Tetrahedron Letters 49 (2008) 7128–7131

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Chejuenolides A and B, new macrocyclic tetraenes from the marine bacterium *Hahella chejuensis*

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ARTICLE INFO

Article history:
Received 10 August 2008
Revised 21 September 2008
Accepted 25 September 2008
Available online 30 September 2008

Keywords:
Macrocyclic tetraenes
Marine bacterium
Hahella chejuensis
Chejuenolides

ABSTRACT

Two new 17-membered carbocyclic tetraenes, chejuenolides A and B (**1** and **2**), have been isolated from the EtOAc extract of the marine bacterium *Hahella chejuensis* by various chromatographic methods. The structures and relative configurations of **1** and **2** were mainly determined by analysis of the NMR spectroscopic data, and their absolute configurations were assigned by application of modified Mosher method.

Marine organisms have been a rich source for new drugs during the last decade.[1] In particular, marine microorganisms have recently been recognized as an important source for structurally diverse bioactive secondary metabolites.[2] As part of our ongoing studies on secondary metabolites from marine microorganisms from Korea, we have investigated chemical constituents of crude extracts obtained from fermentation of the marine bacterium *Hahella chejuensis*, which was isolated from the sediment collected from Gejae Island, Korea. This letter describes the isolation and structure elucidation of two new 17-membered carbocyclic tetraenes, chejuenolides A and B (**1** and **2**), encountered in this investigation.

The marine bacterial strain (MB-1084) was isolated from the marine sediment sample collected from Gejae Island, Korea at inter-tidal zone on January, 2006, and the strain was identified as *H. chejuensis* (KCTC 2396) on the basis of 98.3% 16S rDNA gene sequence identity.[3] Extraction of the filtered fermentation broth (1.6 L) with EtOAc (3 × 1 L) yielded 900 mg of crude extract, which was subjected to C_{18} functionalized silica gel flash column chromatography (4 × 20 cm), eluting with a stepwise gradient of 20–100% MeOH in H_2O (500 mL each). The fraction eluted at 80% MeOH (13.6 mg) was then subjected to semi-preparative reversed-phase HPLC (CH_3CN/H_2O) to yield **1** (2.0 mg; t_R = 13 min) and **2** (1.6 mg; t_R = 16 min).

Chejuenolide A (**1**)[4] showed a pseudo-molecular ion peak at *m*/*z* 410 $(M+Na)^+$ in the ESIMS spectrum, and the molecular formula of $C_{23}H_{33}NO_4$ was revealed by its HRESIMS data [*m*/*z* 388.2488 $(M+H)^+$; Δ 0.0 mmu], indicating eight degrees of unsaturation. This formula was fully supported by ^1H and ^{13}C NMR data (Table 1). The ^1H and DEPT NMR spectra revealed the presence of five methyl groups, seven sp^2 methines, four sp^3 methines (two oxygenated), and two methylene units, requiring the presence of three exchangeable protons. In addition, analysis of DEPT and ^{13}C NMR data (Table 1) revealed the presence of two carbonyl and three quaternary sp^2 carbons. Since seven of eight degrees of unsaturation were accounted for by the presence of two carbonyl and five olefinic groups, chejuenolide A (**1**) was inferred to possess an additional ring. The planar structure of **1** was mainly elucidated on the basis of 2D-NMR data measured in CD_3OD. Analysis of ^1H–^1H COSY data disclosed three proton–proton networks corresponding to the C18–C2–C–4–C19, C6–C10–C20, and C12–C16–C21 substructures. Long-range proton–carbon correlations observed in the HMBC spectrum of **1** provided corroborative evidence to support these subunits deduced from COSY data. The presence of two disubstituted *E*-double bonds at C6–C7 and C12–C13 was implied by *J* (H,H) values [H-6 (δ_H 5.74)/H-7 (δ_H 5.36): 15.9 Hz, H-12 (δ_H 6.04)/H-13 (δ_H 5.42): 15.7 Hz]. All three trisubstituted double bonds at C4–C5, C10–C11, and C16–C17 were assigned the

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doi:10.1016/j.tetlet.2008.09.143

