# 4-Epiclavulones, New Marine Prostanoids from the Okinawan Soft Coral, *Clavularia viridis*

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**Two new marine prostanoids, 4-epiclavulone II and 4-epiclavulone III, 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 conversion.**

**Key words** marine prostanoid; clavulone; soft coral; *Clavularia viridis*

Marine prostanoids such as clavulones have attracted much attention because of their structural features and biological activities.[1—4] During the course of our study on minor chemical congeners of prostanoids from the Okinawan soft coral, *Clavularia viridis* Quoy and Gaimard (Clavulariidae),[5,6] two new prostanoids, 4-epiclavulone II (**1**) and 4-epiclavulone III (**2**), were isolated. These prostanoids are characterized by a (4*S*) configuration on the $\alpha$ side chain unprecedented in the prostanoids of clavulone family.[2] This paper describes structural elucidation of these prostanoids through spectroscopic analysis and chemical conversion.

Specimens of *C. viridis*, collected on the coral reef of Ishigaki Island (Okinawa Prefecture, Japan), were immersed in methanol (MeOH). The MeOH extract was successively partitioned between ethyl acetate (AcOEt) and $H_2O$ to afford AcOEt-soluble portion, and then aqueous layer was extracted with *n*-butanol (BuOH) to afford BuOH- and $H_2O$-soluble portions. The AcOEt-soluble portion was chromatographed on a silica gel column by elution with hexanes, hexan–AcOEt (from 10 : 1 to 1 : 1), AcOEt, and MeOH, in turn, to afford nine fractions. Compound **1** {colorless oil, 4.9 mg, $[\alpha]_D^{25}$ −18.7° ($c$=0.30, $CHCl_3$)} and **2** {colorless oil, 0.9 mg, $[\alpha]_D^{25}$ −10.0° ($c$=0.06, $CHCl_3$)} from the fifth fraction [eluted with hexane–AcOEt (2 : 1)], were isolated by repeated separations using flash column chromatography, medium-pressure liquid chromatography (MPLC) and recycling HPLC.

The molecular formula of **1** was found to be $C_{25}H_{34}O_7$, by high-resolution electron impact MS (HREIMS) [*m/z* 446.2315 (Calcd for $C_{25}H_{34}O_7$, 446.2305)], which was equal to that of clavulones. All 25 carbons appeared in the $^{13}$C-NMR spectrum of **1** (Table 2). Distortionless enhancement by polarization transfer (DEPT) spectrum indicated three methyls, one methoxy, seven $sp^3$ methylenes, one $sp^3$ methine, seven $sp^2$ methines, one $sp^3$ quaternary carbon and five $sp^2$ quaternary carbons. The IR spectrum of **1** showed absorptions due to esters (1738, 1732 $cm^{-1}$) and $\alpha,\beta$-unsaturated cyclopentenone (1704 $cm^{-1}$). The presence of a cross-conjugated system in **1** corresponding to that of clavulones was demonstrated by UV absorptions at 228 (log $\varepsilon$ 4.28) and 291 (log $\varepsilon$ 4.27) nm. The $^{13}$C-NMR spectrum of **1** was quite similar to that of clavulone II (**3**) (Table 2).[2] The $^1$H-NMR spectrum of **1** also showed a quite similar pattern to that of **3,** except for the chemical shift of the signals due to the acetates at $\delta$ 2.03 and 2.09 ppm in **1** instead of $\delta$ 2.06 and 2.07 ppm in **3** (Table 1). Additionally, the signals of the protons at $\delta$ 2.98 (H-13), 5.18 (H-14), and 6.70 (H-6) ppm slightly shifted compared to those of the corresponding protons in **3.** These spectroscopic analyses indicated that compound **1** had to be an epimer of **3** at either of the chiral centers, C-4 or C-12.

Compound **2** has the molecular formula, $C_{25}H_{34}O_7$ by measurement of HREIMS [*m/z* 446.2328 (Calcd for $C_{25}H_{34}O_7$, 446.2305)], the same as that of **1**. $^1$H and $^{13}$C-NMR spectra of **2** were quite similar to those of clavulone III (**4**),[2] as shown in Tables 1 and 2, and the other physical properties of **2** except for optical rotation were also closely related to those of **4,** suggesting that compound **2** was a stereoisomer of **4** as in the case between **1** and **3**.

