Macrolactins O–R, Glycosylated 24-Membered Lactones from *Bacillus* sp. AH159-1

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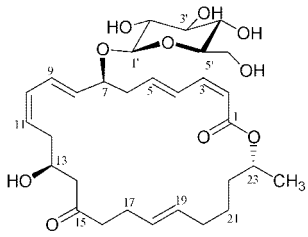
In the course of screening for inhibitors of *Staphylococcus aureus* peptide deformylase, four new glycosylated macrolactin compounds, macrolactins O (**1**), P (**2**), Q (**3**), and R (**4**), along with the known macrolactins B (**5**) and C (**6**), have been isolated from the liquid cultures of *Bacillus* sp. AH159-1. The structures of compounds **1**–**4** were assigned on the basis of MS and NMR data. They inhibited *S. aureus* peptide deformylase and also showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.*

Bacterial genomic studies have revealed a plethora of previously unknown potential targets for use in the discovery of novel antibacterial drugs.[1] Among novel antibacterial targets, the bacterial peptide deformylase (PDF) (EC 3.5.1.31) has received an increasing amount of attention.[2,3] 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 mechanism-based antibacterial agent without toxicity. Recent studies from several research groups have shown that PDF inhibitors act as broad-spectrum antibacterial agents.[2,4,5] Relatively few unique classes of PDF inhibitors, however, have been reported so far, and most of them are peptidic.[6–10]



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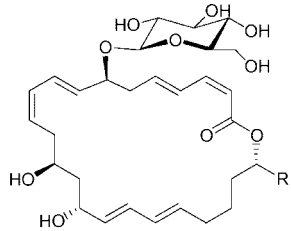
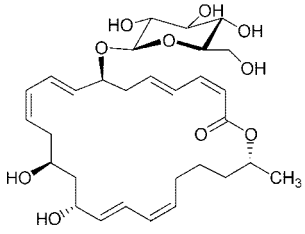


2, R = CH_2CH_3
5, R = CH_3



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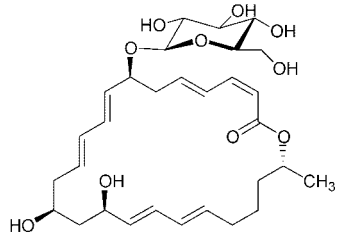

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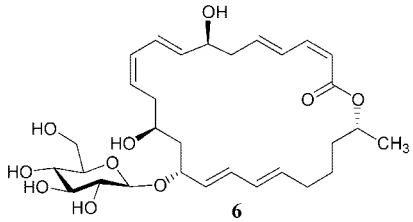

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In the course of our screening for new PDF inhibitors from microbial sources, we have isolated four new glycosylated macrocycles, macrolactins O (**1**), P (**2**), Q (**3**), and R (**4**), together with previously identified macrolactins, macrolactins B (**5**) and C (**6**),[11] from *Bacillus* sp. AH159-1. The macrolactins form a class of 24-membered lactones that have been isolated from an unclassifiable deep sea bacterium,[11] *Actinomadura* sp.,[12] or *Bacillus* sp.[13–15] Of the 17 macrolactins reported to date,[11–16] only macrolactins B and C, both isolated from an unidentified deep sea bacterium,[11] are glycosylated. In this paper, we present the production, isolation, structure determination, and antibacterial activity of **1**–**4**.

The producing strain AH159-1 was isolated from soil collected in Gongju-city, Chungcheongnam-do, Korea. The EtOAc extract of the mycelium from liquid fermentation cultures of strain AH159-1 was fractionated by Si gel chromatography. Final separation of the active fraction by reversed-phase HPLC afforded four new compounds (**1**–**4**), along with two known compounds, **5** and **6**.

