Chejuenolide C: A New Macrocyclic Metabolite from the Marine Bacterium *Hahella chejuensis*

Changon Seo and Hyuncheol Oh*

*College of Medical and Life Sciences, and RIS Center, Industry-Academic Cooperation Foundation, Silla University, Busan 617-736, Korea. *E-mail: hoh@silla.ac.kr*

Received January 29, 2009, Accepted March 12, 2009

Key Words: Macrocyclic metabolite, Marine bacterium, *Hahella chejuensis*, Chejuenolide C

Marine microorganisms have been recognized as important yet relatively unexplored sources for structurally diverse bioactive secondary metabolites.[1-3] In the course of our studies on secondary metabolites from marine microorganisms from Korea, we have recently reported the isolation of chejuenolides A and B from the marine bacterium *Hahella chejuensis*.[4] Continuous studies of *H. chejuensis* scale-up culture have afforded an additional new macrocyclic metabolite named chejuenolide C (**1**). Details of the isolation and structure elucidation of this compound are reported herein.

Chejuenolide C (**1**) was assigned the molecular formula $C_{23}H_{33}NO_4$ as chejuenolides A and B,[4] on the basis of HRESIMS analysis and NMR data (Table 1). Although the ^1H and ^{13}C NMR data for **1** closely resembled those of chejuenolides A and B, close inspection of NMR data with those of chejuenolides A and B revealed some chemical shift differences for the signals corresponding to C-4, C-6, C-19, H-4, H-6, H-10, and H-12. Therefore, chejuenolide C was suggested to be an additional stereoisomer of chejuenolides A and B. Further analysis of ^1H-^1H COSY, HMQC, and HMBC data (Table 1) led to the conclusion that chejuenolide C (**1**) possesses the same planar structure as those of chejuenolides A and B.

The absolute configuration of chejuenolide C (**1**) was assigned by application of the modified Mosher method.[5] Treatment of **1** with (*S*)-MTPACl and (*R*)-MTPACl afforded the bis-(*R*)-MTPA ester (**2a**) and bis-(*S*)-MTPA ester (**2b**), respectively. The differences in chemical shift values ($\Delta\delta = \delta_S - \delta_R$)

Table 1. NMR spectroscopic data for chejuenolide C (**1**) in CD_3OD

No.	δ_H[a] (int., mult., *J* in Hz)[a]	δ_C[b]	COSY	HMBC (H → C#)
1	--	205.9	--	--
2	3.47 (1H, dq, 7.0, 3.9)	44.4	3, 18	1, 3, 18
3	4.92 (1H, m)[c]	50.3	2, 4	1, 2, 4, 5, 18, 1'
4	5.21 (1H, d, 7.7)	128.5	3, 19	6, 19
5	--	135.1	--	--
6	6.19 (1H, d, 15.8)	128.3	7	4, 7, 8, 19
7	5.53 (1H, dd, 15.8, 8.8)	134.6	6, 8	5
8	4.10 (1H, ddd, 11.0, 8.8, 4.4)	75.2	7, 9	10
9a	2.47 (1H, m)	37.1	8, 9b, 10	7, 8, 10, 11
9b	2.31 (1H, m)		8, 9a, 10	7, 8, 10, 11
10	5.35 (1H, dd, 9.9, 7.3)	128.5	9a, 9b, 20	8, 9, 12, 20
11	--	136.3	--	--
12	6.29 (1H, d, 15.8)	135.8	13, 14	10, 11, 14, 20
13	5.51 (1H, dd, 15.8, 5.5)	129.7	12, 14	11, 14, 15
14	4.47 (1H, m)	70.5	13, 15a, 15b	--
15a	2.65 (1H, ddd, 16.5, 8.1, 2.9)	36.7	14, 15b, 16	16
15b	2.52 (1H, m)		14, 15a, 16	13, 14, 16, 17
16	6.84 (1H, br t, 6.2)	141.7	15a, 15b, 21	1, 14, 21
17	--	138.7	--	--
18	1.11 (3H, d, 7.0)	14.7	2	1, 2, 3
19	1.71 (3H, s)	20.1	4	5, 6
20	1.66 (3H, s)	13.0	10	10, 11, 12
21	1.69 (3H, br s)	11.5	16	1, 16, 17
1'	--	172.5	--	--
2'	1.97 (3H, s)	22.7	--	1'

[a]Recorded at 400 MHz. [b]Recorded at 100 MHz. [c]Assigned by HMQC data.

