Journal of Natural Products
Vol. 58, No. 5, pp. 790-793, May 1995

CLAVULOLACTONES, NEW MARINE PROSTANOIDS WITH A γ-LACTONIC MOIETY IN THE α- SIDE-CHAIN FROM THE OKINAWAN SOFT CORAL, *CLAVULARIA VIRIDIS*

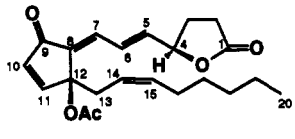
KAZUO IGUCHI,* MAKOTO IWASHIMA, and KINZO WATANABE

Laboratory of Bioorganic Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science,[1] Horinouchi, Hachioji, Tokyo 192-03, Japan

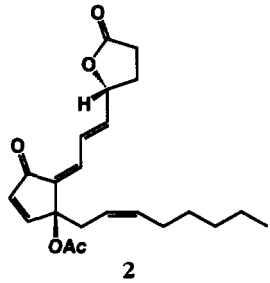
ABSTRACT.—Two new marine prostanoids, clavulolactones II [**1**] and III [**2**], were isolated from the Okinawan soft coral *Clavularia viridis*. Their structures, including absolute configurations, were elucidated by spectroscopic analysis and chemical conversion.

Marine prostanoids have received much attention owing to their structural features and biological activity (1). The Okinawan soft coral *Clavularia viridis* Quoy and Gaimard (Clavulariidae) is a rich source of structurally unique antitumor prostanoids such as the clavulones (2–4) and the chlorovulones (5,6). While conducting investigations on minor congeners of these prostanoids from *C. viridis*, two new marine prostanoids, clavulolactones II [**1**] and III [**2**], were found. These compounds are the first examples of natural prostanoids possessing a γ-lactone moiety in the α- side-chain. The structures of these prostanoids were determined based on spectroscopic and chemical data.

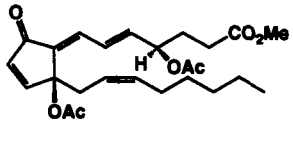
Specimens of *C. viridis* (wet wt 3.3 kg), collected on the coral reef of Ishigaki Island, Okinawa, Japan, in November 1993, were immersed in MeOH. The MeOH solution was diluted with a half volume of H_2O, and the mixture was extracted with hexane. The residual aqueous portion was concentrated to one third the original volume, and extracted with EtOAc. From the hexane-soluble portion (7.6 g), clavulones II [**3**, 185 mg] and III [**4**, 168 mg] were obtained (see Experi-



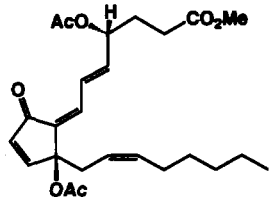
1



2



3



4

[1]Formerly known as the Tokyo College of Pharmacy.

mental).[2] The EtOAc-soluble portion (12.1 g) was chromatographed on a Si gel column by elution, in turn, with hexane-EtOAc (10:1 and then 1:1), EtOAc, and MeOH, to give four fractions. From fraction 2 [eluted by hexane-EtOAc (1:1)], clavulolactones II [**1**, colorless oil, 18 mg, $[\alpha]^{25}D$ −25.6°] and III [**2**, colorless oil, 20 mg, $[\alpha]^{25}D$ −7.3°] were obtained by repeated purification by flash cc, mplc, and hplc.[3]

The molecular formula, $C_{22}H_{28}O_5$, of clavulolactone II [**1**] was established by hreims (see Experimental). The ir spectrum showed absorptions due to γ-lactone (1778 cm^{-1}), ester (1745 cm^{-1}), and conjugated enone moieties (1704 and 1644 cm^{-1}). The presence of a cross-conjugated system in **1** was suggested by characteristic uv absorptions at 292 (ε 16500) nm and 231 (ε 12500) nm, which are similar to those of clavulone II [**3**] (2). Olefinic proton signals in the ^{1}H-nmr spectrum of **1** (Table 1) supported the presence of the cross-conjugated system, with significant signals at δ 6.17 (1H, dd, J=4.9 and 14.7 Hz, H-5), 6.42 (1H, d, J=6.1 Hz, H-10), 6.82 (1H, ddd, J=1.5, 12.0, and 14.7 Hz, H-6), 6.91 (1H, br d, J=12.0 Hz, H-7), and 7.50 (1H, br d, J=6.1 Hz, H-11). Signals due to the γ-lactone moiety were found in the ^{1}H- and ^{13}C-nmr spectra, as follows: δ_H 5.15 (1H, m, H-4); δ_C 78.6 (CH, C-4), 176.2 (CO, C-1). The ^{1}H- and ^{13}C-nmr spectra of **1** also showed signals due to an acetoxy group, a non-conjugated carbon-carbon double bond, four methylenes, a terminal methyl, and a quaternary carbon (Table 1, Experimental). These spectral data for **1** were similar to those of clavulone II [**3**] except for data due to C-1–C-4 in the α- side-chain, indicating

TABLE 1. ^{1}H-Nmr Data (400 MHz, $CDCl_3$) of **1** and **2**.

