New Marine Prostanoid Carboxylate Salts from the Okinawan Soft Coral *Clavularia viridis*

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Two new marine prostanoid carboxylate salts were isolated from the polar fraction of the Okinawan soft coral *Clavularia viridis*. Their structures, including absolute configurations, were determinded based on the results of spectroscopic analysis and chemical conversion to known compounds.

Marine prostanoids have received much attention owing to their structural features and biological activities.[1] The Okinawan soft coral *Clavularia viridis* Quoy and Gaimard (Clavulariidae) is a rich source of structurally unique antitumor prostanoids such as clavulones[2−4] and chlorovulones.[5, 6] Recently the authors reported the isolation and structures of prostanoid γ-lactones, clavulolactone II and III,[7] from the hexanes and EtOAc soluble portions of the MeOH extract of *C. viridis*. Further efforts to find congeners of these prostanoids resulted in discovering two new prostanoid carboxylate salts from the polar portion of the MeOH extract. The structures of these prostanoids were determined based on spectroscopic and chemical data.

Specimens of *C. viridis* (wet wt 3.3 kg), collected on the coral reef of Ishigaki Island (Okinawa Prefecture, Japan) in November 1993, were immersed in MeOH. The MeOH solution was diluted with a half volume of H_2O, and the mixture was extracted with hexanes. The residual aqueous portion was concentrated to one-third the original volume and successively extracted with EtOAc and BuOH to afford EtOAc- and BuOH-soluble portions. A part (5.0 g) of the BuOH-soluble portion (28.3 g) was chromatographed on a silanized Si gel column eluted with H_2O, H_2O−MeOH (1:1 and then 1:2), and MeOH to obtain eight fractions. From the fourth fraction (eluted with H_2O−MeOH, 1:1), compounds **1** (colorless solid, 11.5 mg, $[\alpha]^{25}_D$ −92.3°) and **2** (colorless solid, 1.9 mg, $[\alpha]^{26}_D$ −83.2°) were isolated by repeated separation and purification using reversed-phase flash column chromatography, MPLC, and HPLC.

The ESIMS of **1** showed a pseudomolecular ion peak at *m/z* 371, corresponding to the molecular ion ($C_{20}H_{27}O_5$-Na) plus hydrogen. The IR spectrum showed absorptions due to carboxylate (1567, 1556 cm^{-1}) as well as hydroxyl (3382 cm^{-1}) groups and a conjugated enone (1694, 1633 cm^{-1}) moiety. Compound **1** would thus appear to be a sodium carboxylate salt. This was confirmed by treatment of **1** with iodomethane in DMF to afford methyl ester **3**; in the case of carboxylic acids, such methylation with iodomethane does not proceed.

The presence of a cross-conjugated system in **1** was demonstrated by UV absorptions at 301 (log ϵ 4.01) nm and 233 (log ϵ 4.03) nm. The ^{1}H-NMR spectrum of **1** (Table 1) showed five olefinic protons in the cross-conjugated system at δ 6.30 (1H, dd, J = 5.5, 15.0 Hz, H-5), 6.32 (1H, d, J = 6.0 Hz, H-10), 6.92 (1H, d, J = 11.9 Hz, H-7), 7.09 (1H, dd, J = 11.9, 15.0 Hz, H-6), and 7.40 (1H, dd, J = 0.8, 6.0 Hz, H-11), two olefinic protons on a nonconjugated carbon−carbon double bond at δ 5.16 (1H, ddd, J = 7.4, 8.2, 11.0 Hz, H-14) and 5.45 (1H, dt, J = 7.3, 11.0 Hz, H-15); a hydroxyl-bearing methine group at δ 4.34 (1H, br m, H-4), and a terminal methyl group at δ 0.93 (3H, t, J = 6.9 Hz, H-20). ^{1}H−^{1}H COSY demonstrated sequential ^{1}H−^{1}H correlations from H-2 to H-7 on the α side chain and from H-13 to H-20 on the ω side chain. The ^{13}C-NMR spectrum of **1** showed 18 carbon signals: eight methines, six methylenes, one methyl, and three quaternary carbons, whose assignments were made based on ^{13}C−^{1}H COSY. Signals of the remaining two carbons at C-1 and C-2, unfortunately, could not be observed, owing possibly to a signal broadening.[8] The structure from C-7 to C-13 was confirmed by HMBC correlations shown in Figure 1. These spectroscopic findings suggested **1** to have a structure similar to those of clavulone II and clavulolactone II, though signals of the two carbons were not observed in the ^{13}C-NMR spectrum.

