Pergamon

Tetrahedron Letters, Vol. 38, No. 48, pp. 8319-8322, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10211-8

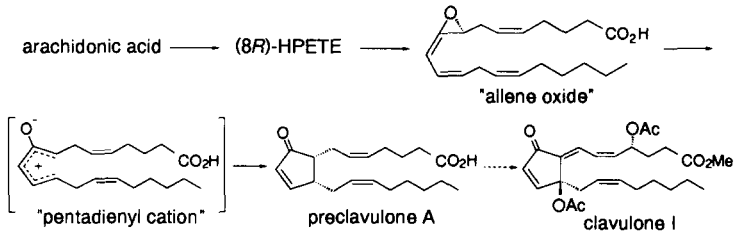
New Marine Prostanoids, Preclavulone Lactones, from the Okinawan Soft Coral *Clavularia viridis*

Makoto Iwashima, Kinzo Watanabe and Kazuo Iguchi*

School of Life Science, Tokyo University of Pharmacy and Life Science, Horinouchi, Hachioji, Tokyo 192-03, JAPAN

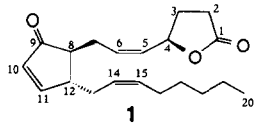
Abstract: Two new marine prostanoids, preclavulone lactones I (1) and II (2) were isolated from the Okinawan soft coral, *Clavularia viridis*. Their structures were determined based on spectroscopic analysis and chemical synthesis from (*S*)-malic acid. Preclavulone lactones with side chains having absolute configurations opposite to those of mammalian prostanoids could be recognized as key intermediates in biosynthesis of clavulones. © 1997 Elsevier Science Ltd.

Clavulones,[1] marine prostanoids isolated from the Okinawan soft coral *Clavularia viridis* Quoy and Gaimard (Clavularidae) are of particular interest because of their unique structural features, remarkable antitumor activity and of unusual biosynthesis from arachidonic acid.[2] The biosynthesis has been reported by Corey *et al.* who identified preclavulone A in cell-free incubation experiments. They proposed a mechanism involving an allene oxide and a pentadienyl cation as intermediates as shown in Scheme 1.[2a] However, the step from preclavulone A to clavulones has not yet been clarified. Furthermore, the absolute configuration of preclavulone A remains to be determined.



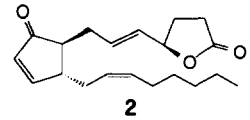
Scheme 1

In our continuing investigation of marine prostanoids from *C. viridis*,[3] we recently discovered two new prostanoids, preclavulone lactone I (**1**) and II (**2**), which may be considered as important intermediates in the biogenesis from preclavulone A to clavulones. We describe here the isolation and structural elucidation of these new prostanoids as well as a possible biogenesis of clavulones. Their chemical structures including absolute configurations were determined based on spectroscopic analysis and the stereo-controlled synthesis of **1** from (*S*)-malic acid. Preclavulone lactones are the first natural prostanoids possessing the side chains oriented to have opposite absolute configurations to those of mammalian prostaglandins.



1

preclavulone lactone I



2

preclavulone lactone II

Wet specimens of *C. viridis* (3.3 kg), collected on a coral reef of Ishigaki Island (Okinawa, Japan) in November 1993, were immersed in methanol. The methanol solution was diluted with a half volume of water, and the mixture was extracted with hexanes. The aqueous portion was concentrated to one-third, and the

remaining mixture was extracted with ethyl acetate. Repeated chromatographic separation of the ethyl acetate soluble portion (12.2 g) afforded preclavulone lactone I (**1**) (2.1 mg, $[\alpha]_D^{25}$ -168.0°) and preclavulone lactone II (**2**) (0.6 mg, $[\alpha]_D^{25}$ -110°). The molecular formula of **1** was assigned as $C_{20}H_{28}O_3$ by HRFABMS (found *m/z* 316.2028, calcd for 316.2038). All 20 carbons appeared in the ^{13}C-NMR spectrum of **1** (Table 1). DEPT indicated one methyl, eight sp^3 methylenes, three sp^3 methines, six sp^2 methines and two sp^2 quaternary carbons. The IR spectrum of **1** showed absorptions due to a five-membered lactone (1775 cm^{-1}) and an α,β-unsaturated cyclopentenone (1706 cm^{-1}). The presence of the conjugated cyclopentenone was also indicated by the UV spectrum; λ_{max} (acetonitrile) 215 nm (ε 5740) and the ^{1}H-NMR spectrum; δ 6.16 (1H, dd) and