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

No.	δ_H[a] (int., mult., *J* in Hz)[a]	δ_C[b]	COSY	HMBC (H→C#)
1	—	205.6	—	—
2	3.40 (1H, dq, 10.3, 6.6)	44.9	3, 18	1, 3, 4, 18
3	4.98 (1H, t, 10.3)	51.0	2, 4	1, 2, 4, 5, 18, 1′
4	5.08 (1H, d, 10.3)	131.6	3, 19	2, 6, 19
5	—	135.5	—	—
6	5.74 (1H, d, 15.9)	136.7	7	5, 7, 8, 19
7	5.36 (1H, dd, 15.9, 8.0)	131.3	6, 8	5, 9
8	4.04 (1H, ddd, 10.6, 8.0, 4.8)	74.8	7, 9a, 9b	6
9a	2.45 (1H, m)	36.2	9b, 8, 10	7, 8, 10, 11
9b	2.17 (1H, ddd, 12.9, 10.6, 8.7)		9a, 8, 10	7, 8, 10, 11
10	5.18 (1H, t, 8.7)	127.7	9a, 9b, 20	8, 9, 12, 20
11	—	135.7	—	—
12	6.04 (1H, d, 15.7)	135.3	13, 14	10, 11, 14, 20
13	5.42 (1H, dd, 15.7, 5.2)	129.6	12, 14	11, 14, 15
14	4.52 (1H, m)	71.3	13, 15a, 15b	—
15a	2.72 (1H, ddd, 14.0, 10.3, 3.2)	37.4	14, 15b, 16	17
15b	2.55 (1H, m)		14, 15a, 16	13, 14, 17
16	6.70 (1H, dd, 10.3, 5.3)	139.2	15a, 15b, 21	1, 21
17	—	140.0	—	—
18	0.99 (3H, d, 6.6)	16.1	2	1, 2, 3
19	1.68 (3H, d, 1.1)	12.8	4	4, 5, 6
20	1.58 (3H, s)	13.6	10	10, 11, 12
21	1.71 (3H, br s)	12.5	16	1, 17
1′	—	172.4	—	—
2′	1.93 (3H, s)	22.7	—	1′

[a] Recorded at 400 MHz.
[b] Recorded at 100 MHz.

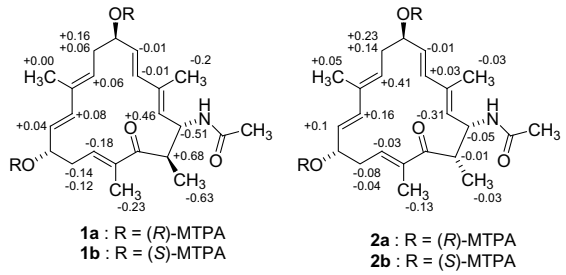

Figure 1. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for the 8,14-bis-(*R*)- and (*S*)-MTPA esters of chejuenolide A (**1a** and **1b**, respectively) and chejuenolide B (**2a** and **2b**, respectively).

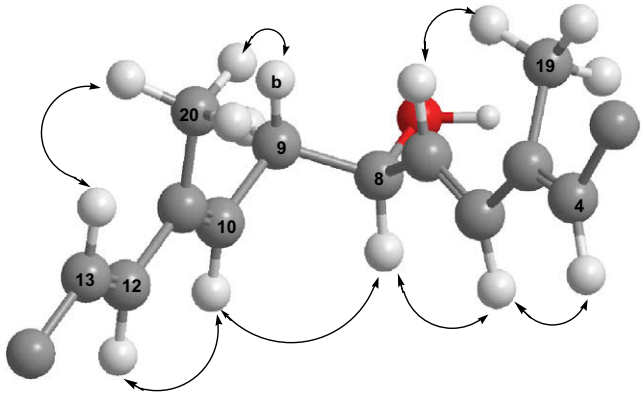

Figure 2. NOESY correlations and relative stereochemistry for C4-C13 portion in chejuenolide A (**1**). NOESY correlations are illustrated by arrows. *J* in Hz (H/H): H-7/H-8: 8.0 Hz, H-8/H-9b: 10.6 Hz.

