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Macrolactin N, a new peptide deformylase inhibitor produced by *Bacillus subtilis*

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Abstract—A new 24-membered ring lactone, macrolactin N, was isolated from a culture broth of *Bacillus subtilis* and its structure was established by various spectral analysis. Macrolactin N inhibited *Staphylococcus aureus* peptide deformylase with an IC_{50} value of 7.5 μM and also showed antibacterial activity against *Escherichia coli* and *S. aureus*.

In recent years, the emergence of antibiotic-resistant bacteria has steadily increased to become a serious threat to the human. This emphasizes the need to discover and develop novel antibacterial drugs with new modes of action.[1] Bacterial genomics has revealed a plethora of previously unknown targets of potential use in the discovery of novel antibacterial drugs.[2] Among novel antibacterial targets, one target that has received an increasing amount of attention lately is the bacterial peptide deformylase (PDF) (EC 3.5.1.31).[3,4] PDF, a unique subclass of metalloenzymes, catalyzes the removal of the formyl group at the N-terminus of bacterial proteins. PDF is essential for bacterial growth but not required by mammalian cells, which potentially makes it possible to identify a selective antibacterial agent without mechanism-based toxicity. Recent studies from several research groups have shown that PDF inhibitors act as broad-spectrum antibacterial agents.[3,5,6] A few skeletons as PDF inhibitors, however, have been reported so far.[7–11]

In the course of our screening for new PDF inhibitors from microbial resources, we have isolated a new potent compound, named macrolactin N (**1**),[12] produced by *Bacillus subtilis* AT29 (Fig. 1). In this paper, we present the isolation, structure determination, and biological activities of **1**.

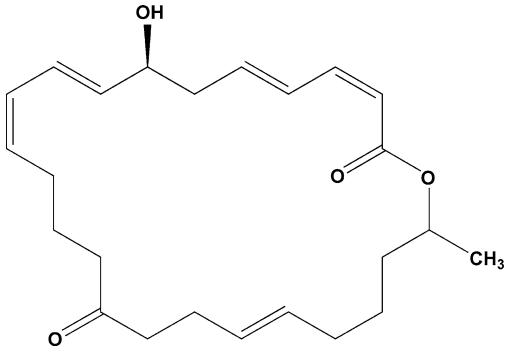

Figure 1. The chemical structure of **1**.

The producing strain AT29 was isolated from a soil sample collected in Daejeon-city, Chungcheongnam-do, Korea, and assigned to the *B. subtilis* AT29. Fermentation was carried out in 1-l Erlenmeyer flasks containing soluble starch 1%, glucose 2%, soybean meal 2.5%, beef extract 0.1%, yeast extract 0.4%, NaCl 0.2%, K_2HPO_4 0.025%, and $CaCO_3$ 0.2% (adjusted to pH 7.2 before sterilization). A piece of strain AT29 from a mature plate culture was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of sterile seed liquid medium with the above composition and cultured on a rotary shaker (150 rpm) at 28 °C for 3 days. For the production of **1**, 5 ml of the seed culture was transferred into 1-l Erlenmeyer flasks containing 100 ml of the above medium and cultivated for 7 days using the same conditions. The culture supernatant obtained from the culture broth (9.6 l) was extracted with an equal volume of EtOAc

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three times and the EtOAc layer was concentrated in vacuo. The resultant residue was subjected to SiO_2 (Merck Art No. 7734.9025) column chromatography followed by elution with $CHCl_3$/MeOH (100:1). The active fractions were pooled and concentrated in vacuo to give an oily residue. The residue was applied again to a Sephadex LH-20 and then eluted with MeOH. Active fraction dissolved in MeOH was further purified by reverse-phase HPLC column (20 × 250 mm, YMC C_{18}) chromatography with a photodiode array detector. The column was eluted with CH_3OH/H_2O (95:5) at a flow rate of 5.5 ml/min to afford **1** (4.6 mg) at a retention time of 13 min as a white powder.