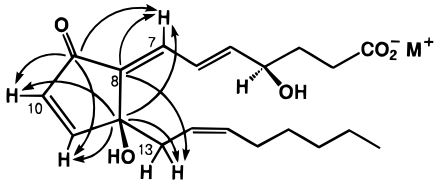

Figure 1. Key HMBC correlations of **1**.

The structure of **1**, including its absolute stereochemistry was determined by chemical conversion. Treatment of **1** with Ac_2O in pyridine at room temperature caused acetylation of the C-12 hydroxyl group and lactonization between carboxylate (C-1) and hydroxyl (C-4) groups with the consequent formation of clavulolactone II (**4**). Physical properties including the optical rotation ($[\alpha]^{28}_D$ −28.3°) of synthetic **4** were identical to those of natural clavulolactone II, whose absolute stereochemistry was previously established.[7]

Compound **2** was found to have the molecular formula $C_{20}H_{27}O_5Na$, the same as that of **1**. The ^{1}H-NMR spectrum of **2** (Table 1) was quite similar to that of **1**, except for the signals of H-5, H-6, and H-7, indicating **2** to be the 7*Z*-isomer of **1**. A comparison of the spectral data of **2** with those of clavulolactone III (**5**) also supports this structure for **2**. The absolute stereochemistry of **2** was determined by comparing the CD spectra

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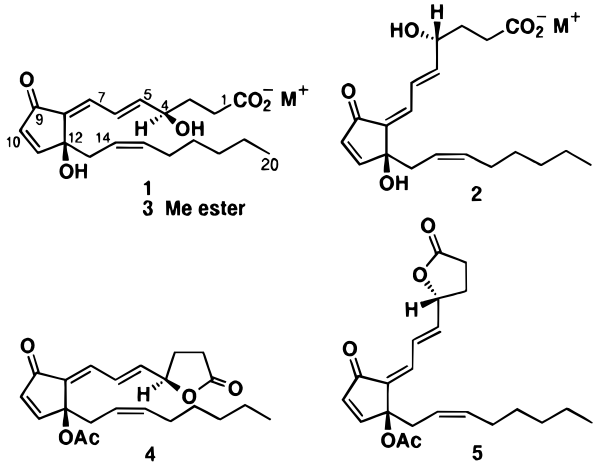
Table 1. ^{1}H-NMR Data (400 MHz, MeOH-d_4) of **1** and **2**

	compound	
no.	1	2
2	2.37 (2H, br m)	2.36 (2H, br m)
3	1.85−1.95 (2H, br m)	1.88 (2H, br m)
4	4.34 (1H, br m)	4.29 (1H, br m)
5	6.30 (1H, dd, J = 5.5, 15.0 Hz)	6.21 (1H, dd, J = 5.5, 15.3 Hz)
6	7.09 (1H, dd, J = 11.9, 15.0 Hz)	7.73 (1H, dd, J = 11.4, 15.3 Hz)
7	6.92 (1H, d, J = 11.9 Hz)	6.70 (1H, d, J = 11.4 Hz)
10	6.32 (1H, d, J = 6.0 Hz)	6.28 (1H, d, J = 6.0 Hz)
11	7.40 (1H, dd, J = 0.8, 6.0 Hz)	7.34 (1H, d, J = 6.0 Hz)
13	2.72 (1H, dd, J = 7.4, 14.0 Hz) 2.84 (1H, dd, J = 8.2, 14.0 Hz)	2.62 (2H, d, J = 7.6 Hz)
14	5.16 (1H, ddd, J = 7.4, 8.2, 11.0 Hz)	5.26 (1H, ttd, J = 1.6, 7.6, 11.0 Hz)
15	5.45 (1H, td, J = 7.3, 11.0 Hz)	5.48 (1H, td, J = 7.3, 11.0 Hz)
16	2.00 (2H, m)	2.02 (2H, m)
17−19	1.25−1.40 (6H, m)	1.25−1.40 (6H, m)
20	0.93 (3H, t, J = 6.9 Hz)	0.93 (3H, t, J = 6.9 Hz)

of **2** and **1**. The CD spectrum of **2** exhibited a Cotton effect at λ_{ext} 257 ($\Delta\epsilon$ = +4.0) and 228 ($\Delta\epsilon$ = −12.3) nm, these values being similar to those of **1** [λ_{ext} 257 ($\Delta\epsilon$ = +5.4) and 230 ($\Delta\epsilon$ = −15.3) nm]. Therefore the absolute configurations at C-4 and C-12 are the same in both compounds.