The ^{1}H and ^{13}C NMR data of **5** and **6** together with their molecular weights suggested that **5** and **6** are members of the macrolactin class. Interpretation of the ^{1}H and ^{13}C NMR together with ^{1}H–^{1}H COSY, HMQC, and HMBC led to the identification of **5** and **6** as macrolactins B and C,[11] respectively. The chirality of the glucose portion in **5** was determined by acidic hydrolysis followed by TLC comparison and optical rotation.[17] The glucose isolated gave a positive specific rotation, $[\alpha]_D$ +38.8 (*c* 0.13, H_2O), indicating that it was D-glucose. The $[\alpha]_D$ values [−30.6 (*c* 3.8, MeOH) and −27.2 (*c* 0.8, MeOH), respectively] of **5** and **6** were also similar to the literature values for these compounds [−42.0 (*c* 3.8, MeOH) and −21.0 (*c* 0.87, MeOH), respecttively].[11] Together with the agreement of the ^{1}H and ^{13}C NMR data in the same solvent with the literature values, these results suggest that **5** and **6** have the same absolute configurations as those of macrolactins B and C.[18]

The molecular formula of **1** was determined to be $C_{30}H_{44}O_{10}$ on the basis of high-resolution ESIMS [$(M + Na)^+$, 587.28424 *m/z* (1.57 mmu error)] in combination with ^{1}H and ^{13}C NMR data. The IR absorption at 1704 and 3423 cm^{-1} suggested the presence of carbonyl and hydroxy moieties, respectively. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) with DEPT, ^{1}H–^{1}H COSY, and HMQC data suggested the presence of 10 olefinic methines, three oxygenated methines, eight methylenes, a methyl, a lactone carbonyl carbon, a ketone carbonyl carbon, and resonances attributable to a hexopyranoside moiety. The ^{1}H–^{1}H COSY spectrum indicated the presence of two partial structures, –^{2}CH=^{3}CH–^{4}CH=^{5}CH–^{6}CH$_2$–^{7}CH(O–)–^{8}CH=^{9}CH–^{10}CH=^{11}CH–^{12}CH$_2$–^{13}CH$_2$(O–)–^{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 connectivity of these two partial structures with the remaining carboxylic and carbonyl carbons was determined by the HMBC spectrum (Figure 1). The olefinic protons at δ 5.55 (H-2) and 6.63 (H-3) were long-range coupled to the carboxylic carbon at δ 168.0

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Table 1. ^{1}H NMR Data (600 MHz) of Compounds **1**–**4** in CD_3OD[a]