Proton	Compound	
	1	**2**
2	2.55 (2H, m)	2.58 (2H, dd, J=6.9, 9.3 Hz)
3	2.04 (1H, m)	2.05 (1H, m)
	2.52 (1H, m)	2.47 (1H, m)
4	5.15 (1H, m)	5.10 (1H, br dd, J=7.3, 7.9 Hz)
5	6.17 (1H, dd, J=4.9, 14.7 Hz)	6.08 (1H, dd, J=7.3, 15.6 Hz)
6	6.82 (1H, ddd, J=1.5, 12.0, 14.7 Hz)	7.82 (1H, br dd, J=11.3, 15.6 Hz)
7	6.91 (1H, br d, J=12.0 Hz)	6.53 (1H, d, J=11.3 Hz)
10	6.42 (1H, d, J=6.1 Hz)	6.38 (1H, d, J=6.1 Hz)
11	7.50 (1H, br d, J=6.1 Hz)	7.49 (1H, d, J=6.1 Hz)
13	2.71 (1H, br dd, J=8.1, 14.2 Hz)	2.65 (1H, br dd, J=7.6, 14.3 Hz)
	2.91 (1H, br dd, J=7.1, 14.2 Hz)	2.84 (1H, br dd, J=7.3, 14.3 Hz)
14	5.15 (1H, m)	5.22 (1H, m)
15	5.52 (1H, m)	5.54 (1H, m)
16	1.94 (2H, br q, J=6.3 Hz)	1.97 (2H, br q, J=7.2 Hz)
17–19	1.20–1.34 (6H, m)	1.22–1.33 (6H, m)
20	0.87 (3H, t, J=7.2 Hz)	0.88 (3H, t, J=7.1 Hz)
CH_3CO	2.02 (3H, s)	2.03 (3H, s)

[2]In several previous isolations of clavulones from *C. viridis*, clavulone II [**3**] and clavulone I (a 5*Z*-isomer of **3**) were usually obtained as the major prostanoids, with clavulone III [**4**] as a minor constituent. From the present specimens of *C. viridis*, however, clavulone I could be detected only in small amounts.

[3]The presence of a lead compound in this series of prostanoids, clavulolactone I (a 5*Z*-isomer of **1**), was indicated by ^{1}H-nmr analysis [δ 7.49 (1H, d, H-11), 7.12 (1H, d, H-7), 6.67 (1H, dd, H-6), 6.44 (1H, d, H-10), 6.03 (1H, dd, H-5), 5.53 (2H, m, H-4, -15), 5.15 (1H, m, H-14)] of chromatographic fractions. It is unfortunate that this compound was not isolated in pure form from the mixture of clavulolactones, being present in too small an amount.

the structure of clavulolactone II to be **1**.

The structure of **1**, including its absolute configuration, was established by chemical conversion. Enzymatic hydrolysis of the two ester groups in the α-side-chain of clavulone II [**3**] was carried out with lipase Amano PS at 40° for 48 h in pH 7.0 phosphate buffer solution (0.067 M KH_2PO_4-Na_2HPO_4) with 0.2% Triton X-100, a non-ionic surfactant used to partially solubilize **3**. The resulting crude 4-hydroxycarboxylic acid was treated with a catalytic amount (0.03 equivalents) of *p*-toluenesulfonic acid in EtOAc at room temperature to give clavulolactone II in 54% yield from **3**. The physical properties including the optical rotation {$[\alpha]^{25}D$ −27.2°} of synthetic clavulolactone II were identical with those of natural clavulolactone II [**1**].

Clavulolactone III [**2**] was found to have the molecular formula $C_{22}H_{28}O_5$, the same as that of clavulolactone II [**1**]. The ^{1}H-nmr spectrum of **2** was quite similar to that of **1** except for signals due to H-5, -6, and -7, indicating clavulolactone III to be the 7*Z*-isomer of **1**. A comparison of the spectral data of **2** with those of clavulone III [**4**, the 7*Z*-isomer of clavulone II] also supported this structure for clavulolactone III. The stereostructure of **2** was determined by chemical conversion. Enzymatic hydrolysis of clavulone III [**4**] using lipase Amano PS followed by acid-catalyzed lactonization provided clavulolactone III {$[\alpha]^{25}D$ −7.8°}, which was identical with natural clavulolactone III [**2**].