The molecular formula of **1** was determined to be $C_{24}H_{34}O_4$ on the basis of high-resolution ESI-MS [$(M+Na)^+$, 409.23355 *m/z* (−1.38 mmu error)] in combination with ^1H and ^{13}C NMR data. The IR data suggested the presence of a carbonyl (1711 cm^{-1}) and a hydroxyl (3449 cm^{-1}) moiety. The ^1H and ^{13}C NMR data (Table 1) with DEPT and HMQC data suggested the presence of 10 olefinic methines, two oxygenated methines, nine methylenes, a methyl, a lactone carbonyl carbon, and a ketone carbonyl carbon. The ^1H–^1H COSY spectrum indicated the presence of two partial structures of $-^2CH{=}^3CH-^4CH{=}\ ^5CH-^6CH_2-^7CH(OH)-^8CH{=}^9CH-^{10}CH{=}^{11}CH-\ ^{12}CH_2-^{13}CH_2-^{14}CH_2-$ and $-^{16}CH_2-^{17}CH_2-^{18}CH{=}^{19}CH-\ ^{20}CH_2-^{21}CH_2-^{22}CH_2-^{23}CH(O-)-^{24}CH_3$. The presence of these two partial structures was confirmed by the HMBC spectrum (Table 1). The connectivity of these two partial structures with the remaining carboxylic and carbonyl carbons was determined by the HMBC spectral data. The olefinic protons at δ 5.60 (2-H) and δ 6.55 (3-H) were long range coupled to the carboxylic carbon at δ 166.5 (C-1). Long range couplings were also observed from the methylene protons at δ 1.65 and δ 1.75 (13-H_2) and δ 2.36 (14-H_2) of the first partial structure to the carbonyl carbon at δ 210.9 (C-15) which was in turn long range coupled with the methylene protons at δ 2.42 (16-H_2) and δ 2.24 (17-H_2) of the second partial structure. Together with the molecular formula, the low-field shift of the proton at δ 5.04 (23-H) suggested the ester linkage of the proton of 23-H with the carboxylic carbon of C-1. This linkage was confirmed by the HMBC spectrum measured at 800 MHz in which the oxygenated proton at 23-H was long range coupled to the carboxylic carbon at δ 66.5 (C-1) (Fig. 2). The geometric configurations of the carbon–carbon double bonds were assigned on the basis of their ^1H coupling constants together with NOESY data. The ^1H coupling constants between 2-H and 3-H were 11.4 Hz, while ^1H coupling constants between 4-H and 5-H was 15.6 Hz, indicating that the geometries of C-2 and C-4 were *Z* and *E* configurations, respectively. The geometry of C-8 was *E* configuration by the ^1H coupling constants of 15.0 Hz between 8-H and 9-H. The geometry of C-10 was *Z* configuration by the ^1H coupling constants (10.8 Hz) and NOE between 10-H and 11-H. The coupling constant between 18-H and 19-H was 15.2 Hz by the decoupling experiment irradiated at δ 2.24 (17-H_2), which indicated that the geometry of C-18 was *E* configuration. The absolute stereochemistry at C-7 was determined to be 7*S* from

Table 1. ^1H (600 MHz) and ^{13}C (125 MHz) NMR spectral data of **1** in $CDCl_3$

Position	δ_H (*J*, Hz)	δ_C	HMBC
1		166.5 C	
2	5.60(1H, d, 11.4)	118.1 CH	C-1, C-4
3	6.55(1H, t, 11.4)	143.3 CH	C-1, C-5
4	7.29(1H, dd, 15.6, 11.4)	130.0 CH	C-3[a], C-6[a]
5	6.11(1H, m)	139.6 CH	C-3, C-6, C-7
6	2.47(2H, m)	41.2 CH_2	C-4, C-5, C-7
7	4.30(1H, m)	72.0 CH	C-5, C-6, C-8, C-9
8	5.73(1H, dd, 15.0, 6.0)	135.5 CH	C-6, C-7, C-10
9	6.45(1H, dd, 15.0, 10.8)	126.1 CH	C-7, C-10, C-11
10	6.05(1H, t, 10.8)	129.3 CH	C-8, C-9, C-12
11	5.42(1H, m)	132.2 CH	C-9, C-12, C-13
12	2.19(2H, m)	26.7 CH_2	C-10, C-11, C-13, C-14
13	1.65(1H, m)	23.0 CH_2	C-11, C-12, C-14, C-15
	1.75(1H, m)		C-11, C-12, C-14, C-15
14	2.36(2H, m)	41.2 CH_2	C-12, C-13, C-15
15		210.9 C	
16	2.42(2H, m)	40.0 CH_2	C-15, C-17, C-18
17	2.24(2H, m)	27.2 CH_2	C-15[a], C-18, C-19
18	5.41(1H, m)	129.3 CH	C-21
19	5.40(1H, m)	131.1 CH	C-17
20	1.97(1H, m)	32.2 CH_2	C-18, C-19, C-21[a], C-22[a]
	2.06(1H, m)		C-18, C-19, C-21[a], C-22[a]
21	1.42(1H, m)	25.3 CH_2	C-19, C-23[a]
	1.50(1H, m)		C-19[a], C-23
22	1.51(1H, m)	35.7 CH_2	C-21[a],
	1.68(1H, m)		C-21, C-20[a], C-23, C-24[a]
23	5.04(1H, m)	70.8 CH	C-1[a], C-21[a]
24	1.27(3H, d, 6.3)	20.1 CH_3	C-22, C-23