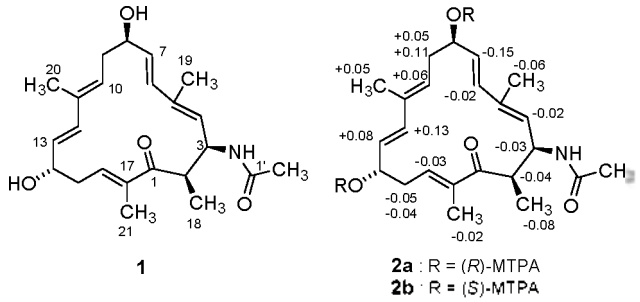

Figure 1. Δδ values [Δδ (in ppm) = $\Delta\delta_S - \delta_R$] obtained for the 8,14-bis-(*R*)- and (*S*)-MTPA esters of chejuenolide C (**2a** and **2b**, respectively).

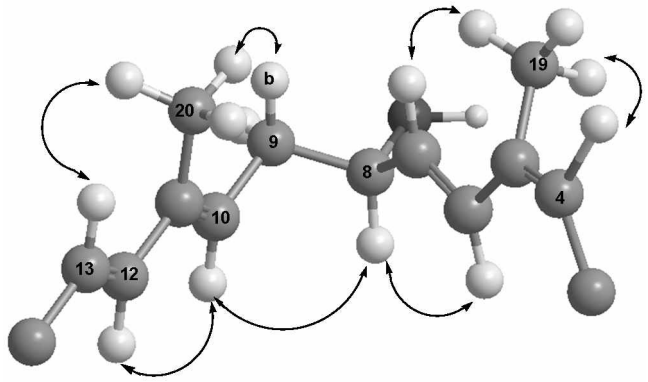

Figure 2. NOESY correlations and relative stereochemistry for C4-C13 portion in chejuenolide C (**1**). NOESY correlations are illustrated by arrows.

for the two diastereomeric esters **2b** and **2a** were calculated in order to assign the absolute configurations at C-8 and C-14 (Figure 1). Calculations for all of the relevant signals suggested the *R* and *S* absolute configurations at C-8 and C-14, respectively.

The relative stereochemistry of four chiral centers in chejuenolide C (**1**) was proposed by detailed analysis of ^{1}H-^{1}H vicinal coupling constants and NOESY correlations. Comparisons of the NOESY data and ^{1}H-^{1}H coupling constants of **1** with those of chejuenolide A led to the assignment of the relative configuration of the macrocyclic ring portion C6-C17 of **1**, as well as the geometries of double bonds at C10/C11, C12/C13, and C16/C17, to match those of chejuenolide A.[4] However, the chemical shifts of the olefinic carbons and protons at C-4 and C-6 as well as the allylic carbon at C-19 were different from the corresponding signals in the NMR data of chejuenolide A. The *J* value (H-6/H-7 = 15.8 Hz) indicated the geometry of the double bond at C6/C7 to be *E*. On the other hand, the geometry of the double bond at C4/C5 was suggested to be reversed as compared to those in chejuenolides A and B based on the chemical shift of C-19 (δ 20.1), which resonated at relatively down-field region from the corresponding methyl groups in chejuenolides A (δ 12.8) and B (δ 12.3). Thus, the geometry of the double bond at C4/C5 position was proposed as *Z*, and this assignment was confirmed by the observation of NOESY correlations for H-3/H-6 and H-4/H$_3$-19 (Figures 2 and 3). The absence of NOESY correlations for H-19/H-3 and H-4/H-6 also supported