E-geometry on the basis of NOESY correlations for H-3/H_3-19, H-9b/H_3-20, and H-15a/H_3-21, respectively. The C16–C17 double bond was shown to be adjacent to the ketone group (C-1) by HMBC correlations for H_3-21 (δ_H 1.71)/C-1 (δ_C 205.6) and H-16 (δ_H 6.70)/C-1. The ketone group was linked to C-2 on the basis of HMBC correlations for H_3-18 (δ_H 0.99)/C-1, H-2 (δ_H 3.40)/C-1, and H-3 (δ_H 4.98)/C-1. HMBC correlations of H_3-19 (δ_H 1.68) with C-6 (δ_C 136.7) and of H-7 (δ_H 5.36) with C-5 (δ_C 135.5) led to the connection of C-5 to C-6. The connection of C-11 to C-12 was revealed by HMBC correlations for H_3-20 (δ_H 1.58)/C-10 (δ_C 127.7), H_3-20/C-11 (δ_C 135.7), and H_3-20/C-12 (δ_C 135.3), thereby completing the 17-membered carbocyclic nature of the compound. The acetyl group was connected to C-3 via a nitrogen atom by HMBC correlations for H-3 (δ_H 4.98)/C-1′ (δ_C 172.4) and H_3-2′ (δ_H 1.93)/C-1′, which was also supported by the chemical shift considerations of C-3 (δ_C 51.0) and H-3 (δ_H 4.98). Considering the NMR chemical shifts of C-8 (δ_C 74.8) and C-14 (δ_C 71.3), together with the presence of three exchangeable protons in **1**, two hydroxyl groups were attached to C-8 and C-14, and the remaining exchangeable proton was assigned to a proton on the nitrogen atom in the amide functionality. On the basis of these data, the planar structure of chejuenolide A was established as depicted in **1**.

The absolute configuration of chejuenolide A (**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 (**1a**) and bis-(*S*)-MTPA ester (**1b**), respectively. The differences in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the two diastereomeric esters **1b** and **1a** were calculated in order to assign the absolute configurations at C-8 and C-14 (Fig. 1). Calculations for all of the relevant signals except two (H-2 and H-4) suggested the *R* and *S* absolute configurations at C-8 and C-14, respectively.

It was expected that the four olefins in the macrocycle would constrain the flexibility, facilitating the stereochemical analysis by detailed analysis of *J* (H,H) values and NOE studies. Thus, the relative stereochemistry of four chiral centers in chejuenolide A (**1**) was proposed by detailed analysis of ^1H–^1H vicinal coupling constants and NOESY correlations, and such analyses revealed that the 17-membered macrocycle adopted an oval-shaped conformation with top- and bottom-faced groups. For the C4–C13 portion (Fig. 2), both *anti*-relationships for H-8-H-9b and H-8-H-7 were suggested by the *J* (H-8/H-9b) and *J* (H-8/H-7) values (10.6 Hz and 8.0 Hz, respectively). Thus, the H-8 was revealed to be in axial position (Fig. 2). Consistent with this observation, H-12, H-10, H-8, H-6, and H-4 (Fig. 2) were sequentially correlated in the NOESY spectrum, defining the periphery of bottom face of the macrocycle. On the other hand, H-7 correlated only to H_3-19, suggesting that these protons are deposited on the other face of the macrocycle. In addition, H-13, H_3-20, and H-9b were sequentially correlated in the NOESY spectrum, defining the periphery of top face of the cycle (Fig. 2). For the C13–C17–C1–C5 portion (Fig. 3), both *gauche* relationships for H-13/H-14 and H-14/H-15a were inferred from the *J* (H,H) values (H-13/H-14: 5.2 Hz, H-14/H-15a: 3.2 Hz). An *anti* relationship for H-15a-H-16 was deduced by the *J* (H-15a/H-16) value (10.3 Hz) and NOESY correlations for H-15a/H_3-21 and H-13/H-15a. Considering NOESY correlations for H-2/H-16 and H-3/H_3-19 as well as *J* (H-3/H-4) value (10.3 Hz), which implied their *anti* relationship, an *anti* relationship for H-2-H-3 was revealed on the basis of large *J* (H-2/H-3) value (10.3 Hz). Therefore, the conformation of the C13-C17–C1–C5 portion of the molecule was assigned as shown in Figure 3. Taken together, the absolute configuration of **1** was assigned as 2*R*, 3*R*, 8*R*, and 14*S*.

The HRESIMS spectrum [*m/z* 388.2486 $(M+H)^+$; Δ −0.2 mmu] of chejuenolide B (**2**)[6] indicated a molecular weight identical to that of **1**. The DEPT and ^{13}C NMR data showed that the numbers of methyls, methylenes, methines, and quaternary carbons was the same as those of **1** (Tables 1 and 2). The ^1H and ^{13}C NMR (Table 2) spectra were similar to those of chejuenolide A (**1**), except for the respective chemical shift differences for C-1, H-16, H_3-19, and H_3-21. This observation implied that chejuenolide B is a