The assignments were aided by ^1H–^1H COSY, DEPT, HMQC, and HMBC.
[a] Detected at 800 MHz.

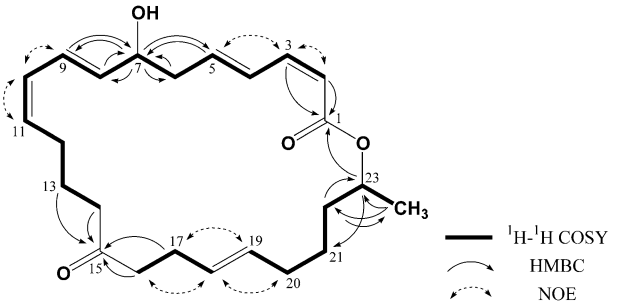

Figure 2. Key ^{1}H–^{1}H COSY, HMBC, and NOE correlations of macrolactin N.

the (*R*)-and (*S*)-MTPA ester[13] of **1** by modified Mosher method,[14] which was the same as that of C-7 of macrolactin A.[15] Thus, the structure of **1** was determined as shown in Figure 1.

Compound **1** is a new derivative dehydroxylated at C-13 of macrolactin F.[16] A class of macrolactin is a 24-membered lactone compound and 15 macrolactin compounds such as macrolactins A-M, 7-*O*-succinoylmacrolactin A, and 7-*O*-succinoylmacrolactin F have been reported so far.[15–17] Compound **1**, however, is the first macrolactin dehydroxylated at C-13. Macrolactin compounds have been isolated from an unclassifiable deep-sea bacterium, *Actinomadura* sp., or *Bacillus* sp. They have been reported to exhibit weak antibacterial activity against *S. aureus* and *B. subtilis* and antiviral activities.[15–17]

The inhibitory activity of **1** against *S. aureus* PDF was evaluated according to our previously reported method[18] with some modifications as follows; assays contained 50 mM Hepes (pH 7.5), 10 mM NaCl, 20 μg/ml bovine serum albumin, 2 mM *N*-formylmethionine-alanine-serine (f-MAS), 20 μM NAD, 0.00025 U formate dehydrogenase, and 54.3 nM *S. aureus* PDF in half-area, 96-well microtiter plates. Compound dissolved in dimethylsulfoxide was added to each well. The rate of increase in the amount of NADH in each reaction well was measured at 340 nm at 30 °C by a microtiter ELISA reader using SOFTmax PRO software (Molecular Devices, California, USA). The inhibitory activity was calculated by the following formula: % of inhibition = $100 \times [1 - (\text{rate in the presence of compound/rate in the untreated control})]$.

Compound **1** inhibited *S. aureus* PDF in a dose-dependent manner with an IC_{50} (μM) value of 7.5 μM. The inhibition pattern of PDF by **1** with respect to the substrate, f-MAS, was examined with a Lineweaver–Burk plot analysis. As shown in Figure 3, **1** exhibited competitive inhibition with f-MAS and its K_i and K_m values for PDF were 2.16×10^{-6} M and 1.8×10^{-4} M, respectively. The antibacterial activity of **1** against *S. aureus* (RN4220), *B. subtilis* (KCTC 1021), and *Escherichia coli* (KCTC 1924) was examined using microdilution broth method. Compound **1** showed stronger antibacterial activity against *E. coli* than *S. aureus* and *B. subtilis*. Compound **1** inhibited bacterial growth against *E. coli*