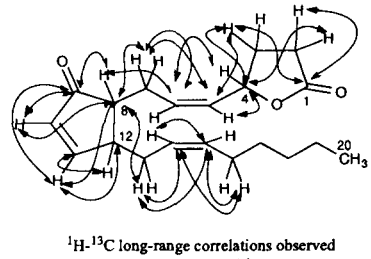
Table 1. NMR Data for Preclavulone Lactones I (**1**) and II (**2**)*

	1			**2**	
No	^{13}C	^{1}H	No	^{13}C	^{1}H
1	177.0 (C)		1	176.8 (C)	
2	28.9 (CH_2)	2.57 (2H, dd, 6.6, 9.7)	2	28.5 (CH_2)	2.52 (2H, m)
3	29.1 (CH_2)#	1.92 (1H, dtd, 8.4, 9.7, 12.9)	3	28.7 (CH_2)	1.96 (1H, m)
		2.41 (1H, qd, 6.6, 12.9)			2.37 (1H, m)
4	76.0 (CH)	5.27 (1H, dt, 6.6, 8.4)	4	80.4 (CH)	4.89 (1H, q, 6.7)
5	129.9 (CH)	5.53 (1H, dd, 8.4, 11.1)	5	130.1 (CH)	5.58 (1H, br dd, 6.7, 15.2)
6	130.9 (CH)	5.56 (1H, td, 7.2, 11.1)	6	131.5 (CH)	5.76 (1H, br td, 7.7, 15.2)
7	27.5 (CH_2)	2.52 (2H, m)	7	33.1 (CH_2)	2.50 (2H, m)
8	50.3 (CH)	2.11 (1H, dt, 2.3, 5.9)	8	50.2 (CH)	2.11 (1H, m)
9	210.6 (C)		9	210.6 (C)	
10	133.4 (CH)	6.16 (1H, dd, 2.3, 5.8)	10	133.0 (CH)#	6.15 (1H, dd, 2.4, 5.8)
11	167.2 (CH)	7.60 (1H, dd, 2.3, 5.8)	11	166.9 (CH)	7.58 (1H, dd, 2.4, 5.8)
12	46.3 (CH)	2.72 (1H, qt, 2.3, 7.0)	12	46.9 (CH)	2.67 (1H, qt, 2.4, 7.0)
13	31.3 (CH_2)	2.27 (1H, br td, 7.0, 14.5)	13	31.3 (CH_2)	2.27 (1H, m)
		2.32 (1H, br td, 7.1, 14.5)			2.32 (1H, m)
14	125.2 (CH)	5.36 (1H, br td, 7.1, 10.8)	14	125.2 (CH)	5.33 (1H, br td, 7.8, 10.9)
15	133.1 (CH)	5.54 (1H, m)	15	133.1 (CH)#	5.52 (1H, br td, 7.3, 10.9)
16	27.3 (CH_2)	2.01 (2H, br q, 7.3)	16	27.3 (CH_2)	2.00 (1H, br q, 7.3)
17-19	22.5 (CH_2)	1.2-1.4 (6H, m)	17-19	22.5 (CH_2)	1.2-1.4 (6H, m)
	29.2 (CH_2)#			29.2 (CH_2)	
	31.5 (CH_2)			31.5 (CH_2)	
20	14.0 (CH_3)	0.89 (3H, t, 7.5)	20	14.0 (CH_3)	0.89 (3H, t, 7.2)

* ^{1}H; 500 MHz in $CDCl_3$. ^{13}C; 125 MHz in $CDCl_3$. *J* in Hz.