Experimental Section

General Experimental Procedures. IR spectra were recorded with a Perkin-Elmer FT-IR 1600 spectrophotometer and UV spectra with a JASCO V-520 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AM-400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) in MeOH-d_4 or $CDCl_3$. 2D-NMR spectra were obtained using a JEOL JNM-A-500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz). ^{1}H−^{1}H COSY, ^{1}H−^{13}C COSY, and HMBC were measured based on standard JEOL pulse sequences. Chemical shifts are given on a δ (ppm) scale with MeOH (^{1}H, 3.34 ppm; ^{13}C, 49.8 ppm) or $CHCl_3$ (^{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). MS were obtained with a Micromass Auto Spec spectrometer. CD spectra were obtained by a JASCO J-720 circular dichrometer. Optical rotation was measured with a JASCO DIP-370 automatic polarimeter. Column chromatography was carried out on a Merck Si gel 60 silanized (70−230 mesh) column, and flash column chromatography was performed on YMC-GEL ODS-A 120−230/70 column. Medium-pressure liquid chromatography (MPLC) was carried out with KHLC-201-43 (Kusano) apparatus using a CIG prepack column (Si gel, CPS-HS-221-05, for the normal phase and ODS Si gel, CPO-HS-221-20, for the reversed phase). HPLC was conducted with a YMC-Pack ODS-AM column (ODS Si gel, SH-343-5AM, reversed phase).

Extraction and Isolation. The soft coral *Clavularia viridis* Quoy and Gaimard was collected from the coral reef of Ishigaki Island (Okinawa Prefecture, Japan) in November 1993 at a depth of 1−2 m. A voucher specimen (no. SC-93-1) is presently on deposit at this laboratory, Tokyo University of Pharmacy and Life Science (Tokyo, Japan). Wet specimens (3.3 kg) were immersed in MeOH (2.5 L). After filtration, the MeOH solution was diluted with a half volume of H_2O, and the mixture was extracted with hexanes. The residual aqueous portion was concentrated to one-third the original volume and then extracted successively with EtOAc and BuOH. Each fraction was concentrated under reduced pressure to give hexanes (7.6 g) and EtOAc- (12.1 g) and BuOH- (28.3 g) soluble portions.

A part of the BuOH-soluble portion (5.0 g) was chromatographed on a silanized Si gel column (100 g). Stepwise elution with H_2O (400 mL), H_2O−MeOH (1:1 and 1:2, each 400 mL), and MeOH (700 mL) gave eight fractions. The fourth fraction (155 mg) (eluted with H_2O−MeOH, 1:1) was subjected to reversed-phase flash column chromatography (H_2O−MeOH, 1:1 and 1:2, as the eluents) to obtain a crude carboxylate salt fraction. The separation and purification of this crude carboxylate salt fraction by reversed-phase MPLC and HPLC (H_2O−MeCN, 3:7, as the eluent) gave **1** (11.5 mg) and **2** (1.9 mg).

Compound 1: obtained as a colorless solid; $[\alpha]^{25}_D$ −92.3° (*c* 0.16, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 301 (4.01), 233 (4.03); CD λ_{ext} (EtOH) ($\Delta\epsilon$) 257 (+5.4), 230 (−15.3); IR ν_{max} cm^{-1} (dry film) 3382, 1694, 1633, 1567, 1556 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR (MeOH-d_4, 100 MHz) δ ppm 198.9 (C, C-9), 165.2 (CH, C-11), 150.3 (CH, C-5), 140.7 (C, C-8), 135.7 (CH, C-10), 135.4 (CH, C-15), 133.2 (CH, C-7), 126.3 (CH, C-6), 124.7 (CH, C-14), 81.1 (C, C-12), 73.6 (CH, C-4), 38.5 (CH_2, C-13), 35.1 (CH_2, C-3), 33.5 (CH_2, C-18), 31.2 (CH_2, C-17), 29.2 (CH_2, C-16), 24.4 (CH_2, C-19), 15.2 (CH_3, C-20); ESIMS *m*/*z*: [M($C_{20}H_{27}O_5Na$) + H]$^+$ 371; HREIMS *m*/*z* calcd for $C_{20}H_{26}O_4$ [M − NaOH]$^+$ 330.1831, found 330.1834.

Compound 2: obtained as a colorless solid; $[\alpha]^{26}_D$ −83.2° (*c* 0.095, MeOH); UV (EtOH) λ_{max} nm (log ϵ) 305 (4.11), 233 (4.14); CD λ_{ext} (EtOH) ($\Delta\epsilon$) 257 (+4.0), 228

(−12.3); IR ν_{max} cm^{-1} (dry film) 3382, 1694, 1574, 1558; ^1H NMR, see Table 1; ^{13}C NMR (MeOH-d_4, 100 MHz) δ ppm 198.7 (C, C-9), 163.0 (CH, C-11), 149.4 (CH, C-5), 140.1 (C, C-8), 137.2 (CH, C-10), 136.7 (CH, C-15), 135.2 (CH, C-7), 126.7 (CH, C-6), 124.9 (CH, C-14), 80.6 (C, C-12), 73.6 (CH, C-4), 39.0 (CH_2, C-13), 35.2 (CH_2, C-3), 33.4 (CH_2, C-18), 31.1 (CH_2, C-17), 29.1 (CH_2, C-16), 24.4 (CH_2, C-19), 15.2 (CH_3, C-20); ESIMS *m/z*: $[M(C_{20}H_{27}O_5Na) + H]^+$ 371; HREIMS *m/z* calcd for $C_{20}H_{26}O_4$ $[M - NaOH]^+$ 330.1831, found 330.1832.