To determine the absolute configurations at C-4 and C-12 in **1** and **2,** the following chemical conversions for each compound were carried out (Chart 1).[5] Selective hydrolysis of the C-4 acetate in **3** (4*R*, 12*S*) by Amano lipase PS, followed by Mitsunobu inversion using acetic acid, triphenylphosphine, and diisopropyl azodicarboxylate gave synthetic **1** in 65% yield. The spectral data including optical rotation {$[\alpha]_D^{25}$ −19.2°} of synthesized **1** were identical to those of natural **1**. The structure of **1** was thus determined to be 4-epiclavulone II (4*S*, 12*S*). Compound **2** was also converted from **4** (4*R*, 12*S*) in the same reaction sequence to reveal the chiral centers. The physical data of synthesized **2** {$[\alpha]_D^{25}$ −11.2°} were identical to those of natural **2**. This result confirmed **2** to be 4-epiclavulone III with (4*S*, 12*S*) configurations.

The possibility that **1** and **2** were derived from clavulone II (**3**) and III (**4**), respectively, by an acid- or base-catalyzed epimerization at the C-4 position could be denied because of the neutral condition through extraction and isolation processes. This was supported by the following experiments

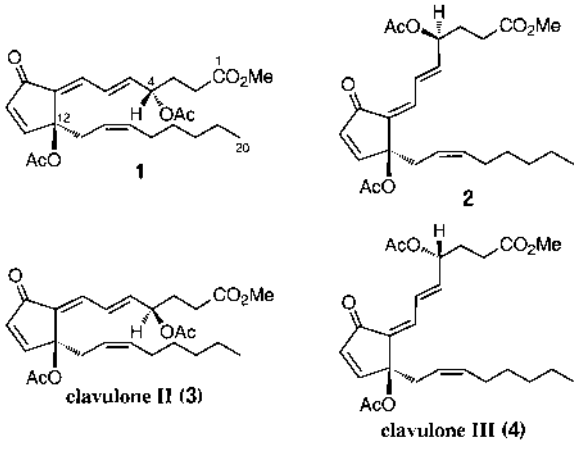


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Table 1. $^1$H-NMR Data of **1**, **2**, Clavulone II (**3**), and III (**4**) (500 MHz, $CDCl_3$, $\delta$ ppm, $J$ in Hz)

| Position | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 2 | 2.37 (2H, t, 7.4) | 2.40 (2H, t, 7.4) | 2.37 (2H, t, 7.1) | 2.39 (2 H, t, 7.5) |
| 3 | 1.98—2.06 (2H, m) | 1.99—2.07 (2H, m) | 1.99—2.06 (2H, m) | 2.00—2.09 (2H, m) |
| 4 | 5.43 (1H, dt, 6.4, 7.0) | 5.45 (1H, dd, 5.4, 5.8) | 5.42 (1H, dt, 6.4, 7.0) | 5.44 (1H, dt, 6.0, 6.9) |
| 5 | 6.07 (1H, dd, 6.4, 11.9) | 6.01 (1H, dd, 5.4, 15.6) | 6.02 (1H, dd, 7.0, 14.9) | 6.01 (1H, dd, 6.0, 15.6) |
| 6 | 6.70 (1H, ddd, 1.0, 11.9, 15.1) | 7.73 (1H, ddd, 1.3, 11.3, 15.6) | 6.75 (1H, dd, 12.0, 14.9) | 7.74 (1H, ddd, 1.3, 11.3, 15.6) |
| 7 | 6.88 (1H, d, 15.1) | 6.51 (1H, d, 11.3) | 6.87 (1H, d, 12.0) | 6.52 (1H, d, 11.3) |
| 10 | 6.41 (1H, d, 6.1) | 6.35 (1H, d, 6.1) | 6.41 (1H, d, 6.1) | 6.36 (1H, d, 6.2) |
| 11 | 7.48 (1H, d, 6.1) | 7.51 (1H, d, 6.1) | 7.47 (1H, d, 6.1) | 7.50 (1H, d, 6.2) |
| 13 | 2.71 (1H, dd, 8.4, 14.1) | 2.63 (1H, dd, 7.3, 14.2) | 2.69 (1H, dd, 8.3, 14.2) | 2.66 (1H, dd, 7.2, 14.4) |
| | 2.98 (1H, dd, 7.0, 14.1) | 2.87 (1H, dd, 8.1, 14.2) | 2.88 (1H, dd, 7.0, 14.2) | 2.86 (1H, dd, 7.5, 14.4) |
| 14 | 5.18 (1H, ddd, 7.0, 8.4, 10.9) | 5.21 (1H, ddd, 7.3, 8.1, 10.9) | 5.22 (1H, ddd, 7.0, 8.3, 10.9) | 5.21 (1H, ddd, 7.2, 7.5, 10.9) |
| 15 | 5.50 (1H, dt, 7.4, 10.9) | 5.53 (1H, dt, 7.3, 10.9) | 5.52 (1H, dt, 7.3, 10.9) | 5.52 (1H, dt, 7.4, 10.9) |
| 16 | 1.94 (2H, q, 6.8) | 1.94 (2H, q, 7.3) | 1.94 (2H, q, 6.9) | 1.94 (2H, q, 7.1) |
| 17—19 | 1.20—1.34 (6H, m) | 1.21—1.34 (6H, m) | 1.20—1.34 (6H, m) | 1.22—1.35 (6H, m) |
| 20 | 0.88 (3H, t, 7.2) | 0.88 (3H, t, 7.1) | 0.88 (3H, t, 6.9) | 0.88 (3H, t, 7.1) |
| $OCH_3$ | 3.67 (3H, s) | 3.68 (3H, s) | 3.68 (3H, s) | 3.67 (3H, s) |
| $CH_3CO$ | 2.03 (3H, s) | 2.03 (3H, s) | 2.06 (3H, s) | 2.02 (3H, s) |
| $CH_3CO$ | 2.09 (3H, s) | 2.09 (3H, s) | 2.07 (3H, s) | 2.10 (3H, s) |