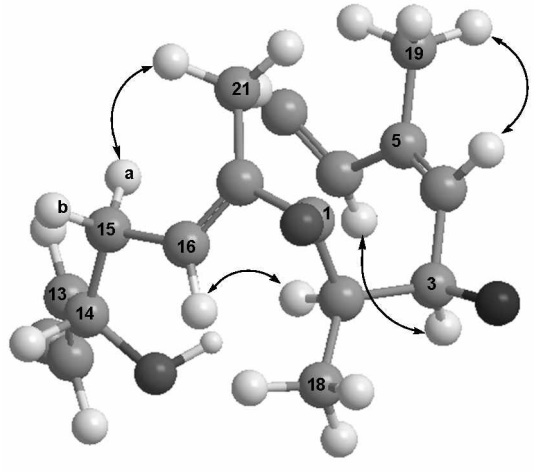

Figure 3. NOESY correlations and relative stereochemistry for C13-C17-C1-C5 portion in chejuenolide C (**1**). NOESY correlations are illustrated by arrows.

this assignment. Therefore, the conformation of the C4- C13 portion of the molecule was assigned as shown in Figure 2. For the C13-C17-C1-C5 portion, the *anti*- relationship for H-3/H-4 and the *gauche*-relationship for H-2/H-3 were deduced from the respective *J* values (H-3/H-4 = 7.7 Hz; H-2/H-3 = 3.9 Hz) and NOESY correlations for H-2/H-16 and H-3/H-6. Accordingly, H-2 and H-3 were deposited on the bottom face of the macrocycle, thereby placing CH$_3$-18 and the *N*-acetyl group to the outside of the macrocycle. Therefore, the conformation of the C13-C17-C1-C5 portion of **1** was assigned as shown in Figure 3. Taken together, the absolute configuration of **1** was assigned as 2*R*, 3*S*, 8*R*, and 14*S*.

Chejuenolide C (**1**) is a new member of 17-membered carbocyclic polyenes, which distinctively differs from the macrocyclic rings of regular macrolide polyketide.[6-10] Among the 17-membered carbocyclic tetraenes and related metabolites, chejuenolide C is the first compound that possesses *Z*-configuration at C4/C5 position.

Experimental Section

General experimental procedures. Optical rotation was recorded on a Perkin Elmer 341 digital polarimeter. ESIMS data were obtained using a Q-tof micro LC-MS/MS instrument (Waters, USA) at the Korea University, Seoul, Korea. NMR spectra (1D and 2D) were recorded in CD_3OD and pyridine-d_5 using a JEOL JNM ECP-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C), and chemical shifts were referenced relative to tetramethylsilane (δ_H/δ_C = 0). HMQC and HMBC experiments were optimized for $^1J_{CH}$ = 140 Hz and $^nJ_{CH}$ = 8 Hz, respectively. Solvents for extractions and open column chromatography were reagent grade and used without further purification. Solvents used for HPLC were analytical grade. Flash column chromatography was carried out using YMC octadecyl-functionalized silica gel (C_{18}). HPLC separations were performed on a Shiseido Capcell Pak® C_{18} column (10 × 250 mm; 5-μm particle size) with a flow rate of 2 mL/min. Compounds were detected by UV absorption at 210 nm.

Biological material. Details of the collection and identification of the bacterium have been described previously.[4]

Fermentation, extraction, and isolation. *Hahella chejuensis* was cultured on eighteen 1 L Elenmeyer flasks, each containing 400 mL of ZoBell broth media [5 g/L of Bacto Peptone (Difco), 1 g/L of yeast extract (Difco), 10 mg/L of $FePO_4$, and 750 g of seawater, pH = 7.2]. Flasks were individually inoculated with 2 mL seed cultures of *H. chejuensis*. Flask cultures were incubated at 28 °C and aerated by agitation on a rotary shaker at 150 rpm for a period of 7 days. Extraction of the filtered fermentation broth with EtOAc (10 × 1 L) provided an organic phase, which was then concentrated using a rotary evaporator to yield 1.9 g of a crude extract. The resulting crude EtOAc extract was subjected to C_{18} functionalized silica gel flash column chromatography (4 × 25 cm), eluting with a stepwise gradient of 20%, 40%, 50%, 60%, 80%, 90%, and 100 % (v/v) MeOH in H_2O (500 mL each). The fraction eluted at 60 % MeOH (24 mg) was then subjected to semi-preparative reversed-phase HPLC using a gradient from 30 to 40% CH_3CN in H_2O (0.1% formic acid) over 40 min to yield chejuenolide A (5.8 mg; t_R = 18 min), chejuenolide B (4.0 mg; t_R = 23 min), and chejuenolide C (**1**; 1.1 mg; t_R = 29 min).