Values in the columns are interchangeable. Assignments of the ^{13}C and ^{1}H signals were made based on ^{13}C-^{1}H COSY.

7.60 (1H, dd). The ^{1}H-NMR spectrum also showed signals due to two nonconjugated carbon-carbon double bonds at δ 5.36 (1H, br td), 5.53 (1H, dd), 5.54 (1H, m) and 5.56 (1H, td), a five-membered lactonic methine proton at δ 5.27 (1H, dt), and a terminal methyl group at δ 0.89 (3H, t). Sequential ^{1}H-^{1}H correlations from H-2 to H-8 on the α side chain, between H-8 and H-12, and from H-10 to H-16 on the ω side chain were observed from ^{1}H-^{1}H COSY data. The structure from C-1 to C16 was confirmed by HMBC correlations. These spectroscopic findings led to the gross structure of preclavulone lactone I (**1**) similar to those of prostaglandin A_2 and clavulolactone II.[3a] The *Z* stereochemistry of the two isolated carbon-carbon double bonds was determined from coupling constants between the corresponding protons ($J_{5,6}$ = 11.1 Hz, $J_{14,15}$ = 10.9 Hz). The coupling constant between H-8 and H-12 (J = 2.3 Hz) suggested the relative configuration of side chains to possibly be *trans*.[4]



^{1}H-^{13}C long-range correlations observed in the HMBC spectrum of **1**

Preclavulone lactone II (**2**) was also found to have the same molecular formula $C_{20}H_{28}O_3$ by HRFABMS. The ^{1}H-NMR

spectrum of **2** (Table 1) was quite similar to that of **1**, except for the signals of protons on the α side chain. The coupling constant of olefinic protons between H-5 and H-6 was 15.2 Hz, indicating the *E* configuration for the double bond. In addition, the ^{13}C chemical shift values of C-4 and C-7 shifted downfield compared to those of **1**, thus confirming *E* stereochemistry.

The absolute configurations of the three chiral centers in **1** were determined based on the following chemical synthesis. At least two diastereomers, (4*S*, 8*R*, 12*S*)-**1** and (4*R*, 8*R*, 12*S*)-**1** should be prepared to confirm the stereostructure of **1** since the *trans* orientation of the side chains has already been suggested but the relative configuration between C-4 and C-8 remained obscure. The strategy toward the synthesis of **1** involves the preparation of cyclopentanone **a** with *trans*-oriented side chains from (*S*)-malic acid and the coupling reaction of the cyclopentane segment **b** having an ω side chain with enantiomeric α side chains (Figure 1).

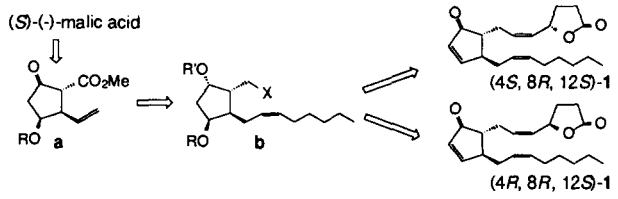
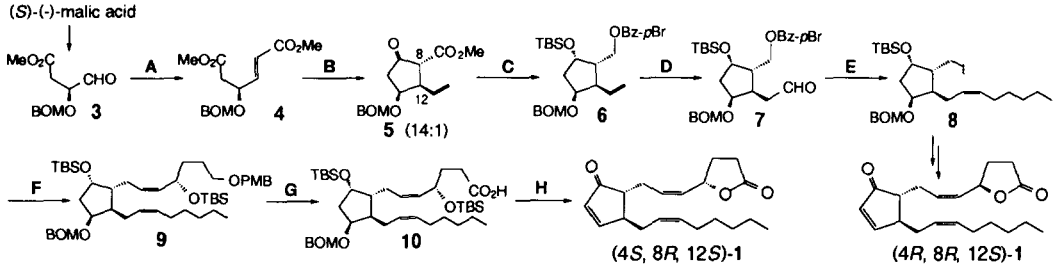