Table 2. $^{13}$C-NMR Data of **1**, **2**, **3**, and **4** (125 MHz, $CDCl_3$, $\delta$ ppm)

| Position | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 172.9 | 173.2 | 172.9 | 173.1 |
| 2 | 29.6[a)] | 29.7[b)] | 29.6[c)] | 29.8[d)] |
| 3 | 29.2[a)] | 29.2[b)] | 29.1[c)] | 29.2[d)] |
| 4 | 72.7 | 72.4 | 72.8 | 72.5 |
| 5 | 141.5 | 141.0 | 141.3 | 141.0 |
| 6 | 126.0 | 126.3 | 126.9 | 126.5 |
| 7 | 129.4 | 133.4 | 129.3 | 133.4 |
| 8 | 136.7 | 136.7 | 137.0 | 136.7 |
| 9 | 193.4 | 194.1 | 193.4 | 194.1 |
| 10 | 135.1 | 135.6 | 135.0 | 135.7 |
| 11 | 157.9 | 156.1 | 158.1 | 156.1 |
| 12 | 85.3 | 85.2 | 85.1 | 85.3 |
| 13 | 35.9 | 35.6 | 36.0 | 35.6 |
| 14 | 121.1 | 121.3 | 121.1 | 121.4 |
| 15 | 135.1 | 134.7 | 135.0 | 134.8 |
| 16 | 27.4 | 27.4 | 27.4 | 27.4 |
| 17 | 29.1[a)] | 29.0[b)] | 29.1[c)] | 29.1[d)] |
| 18 | 31.5 | 31.5 | 31.5 | 31.5 |
| 19 | 22.5 | 22.5 | 22.5 | 22.5 |
| 20 | 14.0 | 14.0 | 14.0 | 14.0 |
| $OCH_3$ | 51.8 | 51.7 | 51.8 | 51.7 |
| $CH_3CO$ | 20.9 | 21.0 | 21.0 | 21.0 |
| $CH_3CO$ | 21.2 | 21.7 | 21.2 | 21.7 |
| $CH_3CO$ | 169.2 | 169.7 | 169.5 | 169.7 |
| $CH_3CO$ | 169.9 | 170.2 | 169.9 | 170.1 |

*a*—*d*) Values with the same superscript in each column are interchangeable.

for **3** and **4** under acidic or basic conditions. For example, treatment of **3** with a catalytic amount of acid (HCl or *p*-TsOH) in MeOH at 65 °C for 6 h gave unreacted **3** and some decomposed compounds, from which epimerized **1** was not detected by $^1$H-NMR and HPLC analysis. A Lewis-acid treatment of **3** gave essentially the same result. Under basic conditions such as KOH or LiOH in aqueous tetrahydrofuran (THF)–EtOH, and NaOMe in MeOH at room temperature for about 4 h, however, many compounds with a small amount of unreacted **3** were detected, but epimerized **1** was not observed. We also conducted the following experiment: **3** was adsorbed on silica gel (both for normal and C-2 reversed phase), allowed to stand for about 50 h at room temperature, and then eluted with MeOH. $^1$H-NMR and HPLC analysis of