Clavulolactones appear to be natural products, since the present specimens of *C. viridis* were preserved at low temperature, and the conditions for extraction and isolation were mild enough to prevent the conversion of clavulones to clavulolactones. This was further supported by the following experiment. Pure clavulones II [**3**] and III [**4**] were each dissolved in MeOH-H_2O (1:1), and the mixtures stirred for 48 h at 40° and concentrated under reduced pressure at room temperature. No change was observed in either experiment, resulting in the recovery of the starting clavulone, in each case.

Clavulolactones could not be obtained from *C. viridis* in several previous studies (2–6), suggesting the present soft coral to be a different chemotype of *C. viridis*.

EXPERIMENTAL

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 $CDCl_3$. 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 taken with a VG Auto Spec spectrometer. Optical rotations were measured with a Jasco DIP-370 automatic polarimeter. Cc was carried out on Merck Si gel 60 (70–230 mesh) and flash cc was performed on Merck Si gel 60 (230–400 mesh), respectively. Mplc was carried out with a KHLC-201-43 (Kusano) apparatus using a CIG prepack column (Si gel, CPS-HS-221-05, normal phase). Hplc was conducted with a YMC-Pack SIL column (Si gel, SH-063-5, normal phase).

EXTRACTION AND ISOLATION.—The soft coral *Clavularia viridis* Quoy and Gaimard was collected from the coral reef of Ishigaki Island, Okinawa, Japan, in November 1993, at a depth of 1–2 m. A voucher specimen (No. SC-93-1) is deposited at Tokyo University of Pharmacy and Life Science, Tokyo, Japan. Wet specimens (3.3 kg) were immersed in MeOH (2.5 liters). After filtration, the MeOH solution was diluted with a half volume of H_2O, and the mixture was extracted with hexane. The residual aqueous portion was concentrated to one-third the original volume, and then extracted with EtOAc. This procedure was repeated two times.

The combined hexane-soluble portion (7.6 g) was chromatographed on a Si gel column (100 g). Stepwise elution with hexane-EtOAc (10:1 and 2:1), EtOAc, and MeOH (each 600 ml) gave seven fractions. Fractions 3 (3.4 g) and 4 (1.7 g) [eluted with hexane-EtOAc (2:1)] were independently purified by Si gel flash cc [hexane-EtOAc (6:1) as eluent], to give crude clavulones (total 400 mg). Further purification was carried out by mplc [hexane-Et_2O (3:1) as eluent] to give clavulone II [**3**, 185 mg, colorless oil] and clavulone III [**4**, 168 mg, colorless oil], respectively.

The combined EtOAc-soluble portion (12.1 g) was chromatographed on a Si gel column (150 g). Stepwise elution with hexane-EtOAc (10:1 and

1:1), EtOAc, and MeOH (each 600 ml) provided four fractions. The 2nd fraction [5.0 g eluted with hexane-EtOAc (1:1)] was subjected to Si gel flash cc [hexane-EtOAc (4:1) as eluent] and then mplc [hexane-Et_2O (3:2) as eluent] to give crude clavulolactones. Further purification was carried out with hplc [hexane-EtOAc (3:1) as eluent] to give, in turn, clavulolactone II [**1**, 18 mg, colorless oil] and clavulolactone III [**2**, 20 mg, colorless oil].

Clavulolactone II [**1**].—Colorless oil; $[\alpha]^{25}D$ −25.6° (c=0.26, $CHCl_3$); uv (EtOH) λ max 292 (ε 16500), 231 (ε 12500) nm; ir (dry film) ν max 1778, 1745, 1704, 1644, 1231 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C nmr ($CDCl_3$, 100 MHz) δ 193.3 (C, C-9), 176.2 (C, C-1), 169.3 (C, CH_3CO), 158.0 (CH, C-11), 140.8 (CH), 137.2 (C, C-8), 135.14 (CH), 135.06 (CH), 128.9 (CH), 124.9 (CH), 121.0 (CH), 85.2 (C, C-12), 78.6 (CH, C-4), 35.8 (CH_2), 31.5 (CH_2), 29.0 (CH_2), 28.2 (CH_2), 27.7 (CH_2), 27.4 (CH_2), 22.5 (CH_2), 21.2 (CH_3, CH_3CO), 14.0 (CH_3, C-20); eims m/z $[M]^+$ 372; hreims m/z calcd for $C_{22}H_{28}O_5$ $[M]^+$ 372.1937, found 372.1920.