Figure 1

The synthesis of **1** was performed starting from the known aldehyde **3** prepared from (*S*)-malic acid in 3 steps[5] (Scheme 2). *E* selective Wittig reaction in benzene gave α,β-unsaturated diester **4** in 93% yield. The synthesis of the crucial cyclopentanone **5** was carried out using Michael-Dieckmann reaction by Saito's method[6] in 71% yield with excellent diastereoselectivity [(8*R*, 12*R*):(8*S*, 12*S*)=14:1]. The separation of these isomers was quite difficult, but purification was possible by flash column chromatography after obtaining the secondary alcohol as a 14:1 mixture by stereoselective $Zn(BH_4)_2$ reduction (89% yield). Further transformation in 3 steps gave **6** in 81% yield. Hydroboration of the vinyl group in **6** followed by oxidation

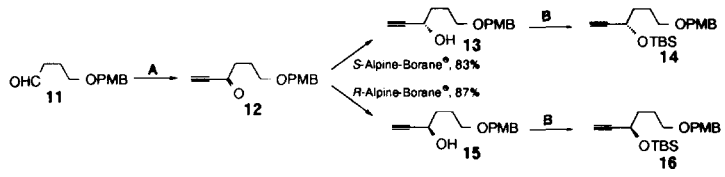


Reagents: **A.** $Ph_3P{=}CHCO_2Me$, 93%; **B.** vinylmagnesium bromide, CuI, -30°C, 71%; **C.** i) $Zn(BH_4)_2$, 89%; ii) TBSCl, imidazole, 92%; iii) DIBAL; iv) *p*-BrBzCl, Py, 88% (2 steps); **D.** i) $(Sia)_2BH$, 90%; ii) DMSO, SO_3·Py, Et_3N, 94%; **E.** i) $[Ph_3P(CH_2)_5CH_3]^+Br^-$, BuLi, HMPA, 93%; ii) DIBAL; iii) MsCl, Et_3N; iv) NaI, 88% (3 steps); **F.** i) **14**, BuLi, HMPA, 95%; ii) H_2, Pd-$BaSO_4$, 91%; **G.** i) DDQ, 92%, ii) Jones ox., 87%; **H.** i) HF, CH_3CN, 87%; ii) Jones ox. ; iii) DBU, 90% (2 steps).

Scheme 2

yielded aldehyde **7** in 85% yield. Wittig olefination (93% yield) for introducing ω side chain regioselectively followed by chemical coversions (3 steps, 88% yield) afforded iodoalkene **8** as a common intermediate for the synthesis of (4*S*, 8*R*, 12*S*)-**1** and (4*R*, 8*R*, 12*S*)-**1**. Optically active α side chains were prepared from aldehyde **11** (Scheme 3). Acetylene addition to **11** followed by oxidation gave **12** (76% yield), which was then enantioselectively reduced with commercially available *R*-Alpine-Borane® to provide propargyl alcohol **13** in 83% yield (94% ee, by MTPA ester). Use of *S*-Alpine-Borane® gave **15** in 87% yield (93% ee). The isomers were protected with TBS to give **14** and **16**, respectively (95% yield). The lithium salt of **14** readily coupled with **8** in the presence of HMPA (95% yield). Reduction of the carbon-carbon triple bond to the corresponding *cis* double bond gave **9** (91% yield). Finally, lactone construction and cyclopentenone

formation afforded (4*S*, 8*R*, 12*S*)-**1** in 5 steps (62% yield from **9**). Diastereomeric (4*R*, 8*R*, 12*S*)-**1** was synthesized in the same manner from **8** and **16** (51% yield from **8**).



Reagents: **A.** i) ethynylmagnesium bromide, 81%; ii) Jones ox., 94%; **B.** TBSCl, imidazole, 95%.