position	1	2	3	4
2	5.55 (1H, d, 12.0)	5.58 (1H, d, 11.5)	5.55 (1H, d, 11.5)	5.54 (1H, d, 11.5)
3	6.63 (1H, t, 12.0)	6.64 (1H, t, 11.5)	6.62 (1H, t, 11.5)	6.58 (1H, t, 11.5)
4	7.24 (1H, dd, 15.0, 12.0)	7.24 (1H, dd, 14.5, 11.5)	7.25 (1H, dd, 15.0, 11.5)	7.32 (1H, dd, 15.0, 11.5)
5	6.23 (1H, m)	6.25 (1H, m)	6.19 (1H, m)	6.03 (1H, m)
6	2.47 (1H, m)	2.41 (1H, m)	2.44 (1H, m)	2.47 (1H, m)
	2.58 (1H, m)	2.60 (1H, m)	2.61 (1H, m)	2.68 (1H, m)
7	4.48 (1H, m)	4.48 (1H, m)	4.52 (1H, m)	4.43 (1H, m)
8	5.62 (1H, dd, 15.6, 7.5)	5.65 (1H, m)	5.63 (1H, m)	5.39 (1H, dd, 15.5, 9.0)
9	6.58 (1H, dd, 15.6, 11.4)	6.68 (1H, dd, 15.0, 10.8)	6.66 (1H, dd, 15.5, 11.0)	6.20 (1H, dd, 15.5, 10.5)
10	6.15 (1H, t, 11.4)	6.18 (1H, t, 10.8)	6.13 (1H, t, 11.0)	6.09 (1H, dd, 15.0, 10.5)
11	5.56 (1H, m)	5.57 (1H, m)	5.59 (1H, m)	5.76 (1H, m)
12	2.35 (1H, m)	2.44 (1H, m)	2.42 (1H, m)	2.21 (1H, m)
	2.49 (1H, m)			2.25 (1H, m)
13	4.12 (1H, m)	3.94 (1H, m)	3.88 (1H, m)	3.68 (1H, m)
14	2.59 (2H, m)	1.54 (1H, m)	1.67 (2H, m)	1.58 (1H, m)
		1.60 (1H, m)		1.67 (1H, m)
15		4.30 (1H, m)	4.36 (1H, m)	4.24 (1H, dd, 13.0, 6.5)
16	2.48 (2H, m)	5.55 (1H, m)	5.72 (1H, dd, 15.2, 6.0)	5.52 (1H, dd, 15.0, 6.5)
17	2.20 (2H, m)	6.15 (1H, dd, 16.2, 10.5)	6.50 (1H, dd, 15.2, 11.0)	6.16 (1H, dd, 15.0, 10.0)
18	5.41 (1H, m)	6.03 (1H, dd, 15.5, 10.5)	6.02 (1H, t, 11.0)	6.01 (1H, dd, 15.0, 10.2)
19	5.41 (1H, m)	5.62 (1H, m)	5.40 (1H, m)	5.65 (1H, m)
20	1.96 (1H, m)	2.10 (1H, m)	2.24 (2H, m)	2.06 (1H, m)
	2.05 (1H, m)	2.17 (1H, m)		2.12 (1H, m)
21	1.41 (2H, m)	1.45 (2H, m)	1.50 (2H, m)	1.42 (2H, m)
22	1.54 (1H, m)	1.59 (2H, m)	1.62 (1H, m)	1.54 (2H, m)
	1.63 (1H, m)		1.66 (1H, m)	
23	5.00 (1H, m)	4.96 (1H, m)	4.93 (1H, m)	5.05 (1H, m)
24	1.23 (3H, d, 6.0)	1.62 (2H, m)	1.23 (3H, d, 6.5)	1.23 (3H, d, 6.0)
25		0.90 (3H, t, 7.5)		
1′	4.32 (1H, d, 7.8)	4.32 (1H, d, 8.2)	4.34 (1H, d, 8.0)	4.31 (1H, d, 8.0)
2′	3.24 (1H, dd, 7.8, 9.0)	3.24 (1H, dd, 8.2, 9.0)	3.25 (1H, m)	3.21 (1H, m)
3′	3.32 (1H, dd, 8.4, 9.0)	3.35 (1H, m)	3.34 (1H, m)	3.32 (1H, m)
4′	3.29 (1H, dd, 8.4, 8.4)	3.30 (1H, m)	3.28 (1H, m)	3.26 (1H, m)
5′	3.21 (1H, m)	3.20 (1H, m)	3.21 (1H, m)	3.19 (1H, m)
6′	3.67 (1H, dd, 6.0, 12.0)	3.66 (1H, dd, 6.0, 12.0)	3.66 (1H, dd, 6.0, 12.0)	3.65 (1H, m)
	3.88 (1H, dd, 2.4, 12.0)	3.87 (1H, dd, 2.0, 12.0)	3.87 (1H, m)	3.87 (1H, dd, 12.0, 2.0)

[a] The assignments were aided by ^{1}H–^{1}H COSY, DEPT, HMQC, and HMBC NMR spectra.

(C-1). Also, the oxymethine proton at δ 4.12 (H-13) and the methylene protons at δ 2.59 (H_2-14) showed HMBC correlations with the carbonyl carbon at δ 212.1 (C-15), which was in turn long-range coupled with the methylene protons at δ 2.48 (H_2-16) of the second partial structure. In addition, an HMBC correlation between the protons of H_2-14 and C-16 was observed. Together with the molecular formula, the low-field shift of the oxymethine proton at δ 5.00 (H-23) suggested the ester linkage. This linkage was confirmed by the HMBC optimized for 6.25 Hz, in which H-23 was long-range coupled to the carboxylic carbon at δ 168.0 (C-1). The relative configuration of the hexopyranose was determined by the ^{1}H NMR coupling patterns. The coupling constants between H-2′ and H-3′, and between H-3′ and H-4′, were 9.0 and 8.4 Hz, respectively, and were determined from decoupling experiments with irradiation at δ 4.32 (H-1′) and 3.24 (H-2′). The typical all-*trans*-diaxial couplings ranging from 7.8 to 9.0 Hz between all of the glycoside ring protons indicated the presence of a β-glucopyranosyl moiety. The postulated axial relationships were supported by NOEs among H-1′, H-3′, and H-5′ (Figure 2). The linkage of the β-glucopyranosyl moiety was determined by the HMBC spectrum. Long-range coupling between the anomeric proton at δ 4.32 (H-1′) and the oxygenated methine at δ 78.4 (C-7) indicated the position of the β-glucopyranosyl moiety at the C-7 hydroxy group of the lactone ring. The geometric configurations of the carbon–carbon double bonds were assigned on the basis of their ^{1}H coupling constants together with NOESY data (Figure 2). The geometries of C-2, C-4, C-8, and C-10 were assigned as *Z, E, E*, and *Z*, respectively, by their respective ^{1}H coupling constants of 12.0, 15.0, 15.6, and 11.4 Hz. The geometry of H-18 and H-19 was determined through NOESY because chemical shift overlap prevented direct measurement of their coupling constants. NOEs from both H-17 and H-20 to H-18 and/or H-19 were observed, but NOEs between H-17 and H-20 were not observed, indicating the olefin configuration to be *E*. Thus, **1** was determined to be a new derivative of macrolactin F[11] with the β-glucopyranosyl moiety positioned at C-7.