Clavulolactone III [**2**].—Colorless oil; $[\alpha]^{25}D$ −7.3° (c=0.21, $CHCl_3$); uv λ max (EtOH) 293 (ε 15500), 229 (ε 17900) nm; ir ν max (dry film) 1778, 1742, 1698, 1643, 1620, 1232 cm^{-1}; ^{1}H-nmr data, see Table 1; ^{13}C nmr ($CDCl_3$, 100 MHz) δ 194.0 (C, C-9), 176.5 (C, C-1), 169.6 (C, CH_3CO), 156.3 (CH, C-11), 139.8 (C, C-8), 139.8 (CH), 136.6 (CH), 135.0 (CH), 132.4 (CH), 127.6 (CH), 121.1 (CH), 85.0 (C, C-12), 80.0 (CH, C-4), 35.7 (CH_2), 31.4 (CH_2), 29.0 (CH_2), 28.6 (CH_2×2), 27.4 (CH_2), 22.5 (CH_2), 21.6 (CH_3, CH_3CO), 14.0 (CH_3, C-20); eims m/z $[M]^+$ 372; hreims m/z calcd for $C_{22}H_{28}O_5$ $[M]^+$ 372.1937, found 372.1961.

CONVERSION OF **3** TO **1**.—To a mixture of **3** (8 mg) in pH 7.0 phosphate buffer solution (0.067 M KH_2PO_4-Na_2HPO_4, 2 ml) and a 0.2% solution of Triton X-100 (0.5 ml) was added lipase Amano PS (1200 unit/ml, 0.8 ml) in phosphate buffer solution (0.067 M KH_2PO_4-Na_2HPO_4) at room temperature. The reaction mixture was vigorously stirred for 48 h at 40°. A 2-ml quantity of EtOH was added to the mixture to terminate the reaction, and the mixture 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-hydroxycarboxylic acid) in EtOAc (1 ml) was added *p*-TsOH (0.2 mg) at room temperature. After stirring for 4 h at this temperature, pyridine (0.02 ml) was added and the mixture was concentrated under reduced pressure. The oily residue was dissolved in hexane-EtOAc (2:1) and the solution was passed through a small plug of Si gel. The eluate was concentrated under reduced pressure to give an oily residue which was purified by mplc [hexane-EtOAc (4:1)] to provide **1** (3.6 mg, 54% yield from **3**) as a colorless oil: $[\alpha]^{25}D$ −27.2° (c=0.18, $CHCl_3$); ir and ^{1}H-nmr spectra of synthetic **1** were identical with those of natural clavulolactone II.

CONVERSION OF **4** TO **2**.—Clavulone III [**4**] (14 mg) was converted to clavulolactone III [**2**] (6.4 mg, 55% yield from **4**) by a procedure essentially the same as that used for the conversion of clavulone II [**3**] to clavulolactone II [**1**]. The ir and ^{1}H-nmr spectra of synthetic clavulolactone III {$[\alpha]^{25}D$ −7.8° (c=0.32, $CHCl_3$)} were identical to those of natural clavulolactone III [**2**].

ACKNOWLEDGMENTS

We thank Prof. Y. Yamada, Tokyo University of Pharmacy and Life Science, for providing spectral data of the clavulones. Thanks are also due to Amano Pharmaceutical Co., Ltd., for furnishing the enzymes used in this investigation.

LITERATURE CITED

1. W.H. Gerwick, *Chem. Rev.*, **93**, 1807 (1993), and references cited therein.
2. H. Kikuchi, Y. Tsukitani, K. Iguchi, and Y. Yamada, *Tetrahedron Lett.*, **23**, 5171 (1982).
3. H. Kikuchi, Y. Tsukitani, K. Iguchi, and Y. Yamada, *Tetrahedron Lett.*, **24**, 1549 (1983).
4. K. Iguchi, Y. Yamada, H. Kikuchi, and Y. Tsukitani, *Tetrahedron Lett.*, **24**, 4433 (1983).
5. K. Iguchi, S. Kaneta, K. Mori, Y. Yamada, A. Honda, and Y. Mori, *Tetrahedron Lett.*, **26**, 5787 (1985).
6. H. Nagaoka, K. Iguchi, T. Miyakoshi, N. Yamada, and Y. Yamada, *Tetrahedron Lett.*, **27**, 223 (1986).

Received 8 November 1994