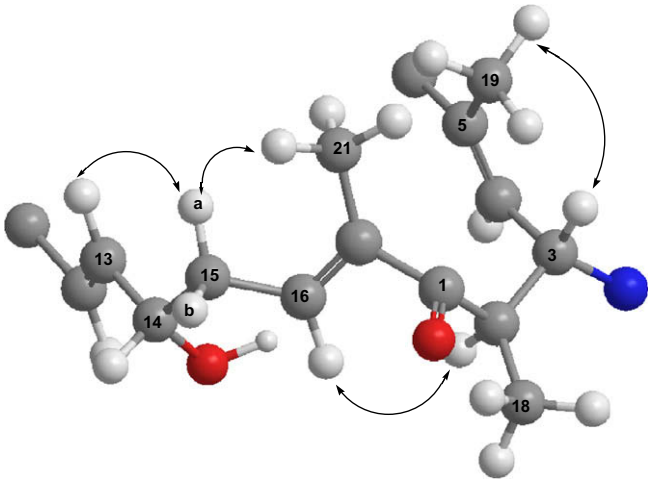

Figure 3. NOESY correlations and relative stereochemistry for C13–C17–C1–C5 portion in chejuenolide A (**1**). NOESY correlations are illustrated by arrows. *J* in Hz (H/H): H-2/H-3: 10.3 Hz, H-3/H-4: 10.3 Hz, H-13/H-14: 5.2 Hz, H-14/H-15a: 3.2 Hz, H-15a/H-16: 10.3 Hz.

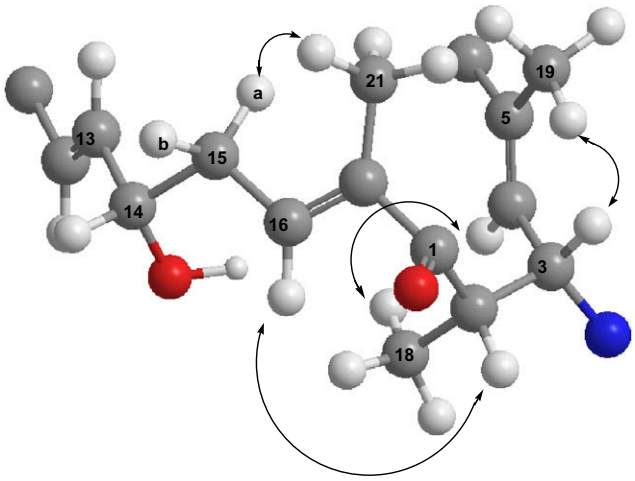

Figure 4. NOESY correlations and relative stereochemistry for C13–C17–C1–C5 portion in chejuenolide B (**2**). NOESY correlations are illustrated by arrows. *J* in Hz (H/H): H-2/H-3: 3.7 Hz, H-3/H-4: 9.5 Hz, H-13/H-14: 5.5 Hz, H-14/H-15a: 7.7 Hz, H-15a/H-16: 7.7 Hz.

Table 2
NMR spectroscopic data for chejuenolide B (**2**) in CD_3OD

No.	δ_H[a] (int., mult., *J* in Hz)[a]	δ_C[b]	COSY	HMBC (H→C#)
1	—	208.9	—	—
2	3.43 (1H, dq, 7.0, 3.7)	43.0	3, 18	—
3	4.90 (1H, m)[c]	51.5	2, 4	1, 2, 4, 5, 1′
4	5.05 (1H, d, 9.5)	131.3	3, 19	6, 19
5	—	135.8	—	—
6	5.70 (1H, d, 15.8)	136.4	7	5, 7, 8, 19
7	5.42 (1H, dd, 15.8, 8.8)	131.6	6, 8	5
8	4.05 (1H, ddd, 10.2, 8.8, 5.2)	75.3	7, 9a, 9b	
9a	2.49 (1H, m)	36.9	9b, 8, 10	7, 8, 10, 11
9b	2.27 (1H, dt, 12.4, 10.2)		9a, 8, 10	7, 8, 10, 11
10	5.24 (1H, dd, 9.5, 6.2)	128.8	9a, 9b, 20	12, 20
11	—	135.9	—	—
12	6.13 (1H, d, 15.7)	136.1	13	10, 11, 14, 20
13	5.53 (1H, dd, 15.8, 5.5)	128.9	12, 14	11, 14
14	4.47 (1H, m)	71.9	13, 15a, 15b	—
15a	266 (1H, dt, 15.4, 7.7)	37.8	14, 15b, 16	13, 14, 17
15b	2.53 (1H, m)		14, 15a, 16	14, 17
16	6.47 (1H, br t, 7.7)	140.14	15a, 15b, 21	1, 21
17	—	140.18	—	—
18	1.07 (3H, d, 7.0)	16.3	2	1, 2, 3
19	1.79 (3H, d, 1.1)	12.3	4	4, 5
20	1.64 (3H, s)	13.1	10	10, 11
21	1.80 (3H, s)	12.5	16	1, 17
1′	—	172.5	—	—
2′	1.99 (3H, s)	22.9	—	1′

