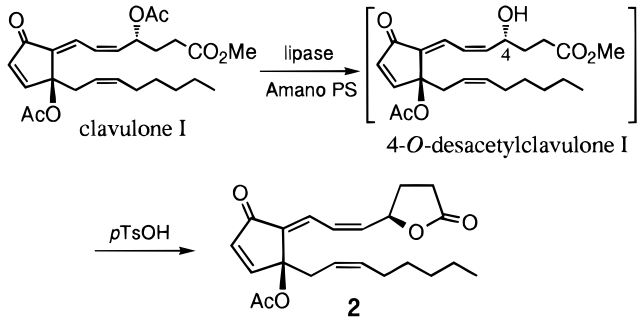


Figure 1. Key HMBC correlations of **1**.

Scheme 1. Chemical Conversion from Clavulone I to **2**



bonds in the α side chain, is a geometrical isomer of clavulolactone II (5*E*,7*E*) and clavulolactone III (5*E*,7*Z*).[8]

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 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz) in $CDCl_3$. ^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY, and HMBC NMR 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; ^{13}C, 77.0 ppm) as the internal standard. MS were obtained on a Micromass Auto Spec spectrometer. Column chromatography was carried out on Merck Si gel 60 (70−230 mesh) and Merck Si gel 60 silanized (C_2 Si gel, 70−230 mesh). Flash column chromatography was performed on Merck Si gel 60 (230−400 mesh). MPLC was carried out with a KHLC-201−43 (Kusano) apparatus using a CIG prepack column (Si gel, CPS−HS-221−05, for normal phase and ODS Si gel, CPO−HS-221−20, for reversed phase). HPLC with a recycle loop was conducted with a YMC-Pack SIL-06 column (Si gel, SH-043−5−06, normal phase) and a YMC-Pack ODS-AM column (ODS Si gel, SH-343−5AM, reversed phase).

Animal Material. The soft coral, *Clavularia viridis* Quoy and Gaimard (order Stolonifera, family Clavularidae), was collected from a coral reef of Ishigaki Island, Okinawa Prefecture, Japan, in December 1995, at a depth of 1−2 m. A voucher specimen (no. SC-95-1) has been on deposit at Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Extraction and Isolation. Wet specimens (17.1 kg) were immersed in MeOH (3 × 19 L) and then EtOAc (3 × 6 L). After filtration, the combined extracts were concentrated under reduced pressure. The MeOH extract (644 g) was partitioned between EtOAc and H_2O, and then the aqueous layer was extracted with *n*-BuOH. Each layer was concentrated under reduced pressure to give, in turn, EtOAc- (123.5 g), *n*-BuOH- (39.6 g), and H_2O- (379.0 g) soluble portions. An aliquot of the EtOAc-soluble portion (50 g) was chromatographed on a Si gel column (600 g). Stepwise elution with hexanes (400 mL), hexane−EtOAc (10:1, 6:1, 4:1, 2:1, 3:2, and 1:1, each 400 mL), EtOAc (400 mL), and MeOH (400 mL) gave nine fractions. The sixth fraction (5.90 g) (eluted with hexane−EtOAc, 3:2) was subjected to a Si gel column chromatography [silanized Si gel; H_2O−MeOH (3:1 and 1:2) then 1,4-dioxane as eluents] to obtain a crude prostanoid fraction eluted with H_2O−MeOH (3:1). The separation and purification of this fraction by MPLC and HPLC (hexane−EtOAc, 7:2, as eluent) gave **1** (6.3 mg). The seventh fraction (2.14 g) (eluted with hexane−EtOAc, 3:2) was subjected to a Si gel column chromatography [silanized Si gel; H_2O−MeOH (3:1), MeOH and 1,4-dioxane as eluents] to afford a crude prostanoid fraction present in the H_2O−MeOH (3:1) fraction. Further purification by MPLC and recycle reversed-phase HPLC (H_2O−MeOH, 2:7) afforded **2** (9.2 mg).

17,18-Dehydroclavulone I (1): colorless oil: $[\alpha]^{25}_D$ −27.1°

(*c* 0.31, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 228 (3.98), 293 (4.01) nm; IR ν_{max} (dry film) 1738, 1714, 1643, 1233 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 444 (M^+, $C_{25}H_{32}O_7$); HREIMS *m*/*z* 384.1925 [calcd for $C_{23}H_{28}O_5$, 384.1937 ($M - CH_3CO_2H)^+$].

Clavulolactone I (2): colorless oil: $[\alpha]^{25}_D$ −7.8° (*c* 0.26, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 231 (4.14), 292 (4.20) nm; IR ν_{max} (dry film) 1770, 1732, 1704, 1643, 1230 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 372 (M^+, $C_{22}H_{28}O_5$); HREIMS *m*/*z* 372.1916 [calcd for $C_{22}H_{28}O_5$, 372.1937 $(M)^+$].

Conversion of Clavulone I to 2. To a mixture of clavulone I[2] (14 mg) in pH 7.0 phosphate buffer solution (0.067 M KH_2PO_4−Na_2HPO_4, 2 mL) and 0.2% solution of Triton X-100 (0.5 mL), was added lipase Amano PS (1200 units/mL, 1 mL) containing 0.067 M KH_2PO_4−Na_2HPO_4, at room temperature. The reaction mixture was vigorously stirred for 48 h at 40 °C, and EtOH (2 mL) was added to terminate the reaction; then the product was concentrated under reduced pressure. The residue was extracted twice with EtOAc (25 mL), and the combined extracts were washed with saturated NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. To a solution of the crude products (mainly 4-*O*-desacetylclavulone I) in EtOAc (3 mL) was added *p*-toluenesulfonic acid (0.3 mg) at room temperature. After stirring for 8 h, pyridine (0.03 mL) was added, and the mixture was concentrated under reduced pressure. The oily residue was purified by passing over a small plug of Si gel (hexane−EtOAc, 2:1), followed by MPLC separation (hexane−EtOAc, 4:1) to provide **2** (9.2 mg, 79% yield) as a colorless oil: $[\alpha]^{25}_D$ −7.9° (*c* 0.92, $CHCl_3$). The spectral data obtained for the product were identical to those of natural **2**.

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References and Notes

(1) Gerwick, W. H. *Chem. Rev.* **1993**, *93*, 1807−1823, and references therein.
(2) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1982**, *23*, 5171−5174.
(3) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1983**, *24*, 1549−1552.
(4) Iguchi, K.; Yamada, Y.; Kikuchi, H.; Tsukitani, Y. *Tetrahedron Lett.* **1983**, *24*, 4433−4434.
(5) Iguchi, K.; Kaneta, S.; Mori, K.; Yamada, Y.; Honda, A.; Mori, Y. *Tetrahedron Lett.* **1985**, *26*, 5787−5790.
(6) Nagaoka, H.; Iguchi, K.; Miyakoshi, T.; Yamada, N.; Yamada, Y. *Tetrahedron Lett.* **1986**, *27*, 223−226.
(7) Iwashima, M.; Watanabe, K.; Iguchi, K. *Tetrahedron Lett.* **1997**, *38*, 8319−8322.
(8) Iguchi, K.; Iwashima, M.; Watanabe, K. *J. Nat. Prod.* **1995**, *58*, 790−793.

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