Conversion of 1 to 3. To a solution of **1** (5.6 mg) in DMF (0.3 mL) was added methyliodide (0.2 mL) at room temperature. The reaction mixture was allowed to stand for 20 h. The reaction mixture was then concentrated under reduced pressure, and the residual material was subjected to reversed-phase HPLC (MeCN−H_2O, 1:1, as the eluent) to obtain methyl ester **3** (3.2 mg, 60% yield.

Compound 3: colorless oil; $[a]^{25}D$ −98.8° (*c* 0.16, $CHCl_3$); IR ν_{max} cm^{-1} (dry film) 3418, 1738, 1694, 1634; ^1H NMR ($CDCl_3$, 400 MHz) d ppm 7.32 (1H, dd, *J* = 0.5, 6.0 Hz, H-11), 6.96 (1H, ddd, *J* = 1.2, 11.8, 14.0 Hz, H-6), 6.91 (1H, d, *J* = 11.8 Hz, H-7), 6.33 (1H, d, *J* = 6.0 Hz, H-10), 6.19 (1H, dd, *J* = 5.8, 14.0 Hz, H-5), 5.52 (1H, ttd, *J* = 1.6, 7.3, 10.9 Hz, H-15), 5.22 (1H, ttd, *J* = 1.6, 7.7, 10.9 Hz, H-14), 4.38 (1H, br m, H-4), 3.68 (3H, s, CO_2CH_3), 2.79 (1H, dd, *J* = 7.7, 14.1 Hz, H-13), 2.67 (1H, dd, *J* = 7.7, 14.1 Hz, H-13), 2.47 (2H, m, H-2), 2.39 (2H, br, OH × 2) 1.96 (2H, m, H-16), 1.89 (2H, m, H-3), 1.20−1.35 (6H, m, H-17, 18 and 19), 0.87 (3H, t, *J* = 7.0 Hz, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm 195.2 (C, C-9), 174.3 (C, C-1), 161.2 (CH, C-11), 146.8 (CH, C-5), 138.7 (C, C-8), 134.8 (CH, C-10 or C-15), 134.6 (CH, C-15 or C-10), 131.1 (CH, C-7), 124.6 (CH, C-6), 122.1 (CH, C-14), 79.5 (C, C-12), 71.3 (CH, C-4), 51.8 (CH_3, $CO_2\underline{C}H_3$), 36.7 (CH_2, C-13), 31.6 (CH_2, C-3 or C-18), 31.5 (CH_2, C-18 or C-3), 29.9 (CH_2, C-17), 29.1 (CH_2, C-2), 27.4 (CH_2, C-16), 22.5 (CH_2, C-19), 14.0 (CH_3, C-20); CIMS *m/z* $M(C_{21}H_{30}O_5)^+$ 362; HREIMS *m/z* calcd for $C_{21}H_{30}O_5$ (M^+) 362.2093 found 362.2090.

Conversion of 1 to 4. To a mixture of **1** (3.7 mg) in pyridine (1.0 mL) was added Ac_2O (1.0 mL) at room temperature. The reaction mixture was allowed to stand for 4 h. The reaction mixture was concentrated under reduced pressure and the residue was passed through a Si gel (5g) short column (hexanes−EtOAc, 1:1, as the eluent). The crude product was purified by normal-phase MPLC (hexanes−EtOAc, 3:2, as the eluent) to provide **4** (1.2 mg, 32% yield).

Compound 4: a colorless solid; $[\alpha]^{28}D$ −28.3° (*c* 0.06 $CHCl_3$); ^1H- and ^{13}C-NMR spectra of synthetic **4** were identical with those of natural clavulolactone II.[7]

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References and Notes

(1) Gerwick, W. H. *Chem. Rev.* **1993**, *93*, 1807−1823 and references cited 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) Iguchi, K.; Iwashima, M.; Watanabe, K. *J. Nat. Prod.* **1995**, *58*, 790−793.
(8) Chadwick, D. J.; Dunitz, J. D. *J. Chem. Soc., Perkin Trans. 2* **1979**, 276−284.

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