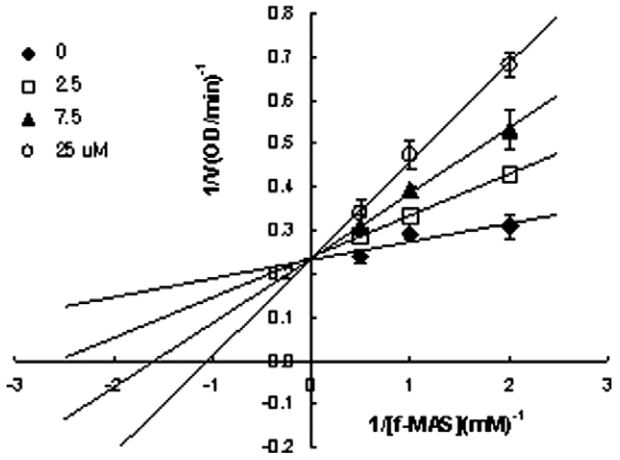

Figure 3. The mechanism of inhibition of *Staphylococcus aureus* PDF by **1** with respect to f-MAS. The values are represented as means ± SD in triplicate.

with a MIC (μg/ml) of 100, while inhibiting weaker bacterial growth against *S. aureus* and *B. subtilis* with a MIC_{50} (μg/ml) of 100, respectively.

In summary, macrolactin N is a new 24-membered lactone compound isolated from *B. subtilis*. Macrolactin N strongly inhibited *S. aureus* PDF and also showed antibacterial activity against *E. coli*, *B. subtilis*, and *S. aureus*. Macrolactin N may serve as a new class of PDF inhibitors for development of antibacterials.

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12. Compound **1**: a white powder; λ_{max} nm ($\log\varepsilon$) in MeOH: 229 (4.03), 235 (4.03), 262 (3.96); IR (KBr): 3449, 2932, 1711, 1273, 1190 cm^{-1}; $[\alpha]_D = -26.7°$ (*c* 0.2, MeOH); ESI-MS: *m/z* 409.23355 $(M+Na)^+$, $C_{24}H_{34}O_4Na$ requires 409.23493.
13. Preparation of (*R*)- and (*S*)-MTPA ester of **1**: To a solution of 4-(dimethylamino)pyridine (1.3 mg, 10.6 μmol) and triethylamine (2.0 μL, 14.3 μmol) in chloroform (0.2 mL) at room temperature were added **1** (1.0 mg, 2.7 μmol) and (*R*)-MTPA chloride (2.0 μL, 10.7 μmol) successively. The reaction mixture was stirred for 16 h and 3-(dimethylamino)propylamine (2.0 μL, 15.9 μmol) was added. The mixture was subjected to flash chromatography (SiO_2, 25% ethyl acetate/hexane) to afford (*R*)-MTPA ester of **1**. The ^1H NMR (500 MHz, $CDCl_3$) of (*R*)-MTPA ester: δ 5.61 (2-H), δ 6.42 (3-H), δ 7.25 (4-H), δ 5.79 (5-H), δ 2.58 (6-H), δ 4.38 (7-H), δ 5.67 (8-H), δ 6.57 (9-H), δ 6.02 (10-H), δ 5.49 (11-H), δ 2.24 and 2.13 (12-H), δ 1.74 and 1.66 (13-H), δ 2.36 (14-H), δ 2.40 (16-H), δ 2.24 (17-H), δ 5.39 (18-H, 19-H), δ 2.05 (20-H), δ 1.51 and δ 1.40 (21-H), δ 1.67 and 1.52 (22-H), δ 5.02 (23-H), δ 1.26 (24-H). The ^1H NMR (500 MHz, $CDCl_3$) of (*S*)-MTPA ester: δ 5.62 (2-H), δ 6.50 (3-H), δ 7.30 (4-H), δ 5.95 (5-H), δ 2.648 (6-H), δ 4.38 (7-H), δ 5.57 (8-H), δ 6.47 (9-H), δ 5.99 (10-H), δ 5.46 (11-H), δ 2.16 and 2.05 (12-H), δ 1.70 and 1.63 (13-H), δ 2.33 (14-H), δ 2.40 (16-H), δ 2.24 (17-H), δ 5.39 (18-H, 19-H), δ 2.05 (20-H), δ 1.51 and δ 1.40 (21-H), δ 1.67 and 1.52 (22-H), δ 5.02 (23-H), δ 1.26 (24-H).
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