Scheme 3

Spectral data of synthetic (4*S*, 8*R*, 12*S*)-**1** were completely identical with those of natural preclavulone lactone I (**1**), though the sign of optical rotation of the synthetic compound was opposite ($[\alpha]_D^{25}$ +150.0°), indicating the absolute stereochemistry of natural **1** to be 4*R*, 8*S* and 12*R*. Preclavulone lactone II (**2**) is assumed to have the same absolute configuration.

Preclavulone lactone I may possibly be biosynthesized from preclavulone A as follows (Figure 2). First the isomerization of either side chain in preclavulone A to *trans* stereochemistry provides **A**, and then oxygenation at C-4 occurs to produce a 4*R*-hydroxy acid intermediate **B**, which is lactonized to give preclavulone lactones. Clavulones may be biosynthesized from preclavulone lactones *via* clavulolactones[3] by oxygenation (or acetoxygenation) at C-12, dehydration between C-7 and C-8, and esterification at C-1 and C-4. Clavulones may be also yielded from a diester **C** by oxygenation at C-12 and dehydration between C-7 and C-8. We are now investigating on new related prostanoids from *C. viridis* to promote the biogenesis.

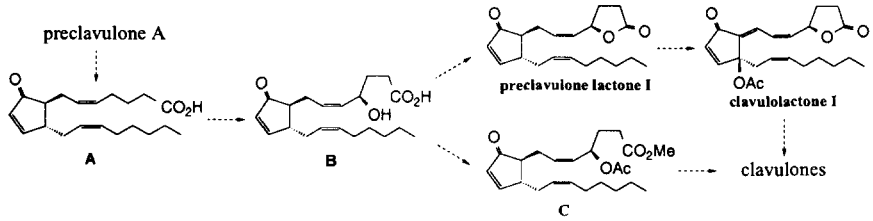


Figure 2

Acknowledgments. The authors express their appreciation to Prof. Satoshi Iwashima (Meisei Univ.) for assistance in performing 2D-NMR and Dr. Yasuo Shida (TUPLS) for measuring mass spectra. This work was supported in part by Grants-in-Aid for Scientific Research (08772037) from the Ministry of Education, Science and Culture of Japan.

References

1. (a) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1982**, *23*, 5171. (b) Kikuchi, H.; Tsukitani, Y.; Iguchi, K.; Yamada, Y. *Tetrahedron Lett.* **1983**, *24*, 1549.
2. (a) Corey, E. J.; Matsuda, S. P. T. *Tetrahedron Lett.* **1987**, *28*, 4247. (b) Corey, E. J.; d'Alarcao, M.; Matsuda, S. P. T.; Lansbury, P. T., Jr; Yamada, Y. *J. Am. Chem. Soc.* **1987**, *109*, 289. (c) Brash, A. R.; Baertschi, S. W.; Ingram, C. D.; Harris, T. M. *J. Biol. Chem.* **1987**, *262*, 15829. (d) Gerwick, W. H. *Chem. Rev.*, **1993**, *93*, 1807.
3. (a) Iguchi, K.; Iwashima, M.; Watanabe, K. *J. Nat. Prod.*, **1995**, *58*, 790. (b) Watanabe, K.; Iwashima, M.; Iguchi, K. *J. Nat. Prod.*, **1996**, *59*, 980.
4. Mikolajczyk, M. private communication.
5. (a) Maeda, H.; Kraus, G. A. *J. Org. Chem.* **1996**, *61*, 2986. (b) Saito, S.; Hasegawa, T.; Inaba, M.; Nishida, R.; Fujii, T.; Nomizu, S.; Moriwake, T. *Chem.Lett.* **1984**, 1389.
6. Saito, S.; Hirohara, Y.; Narahara, O.; Moriwake, T. *J. Am. Chem. Soc.* **1989**, *111*, 4533.

(*Received in Japan* 22 *August* 1997; *accepted* 18 *September* 1997)