The molecular formula of **2** was determined to be $C_{31}H_{46}O_{10}$ on the basis of high-resolution ESIMS [$(M + Na)^+$, 601.29657 *m/z* (-1.74 mmu error)] in combination with ^{1}H and ^{13}C NMR data. The IR absorption at 1703 and 3412 cm^{-1} suggested the presence of carbonyl and hydroxy moieties, respectively. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **2** with ^{1}H–^{1}H COSY and HMQC data were similar to those of **5**. The major difference was that an ethyl group (δ 1.62, 2H, m; δ 28.1 and 0.90, 3H, t, $J = 7.5$ Hz; δ 10.2) in **2** replaced the resonances for the methyl group of C-24 in **5**. The methylene protons of the ethyl group were correlated with the oxygenated methine at δ 4.96 (H-23) in the ^{1}H–^{1}H COSY spectrum. This data suggested the presence of the ethyl group at C-23. This was determined by the HMBC spectrum. Both methyl and methylene protons of the ethyl group were long-range coupled to the oxygenated methine at δ 76.3 (C-23), the attached proton of which was in turn long-range coupled with the carboxylic carbon at δ 168.2 (C-1). In addition, HMBC correlations from the protons at δ 1.59 (H_2-22) and 1.45 (H_2-21) to the carbon at δ 76.3 (C-23) were observed. The position of the β-glucopyranosyl moiety at C-7 was confirmed by the HMBC correlation between the anomeric proton at δ 4.32 (H-1′) and the carbon at δ 78.3 (C-7). The presence of the hydroxy group at C-15 was also corroborated by HMBC correlations and by ^{1}H–^{1}H COSY correlations among the methylene protons of H_2-14, the oxymethine proton of H-15, and the olefinic proton of H-16. The remaining structure was also confirmed by the HMBC spectrum. The geometric configurations of the carbon–carbon double bonds were assigned by the same protocol as applied for **1**. The coupling constant between H-16 and H-17 was

Table 2. ^{13}C NMR Data (150 MHz) of Compounds **1**–**4** in CD_3OD[a]

position	1	2	3	4
1	168.0 C	168.2 C	168.1 C	167.7 C
2	117.9 CH	117.8 CH	117.9 CH	117.4 CH
3	145.5 CH	145.7 CH	145.6 CH	146.4 CH
4	130.3 CH	130.0 CH	130.6 CH	130.3 CH
5	141.8 CH	142.4 CH	140.9 CH	141.6 CH
6	41.1 CH_2	41.8 CH_2	41.2 CH_2	41.6 CH_2
7	78.4 CH	78.3 CH	78.0 CH	78.0 CH
8	134.3 CH	134.2 CH	134.0 CH	131.8 CH
9	129.9 CH	129.1 CH	129.6 CH	136.1 CH
10	131.8 CH	131.1 CH	131.3 CH	133.3 CH
11	128.9 CH	129.2 CH	129.2 CH	133.1 CH
12	35.8 CH_2	36.7 CH_2	36.7 CH_2	42.0 CH_2
13	68.8 CH_2	69.8 CH	69.4 CH	69.6 CH
14	49.5 CH_2	43.9 CH_2	44.4 CH_2	44.7 CH_2
15	212.1 C	69.9 CH	70.0 CH	70.1 CH
16	44.4 CH_2	135.5 CH	137.8 CH	135.1 CH
17	28.1 CH_2	131.0 CH	126.0 CH	131.7 CH
18	130.5 CH	131.9 CH	130.9 CH	131.8 CH
19	132.1 CH	134.9 CH	132.7 CH	135.2 CH
20	33.1 CH_2	32.9 CH_2	28.3 CH_2	31.3 CH_2
21	26.1 CH_2	25.6 CH_2	26.4 CH_2	26.1 CH_2
22	36.4 CH_2	33.9 CH_2	36.1 CH_2	36.5 CH_2
23	72.0 CH	76.3 CH	72.2 CH	71.2 CH
24	20.6 CH_3	28.1 CH_2	20.6 CH_2	20.2 CH_2
25		10.2 CH_3		
1′	101.2 CH	101.1 CH	101.6 CH	100.5 CH
2′	75.2 CH	75.3 CH	75.2 CH	75.3 CH
3′	78.3 CH	78.3 CH	78.2 CH	78.3 CH
4′	71.9 CH	72.0 CH	72.0 CH	72.0 CH
5′	78.2 CH	78.2 CH	78.1 CH	78.2 CH
6′	63.1 CH	63.0 CH	63.0 CH	63.0 CH