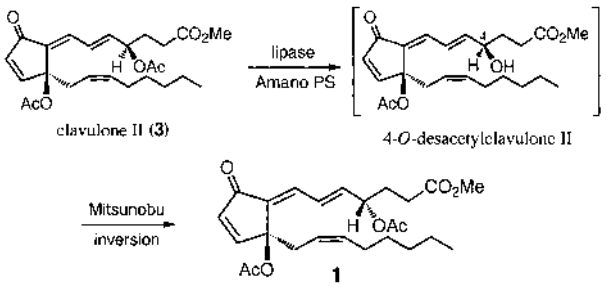


Chart 1

the eluate showed the presence of only **3**. The possibility of epimerization for **2** and **4** were also confirmed by the above methods. No epimerization at the C-4 position in both compounds were detected.

Biological activity and biosynthesis for **1** and **2** are currently under investigation.

**Experimental**

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. NMR spectra were recorded with a Bruker DRX-500 and a JEOL $\alpha$-500 spectrometer ($^1$H, 500 MHz; $^{13}$C, 125 MHz) in $CDCl_3$. $^1$H–$^1$H correlation spectroscopy (COSY) and $^1$H–$^{13}$C COSY NMR spectra were measured with a Bruker DRX-500 using standard Bruker pulse sequences. Chemical shifts are given on a $\delta$ (ppm) scale with $CHCl_3$ ($^1$H, 7.26 ppm; $^{13}$C, 77.0 ppm) as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Mass spectra were taken with a Micromass Auto Spec spectrometer. Column chromatography was carried out on Merck silica gel 60 (70—230 mesh) and Merck silica gel 60 silanized ($C_2$ silica gel, 70—230 mesh). Flash column chromatography was performed on Merck silica gel 60 (230—400 mesh). Medium-pressure liquid chromatography (MPLC) was carried out with a Kusano KHLC-201-43 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 with a recycling loop was conducted with 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-343-5AM, reversed phase).

**Animal and Material** The soft coral, *Clavularia viridis* Quoy and Gaimard (order Stolonifera, family Clavularidae), was collected from the 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 AcOEt (3×6 l). After filtration, the combined extracts were concentrated under reduced pressure. The MeOH extract (644.0 g) was partitioned between AcOEt and $H_2O$, and then the aqueous layer was extracted with *n*-BuOH. Each layer was concentrated under reduced pressure to give, in turn, AcOEt- (123.5 g), *n*-BuOH- (39.6 g), and $H_2O$- (379.0 g) soluble portions. An aliquot of the AcOEt-soluble portion (50.0 g) was chromatographed on a silica gel column (600.0 g). Stepwise elution with hexanes (400 ml), hexane–AcOEt (10 : 1, 6 : 1, 4 : 1, 2 : 1, 3 : 2 and 1 : 1, 400 ml of each), AcOEt (400 ml), and MeOH (400 ml) gave nine fractions. The fifth fraction (8.4 g) [eluted with hexane–AcOEt (3 : 2)] contained prostanoids along with a mixture of fatty acids and steroids. A part (5.6 g) of the fifth fraction was subjected to silica gel column chromatography [silanized silica gel; $H_2O$–MeOH (3 : 1 and 1 : 2) then 1,4-dioxane as eluants] to obtain a mixture of clavulones eluted with $H_2O$–MeOH (3 : 1). The separation and purification of this fraction by MPLC and recycled HPLC [hexane–AcOEt (7 : 3) as eluant] gave **1** (4.9 mg) and **2** (0.9 mg). Clavulone II (**3**, 1090.0 mg) and clavulone III (**4**, 242.0 mg) were also obtained in this experiment.[2] These were used for chemical conversion to determine the absolute configuration of the above new compounds.

**4-Epiclavulone II (1)** Colorless oil. $[\alpha]_D^{25}$ −18.7° ($c$=0.30, $CHCl_3$). UV $\lambda_{max}$ (EtOH) nm (log $\varepsilon$): 228 (4.28), 291 (4.27). IR (dry film) $cm^{-1}$: 1738, 1732, 1704, 1644, 1232. $^1$H-NMR and $^{13}$C-NMR, see Tables 1 and 2. MS *m*/*z*: 446 ($M^+$). HREIMS *m*/*z*: 446.2315 [Calcd for $C_{25}H_{34}O_7$: 446.2305 $(M)^+$].