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

stereoisomer of **1**, and detailed analysis of the 1D- and 2D-NMR data revealed that **2** possessed the same planar structure as **1** (Table 2). Comparisons of the NOESY data and *J* (H,H) values of **2** with those of **1** suggested that the relative stereochemistry of the macrocyclic ring portion C4–C13 of **2** is analogous to that of **1** (Fig. 2). For the C13–C17–C1–C5 portion (Fig. 4), most NOESY correlations and the *J* (H,H) values were similar to those of **1**, except for the *J* value for H-2/H-3. The relatively small *J* (H-2/H-3) value (3.7 Hz) implied a *gauche* relationship. Considering this relationship, NOESY correlations for H-16/H-2, $H3/H_3$-19, and H-15a/H_3-21 were suggestive of pseudo-equatorial deposition of H-2, thereby placing the methyl group (CH_3-18) inside the macrocycle ring. This was also supported by NOESY correlation of H_3-18/H-4 and the relatively large *J* (H-15a/H-16) value (7.7 Hz). Thus, the conformation of the C13–C17–C1–C5 portion of **2** was assigned as shown in Figure 4.

The absolute configuration of chejuenolide B (**2**) was also assigned by application of the modified Mosher method.[5] Bis-(*R*)-MTPA ester (**2a**) and bis-(*S*)-MTPA ester (**2b**) of chejuenolide B (**2**) demonstrated the absolute configurations at C-8 and C-14 to be *R* and *S*, respectively (Fig. 1). Therefore, the absolute configuration of **2** was assigned as 2*S*, 3*R*, 8*R*, and 14*S*.

Chejuenolides A and B (**1** and **2**) are new members of the 17-membered carbocyclic tetraenes, which distinctively differ from the macrocyclic rings of regular macrolide polyketides.[7] The lankacidins are the only precedents of the 17-membered carbocyclic antibiotics isolated from *Streptomyces griseofuscus*, *S. violaceoniger*, and *S. rochei var. volubilis*.[8–11] The combination of the potential chemotherapeutic value and the stereochemically complex structure makes the lankacidins attractive biosynthetic and synthetic targets. Several reports have described the approaches to their total syntheses,[12–14] and the identification of genes playing important roles in the producing strain.[15] Chejuenolides A and B differ from most known lankacidins by virtue of the presence of an ethamide rather than that of a 2-hydroxypropamide branch at C-3 position, and the lack of the six-membered δ-lactone ring, which seems most likely to arise from the decarboxylation step. Lankacyclinol,[16] a metabolic product of lankacidin C found in rat bile, and lankacyclinol A,[17] a 12-O-acetylated derivative of lankacyclinol, are only precedents of the 17-membered macrocyclic tetraenes without the six-membered δ-lactone ring moiety. It is also noteworthy that chenjuenolides A and B are the first examples of the 17-membered macrocyclic tetraenes encountered from Gram-negative bacteria.

Chejuenolides A and B were evaluated for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans* in standard agar disk diffusion assays,[18,19] and were inactive at 200 μg/disk. This was somewhat unexpected given the structural resemblance of chejuenolides to other known 17-membered macrocyclic tetraenes with antibiotic effects. Chejuenolides A and B were also evaluated for their inhibitory effects on the activity of PTP1B, and showed weak inhibitory activity of 65% and 75%, respectively, at the concentration level of 150 μg/ml. Inhibitors of PTP1B are considered as potential agents in efforts to develop new treatments for type 2 diabetes and related metabolic syndromes.[20–22]

Acknowledgment

This work was supported by a Grant (M2007-06) from Marine Bioprocess Research Center of the Marine Bio 21 Center funded by the Ministry of Maritime Affairs and Fisheries, Republic of Korea.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.09.143.

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4. Chejuenolide A (1): colorless gum; $[\alpha]_D^{25}$ +239 (*c* 0.23, CH_3OH); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* $[M+H]^+$ 388.2488 (calc for $C_{23}H_{34}NO_4$, 388.2488).
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