[a] The assignments were aided by ^1H–^1H COSY, DEPT, HMQC, and HMBC NMR spectra.

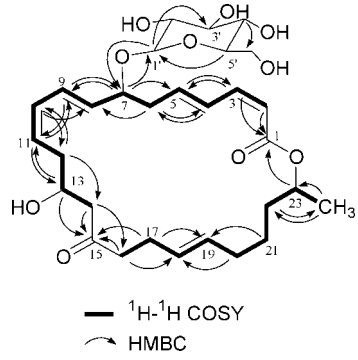

Figure 1. Key HMBC and ^1H–^1H COSY correlations of compound **1**.

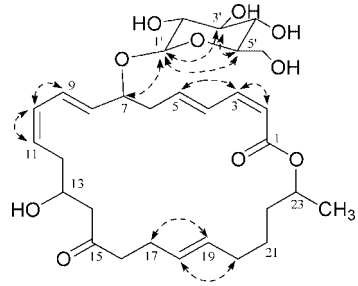

Figure 2. Key NOEs of compound **1**.

16.2 Hz by the decoupling experiment irradiated at δ 6.03 (H-18). Thus, the geometries of C-2, C-4, C-8, C-10, C-16, and C-18 were *Z, E, E, Z, E*, and *E*, respectively. These NMR data indicated that **2** was a new derivative of macrolactin B with the ethyl group instead of the methyl group at C-23. The specific rotation of **2** (−38.4, *c* 0.1, MeOH) was also similar to the literature value of macrolactin B (−42.0, *c* 3.8, MeOH),[14] as expected. Thus, **2** was determined to be a new derivative of macrolactin B with the ethyl group at C-23.

The molecular formula of **3** was determined to be $C_{30}H_{44}O_{10}$ on the basis of high-resolution ESIMS [$(M + Na)^+$, 587.2805 *m/z* (−2.1 mmu error)] in combination with ^1H and ^{13}C NMR data. The IR absorption suggested the presence of carbonyl (1697 cm^{-1}) and hydroxy (3411 cm^{-1}) moieties, respectively. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **3** with ^1H–^1H COSY and HMQC data were similar to those of **2**. The major differences were that the ^{13}C NMR chemical shifts of C-17 and C-20 were upfield from δ 131.0 and 32.9 to δ 126.0 and 28.3, respectively, and a methyl group was present in **3** instead of resonances for the ethyl group of C-24 in **2**. The upfield chemical shifts of C-17 and C-20 appear to be due to a γ-effect, suggesting that the configuration of the double bond at C-18 could be *Z*. The geometric configurations of the carbon–carbon double bonds were also assigned by the same protocol as applied for **1**. The configuration of C-18 was determined as *Z*, while those of the others were the same as that of **2**. The remaining structure was also confirmed by the HMBC spectrum. Thus, **3** was determined to be a new geometric isomer of macrolactin B with *cis*-configuration at C-18.