**4-Epiclavulone III (2)** Colorless oil. $[\alpha]_D^{25}$ −10.0° ($c$=0.06, $CHCl_3$). UV $\lambda_{max}$ (EtOH) nm (log $\varepsilon$): 229 (4.23), 295 (4.14). IR (dry film) $cm^{-1}$: 1738, 1698, 1229. $^1$H-NMR and $^{13}$C-NMR, see Tables 1 and 2. MS *m*/*z*: 446 ($M^+$). HREIMS *m*/*z*: 446.2328 [Calcd for $C_{25}H_{34}O_7$: 446.2305 ($M^+$)].

**Conversion of 3 to 1** To a mixture of **3** (14.0 mg, 0.031 mmol) in pH 7.0 phosphate buffer solution (0.067 M $KH_2PO_4$–$Na_2HPO_4$, 5 ml) and 0.2% solution of Triton X-100 (0.5 ml), was added lipase Amano PS (1200 unit/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, ethanol (2 ml) was added to terminate the reaction, and the product was concentrated under reduced pressure. The residue was extracted twice with AcOEt (25 ml) and the combined extracts were washed with saturated NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude products (15.6 mg, mainly 4-*O*-desacetylclavulone II) were obtained as a colorless oil. To a solution of the product (6.0 mg) in THF (1 ml) was added, in turn, triphenylphosphine (40.0 mg, 0.15 mmol), acetic acid (0.020 ml, 0.35 mmol), and diisopropyl azodicarboxylate (0.023 ml, 0.12 mmol) at room temperature, and then the reaction temperature was raised to 40 °C. After stirring for 40 min, pyridine (0.3 ml) was added and the mixture was concentrated under reduced pressure. The oily residue was purified by passing over a small plug of silica gel [hexane–AcOEt (2 : 1)], followed by MPLC separation [reversed phase, acetonitrile–$H_2O$ (3 : 1)] to afford **2** (3.5 mg, 65% yield) as a colorless oil: $[\alpha]_D^{25}$ −19.2° ($c$=0.17, $CHCl_3$). The spectral data obtained for the product were identical to those of natural **1**.

**Conversion of 4 to 2** Compound **2** was prepared by a procedure similar to that mentioned above; **4** (15.2 mg, 0.034 mmol) was treated with lipase Amano PS (1 ml) in pH 7.0 buffer solution (5 ml) and Triton X-100 (0.5 ml) for 24 h at 40 °C for hydrolysis, and then with triphenylphosphine (157.0 mg, 0.60 mmol), acetic acid (0.069 ml, 1.20 mmol), and diisopropyl azodicarboxylate (0.095 ml, 0.48 mmol) in THF (1 ml) for 2 h at 40 °C for Mitsunobu reaction. The crude product was purified in the same manner to give **2** (1.5 mg, 10 % yield) as a colorless oil: $[\alpha]_D^{25}$ −11.2° ($c$=0.11, $CHCl_3$). 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.*, **93**, 1807—1823 (1993), and references cited therein.
2) Kikuchi H., Tsukitani Y., Iguchi K., Yamada Y., *Tetrahedron Lett.*, **23**, 5171—5174 (1982); *idem*, *ibid.*, **24**, 1549—1552 (1983).
3) Kobayashi M., Yasuzawa T., Yoshihara M., Akutsu H., Kyogoku Y., Kitagawa I., *Tetrahedron Lett.*, **23**, 5331—5334 (1982); Kitagawa I., Kobayashi M., Yasuzawa T., Son B. W., Yoshihara M., Kyogoku Y., *Tetrahedron*, **41**, 995—1005 (1985).
4) Iguchi K., Yamada Y., Kikuchi H., Tsukitani Y., *Tetrahedron Lett.*, **24**, 4433—4434 (1983); Iguchi K., Kaneta S., Mori K., Yamada Y., Honda A., Mori Y., *ibid.*, **26**, 5787—5790 (1985); Nagaoka H., Iguchi K., Miyakoshi T., Yamada N., Yamada Y., *ibid.*, **27**, 223—226 (1986).
5) Iguchi K., Iwashima M., Watanabe K., *J. Nat. Prod.*, **58**, 790—793 (1995).
6) Iwashima M., Watanabe K., Iguchi K., *Tetrahedron Lett.*, **38**, 8319—8322 (1997); Iwashima M., Okamoto K., Konno F., Iguchi K., *J. Nat. Prod.*, **62**, 352—354 (1999).