Chejuenolide C (1): colorless gum; $[\alpha]_D^{25}$ + 83 (*c* 0.06, CH_3OH); ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* $[M - H]^-$ 386.2331 (calc for $C_{23}H_{32}NO_4$, 386.2331).

Preparation of bis-(*R*)-MTPA ester (2a) and bis-(*S*)-MTPA ester (2b). A sample of **1** (0.5 mg, 0.001 mmol), (*S*)-MPTACl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 24 h. The ^1H NMR data of the bis-*R*-MTPA ester derivative (**2a**) were obtained directly on the reaction mixture by analysis of the ^1H NMR and COSY spectra: ^1H NMR (pyridine-d_5, 400 MHz) δ (integration, multiplicity, *J* in Hz, assignment): 3.78 (1H, m, H-2), 5.47 (1H, t, 8.1, H-3), 5.71 (1H, d, 7.0, H-4), 6.87 (1H, d, 15.0, H-6), 5.71 (1H, m, H-7), 5.79 (1H, m, H-8), 2.82 (1H, m, H-9a), 2.48 (1H, m, H-9b), 5.45 (1H, m, H-10), 6.48 (1H, d, 15.7, H-12), 5.79 (1H, m, H-13), 6.03 (1H, m, H-14), 2.92 (1H, m, H-15a), 2.87 (1H, m, H-15b), 7.12 (1H, br s, H-16), 1.31 (3H, d, 7.0, H-18), 1.62 (3H, s, H-19), 1.78 (3H, s, H-20), 1.85 (3H, s, H-21).

Similarly, the reaction mixture from another sample of **1** (0.5 mg, 0.001 mmol), (*R*)-MPTACl (2.0 μL, 0.011 mmol), and pyridine-d_5 (0.5 mL) was processed as described above for **2a** to afford **2b**: ^1H NMR (pyridine-d_5, 400 MHz) δ (integration, multiplicity, *J* in Hz, assignment): 3.74 (1H, m, H-2), 5.44 (1H, t, 8.1, H-3), 5.69 (1H, d, 7.0, H-4), 6.85 (1H, d, 15.4, H-6), 5.56 (1H, dd, 15.8, 9.9, H-7), 5.79 (1H, m, H-8), 2.87 (1H, m, H-9a), 2.59 (1H, m, H-9b), 5.51 (1H, m, H-10), 6.61 (1H, d, 15.8, H-12), 5.87 (1H, dd, 15.8, 5.5, H-13), 6.03 (1H, m, H-14), 2.87 (1H, m, H-15a), 2.83 (1H, m, H-15b), 7.07 (1H, br s, H-16), 1.23 (3H, d, 7.0, H-18), 1.56 (3H, s, H-19), 1.83 (3H, s, H-20), 1.827 (3H, s, H-21).

Acknowledgments. Mass data were obtained at the Lab of Natural Products and Metabolomics, Division of Biotechnology of Korea University.

References

1. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216.
2. Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666.
3. Prudhomme, J.; McDaniel, E.; Ponts, N.; Bertani, S.; Fenical, W.; Jensen, P.; Le Roch, K. *PLoS ONE.* **2008**, *3*, e2335.
4. Choi, Y.-H.; Sohn, J.-H.; Lee, D.; Kim, J. K.; Kong, I. S.; Ahn, S. C.; Oh, H. *Tetrahedron Lett.* **2008**, *49*, 7128.
5. Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17.
6. Kakinuma, K.; Uzawa, J.; Uramoto, M. *Tetrahedron Lett.* **1982**, *23*, 5303.
7. Gaumann, E.; Huter, R.; Keller-Shierlein, W.; Neipp, L.; Prelog, V.; Zahner, H. *Helv. Chim. Acta* **1960**, *43*, 601.
8. Sakamoto, J. M. J.; Kondo, S.; Yumoto, H.; Arishima, M. *J. Antibiot.* **1962**, *15*, 98.
9. Harada, S.; Yamazaki, T.; Hatano, K.; Tsuchiya, K.; Kishi, T. *J. Antibiot.* **1973**, *26*, 647.
10. Harada, S.; Kishi, T. *Chem. Pharm. Bull.* **1974**, *22*, 99.