The molecular formula of **4** was determined to be $C_{30}H_{44}O_{10}$ on the basis of high-resolution ESIMS [$(M + Na)^+$, 587.2830 *m/z* (+0.4 mmu error)] in combination with ^1H and ^{13}C NMR data. The IR absorption suggested the presence of carbonyl (1706 cm^{-1}) and hydroxy (3427 cm^{-1}) moieties, respectively. The ^1H and ^{13}C NMR data (Table 1 and 2) of **4** with ^1H–^1H COSY and HMQC data were also similar to those of **2**. The major differences were that the ^{13}C NMR chemical shifts of C-9 and C-12 were shifted downfield from δ 129.1 and 36.7 to δ 136.1 and 42.0, respectively, and a methyl group was present in **4** instead of resonances for the ethyl group of C-24 in **2**. The low-field chemical shifts of C-9 and C-12 suggested that the configuration of the double bond at C-10 is *E*. The configuration of C-10 was determined to be *E* by the coupling constant (J = 15.0 Hz) between H-10 and H-11. The remaining structure was also confirmed by the HMBC spectrum. Thus, **4** was determined to be a new geometric isomer of macrolactin B with *trans*-configuration at C-10.

Compound **1** is a new derivative of macrolactin F with the β-glucopyranosyl moiety positioned at C-7, and compound **2** is a new derivative of macrolactin B with the ethyl group at C-23. Compounds **3** and **4** are new geometric isomers of macrolactin B with the *Z* configuration at C-18 and the *E* configuration of C-10, respectively. In this study, macrolactin compounds produced by the strain *Bacillus* sp. AH159-1 were primarily glycosylated, and nonglycosylated macrolactins were obtained in too small a quantity for structure determination. Thus, it seems that the producing strain *Bacillus* sp. AH159-1 is very active in the glycosylation process.

Macrolactin A shows antibacterial activity, inhibits B16-F10 murine melanoma cancer cells in *in vitro* assays, shows significant inhibition of mammalian Herpes simplex viruses (types I and II),[11] and prevents glutamate neurotoxicity in N18-RE-105 cells.[12] In addition, macrolactins F–N, 7-*O*-succinoylmacrolactin A, 7-*O*-succinoylmacrolactin F, and 7-*O*-malonylmacrolactin A have been reported to exhibit antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*.[13–16] However, there have not been any reports of biological activity for glycosylated macrolactins.

The inhibitory activities of **1**–**4** against *S. aureus* PDF was evaluated according to our previously reported method.[15] The antibacterial activities of **1**–**4** against *S. aureus* (RN4220), *B. subtilis* (KCTC 1021), and *E. coli* (KCTC 1924) were examined using the microdilution broth method.[19] The MIC was the lowest antibiotic concentration that complelety prevented visible growth after incubation for 18 h; the minimum restrictive concentration (MRC) was defined as the lowest antibiotic concentration that caused at least 50% reduction of growth.[16]

Compounds **1**–**4** inhibited *S. aureus* PDF in dose-dependent manners with IC_{50} (μM) values of 53.5, 57.7, 12.1, and 61.5. Compounds **1**–**4** also showed antibacterial activity against *E. coli*, *S. aureus*, and *B. subtilis*. They all inhibited bacterial growth against *E. coli* with an MIC of 100 μg/mL, and all showed antibacterial activity against *S. aureus* and *B. subtilis* with an MRC of 100 μg/mL.

In summary, macrolactins O–R are new glycosylated 24-membered lactones from *Bacillus* sp. AH159-1, which are rare microbial metabolites. Macrolactins show antitumor, antiviral, and antibacterial activity, but biological activity of glycosylated macrolactins is reported in this study for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-1601 UV–visible spectrophotometer. IR spectra were obtained using a Bruker EQUINOX 55 spectrophotometer. NMR spectra were recorded on a Bruker Biospin DMX 600 spectrometer. HRESIMS data were recorded on a JEOL JMS-HX110/110A mass spectrometer.

Bacterial Material. The bacterial strain AH159-1 was isolated from a soil sample collected in October 2003 near Gongju-city, Chungnam Province, Korea. The strain was identified as *Bacillus* sp. on the basis of 16S rDNA sequence by staff at the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea.

Fermentation and Isolation. Fermentation was carried out in 1 L Erlenmeyer flasks containing GSS medium (1% soluble starch, 2% glucose, 2.5% soybean meal, 0.1% beef extract, 0.4% yeast extract, 0.2% NaCl, 0.025% K_2HPO_4, and 0.2% $CaCO_3$, pH 7.2 before sterilization). An inoculum of strain AH159-1 from a mature plate culture was inoculated into a 500 mL Erlenmeyer flask containing 80 mL of sterile seed liquid medium and cultured on a rotary shaker (150 rpm) at 28 °C for 3 days. For the production of **1**–**4**, 5 mL of the seed culture was transferred into 1 L Erlenmeyer flasks containing 100 mL of the GSS medium and cultivated for 7 days at 28 °C. The culture supernatant obtained from the culture broth (13 L) was extracted with an equal volume of EtOAc (×3), and the EtOAc layer was concentrated *in vacuo*. The resultant residue was subjected to Si gel (Merck Art No. 7734.9025) column chromatography followed by stepwise elution with $CHCl_3$–MeOH (20:1, 10:1, 5:1, 1:1). The active fractions eluted with $CHCl_3$–MeOH (1:1) were pooled and concentrated *in vacuo* to give an oily residue. The residue was applied to a Sephadex LH-20 column and then eluted with $CHCl_3$–MeOH (1:1). The active fraction dissolved in $CHCl_3$–MeOH (1:1) was further purified on an RP-HPLC column (YMC C_{18} 20 × 250 mm) with a photodiode array detector. The column was eluted with CH_3OH–H_2O (70:30) at a flow rate of 5 mL/min to afford **1** (3.6 mg), **2** (2.1mg), **3** (5.6 mg), **4** (12.7 mg), **5** (139 mg), and **6** (8.2 mg), with retention times of 33.3, 32.1, 26.2, 20.4, 22.5, and 25.1 min, respectively.

Macrolactin O (1): white powder; $[\alpha]_D$ −56.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.67), 262 (4.27) nm; IR (KBr) ν_{max} 3423 (OH), 2927, 1704 (CO), 1193, 1076 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS $[M + Na]^+$ *m/z* 587.2842 (calcd for $C_{30}H_{44}O_{10}$ + Na, 587.2826).

Macrolactin P (2): white powder; $[\alpha]_D$ −38.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 233 (4.52), 262 (4.30) nm; IR (KBr) ν_{max} 3412 (OH), 2928, 1703 (CO), 1191, 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS $[M + Na]^+$ *m/z* 601.2965 (calcd for $C_{31}H_{46}O_{10}$ + Na, 601.2983).

Macrolactin Q (3): white powder; $[\alpha]_D$ −56.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.72), 263 (4.27) nm; IR (KBr) ν_{max} 3411 (OH), 2927, 1697 (CO), 1191, 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS $[M + Na]^+$ *m/z* 587.2805 (calcd for $C_{30}H_{44}O_{10}$ + Na, 587.2826).

Macrolactin R (4): white powder; $[\alpha]_D$ −60.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 235 (4.53), 265 (4.24) nm; IR (KBr) ν_{max} 3427 (OH), 2927, 1706 (CO), 1195, 1076 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRESIMS $[M + Na]^+$ *m/z* 587.2830 (calcd for $C_{30}H_{44}O_{10}$ + Na, 587.2826).

Macrolactin B (5): white powder; $[\alpha]_D$ −30.6 (*c* 3.8, MeOH) [lit.14 $[\alpha]_D$ −42.0 (*c* 3.8, MeOH)]; ^1H, ^{13}C NMR, and MS data in accordance with those of macrolactin B.

Macrolactin C (6): white powder; $[\alpha]_D$ −27.2 (*c* 0.8, MeOH) [lit.14 $[\alpha]_D$ −21.0 (*c* 0.87, MeOH)]; ^1H, ^{13}C NMR, and MS data in accordance with those of macrolactin C.

Acidic Hydrolysis of 5. Compound **5** (5 mg) was refluxed in 1 N HCl (1 mL) at 100 °C for 1 h. After cooling, the reaction mixture was extracted with EtOAc (3 × 1 mL), and the aqueous phase was neutralized with 1 N NaOH and dried. The residue was subjected to Si gel column chromatography with $CHCl_3$–MeCN (3:1) to afford D-glucose [1.3 mg, $[\alpha]_D$ +38.8 (*c* 0.13, H_2O)]. Glucose identification was carried out via Si gel TLC with $CHCl_3$–MeOH (1:1) comparison with an authentic glucose sample.

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