NOTE

Aldgamycin I, an antibacterial 16-membered macrolide from the abandoned mine bacterium, *Streptomyces* sp. KMA-001

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Abandoned mines are often extremely contaminated by heavy metals and acid, creating a unique nutrient environment. Nonetheless, it has been recognized that microorganisms have adapted to life in this unique bioregion.¹ In the harsh conditions of an abandoned mine, microorganisms are likely to be subjected to substantial levels of competition, which could lead them to develop unique chemical arsenals, such as antibiotics.^{2,3} In the course of our study on the production of secondary metabolites by the mine actinomycete, *Streptomyces* sp. KMA-001 was isolated from a heat-treated soil sample (55 °C, 5 min) collected at the Yeonhwa abandoned zinc mine in Korea. From a pure culture of this strain, we have isolated a new aldgamycin derivative, aldgamycin I (1), along with four earlier reported 16-membered macrolides, aldgamycin E (2),^{4,5} aldgamycin F (3),⁶ aldgamycin G (4)⁷ and chalcomycin (5)⁸ (Figure 1).

A stock culture of the strain KMA-001 was maintained on Yeast–Malt extract (YM) agar containing nystatin (50 µg ml⁻¹) to minimize fungal contamination. The YM agar medium consisted of a yeast extract (Difco, Detroit, MI, USA) 0.5%, a malt extract (Difco) 3.0% and agar 1.5%. The strain KMA-001 was grown on the YM agar medium for 7 days and was then cultured in 25 ml YM broth medium (0.5 g yeast extract (Difco) and 3 g malt extract (Difco) in 100 ml deionized water) in a 100-ml erlenmeyer flask for 3 days. The seed culture broth (25 ml) was transferred to a 1-l erlenmeyer flask containing 500 ml A1BFe broth (pH 6.4 before sterilization) consisting of starch (Difco, 10 g), yeast extract (Difco, 4 g), peptone (Difco, 2 g), FeSO₄·7H₂O (0.08 g) and KBr (0.1 g) in 1 l of deionized water for the second culture step. After 3 days, each 25 ml broth from the second culture was inoculated into 25 erlenmeyer flasks of 1 l, each containing 500 ml A1BFe medium, and was cultured at 28 °C with shaking at 200 r.p.m. for 7 days.

Total genomic DNA preparation of the KMA-001 strain was carried out according to the G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea). PCR amplification of the 16S

rDNA was performed using two universal primers 27f (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGH TAC CTT GTT ACG ACT T-3').⁹ The PCR mixture consisted of 30 picomoles of each primer, 100 ng of chromosomal DNA, 200 µM dNTPs and 2.5 U of *Taq* polymerase in 50 µl of total volume. The amplification was carried out for 35 cycles at 94 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 1.5 min. The PCR product was analyzed by agarose gel electrophoresis and the remaining mixture was purified using a PCR purification kit (Solgent Co., Ltd, Daejeon, Korea). The 16S rDNA PCR product was sequenced by Solgent Co., Ltd. BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to access the DNA similarities. The 16S rDNA sequence of KMA-001 strain showed a homology of 99.3% with *Streptomyces goshikiensis* NBRC 12868, *S. citricolor* NBRC 13005 and *S. sporoverrucosus* NBRC 15458. The cultured strain, KMA-001, was deposited with the Korean Culture Center of Microorganisms (KCCCM42921).

The secondary metabolites of this bacterium were analyzed by HPLC-MS using a gradient solvent system, 10–100% CH₃CN/water, for 30 min (flow rate 0.7 ml min⁻¹, column agilent eclipse XDB-C18, 4.6×150 mm, 5 µm) daily for 7 days to observe temporal production. Five chromatographic peaks were selected for purification after comparison analysis with our in-house HPLC-MS-UV database. After 7 days of fermentation, 20 g l⁻¹ Amberlite XAD-7 adsorbent resin (Supelco, Bellefonte, PA, USA) was added into each 1-l culture flask (the volume of broth 500 ml×25), and the mixture was shaken for an additional hour. The resin was then collected by filtration through cheesecloth, washed with deionized water and eluted twice with Me₂CO. The crude extract was fractionated by C18 flash column chromatography using mixtures of MeOH–water (20, 40, 60, 80 and 100% MeOH in water, each 200 ml, flow rate 20 ml min⁻¹) as elution solvents. The 60% MeOH fraction contained all the HPLC peaks of interest and was repurified by prep-HPLC with a Luna C18 (2) column (21.2×250 mm, 15 µm, Phenomenex Inc., Torrance, CA, USA).

Figure 1 Structures of compounds 1–6.

using an acetonitrile–water (1.6:1 v/v) isocratic condition (flow rate 10 ml min^{−1}) to yield five 16-membered macrolides, aldgamycin I (1, 16.0 mg), aldgamycin E (2, 5.4 mg), aldgamycin F (3, 4.0 mg), aldgamycin G (4, 3.5 mg) and chalcomyacin (5, 12.0 mg).

Aldgamycin I (1) was obtained as a white amorphous powder. The molecular formula was assigned to be C₃₆H₅₈O₁₅ by HR-FAB-MS ((M+Na)⁺=753.3668), ¹H and ¹³C NMR data (Table 1). The observed IR absorptions at 1709 and 1235 cm^{−1} showed the presence of α,β-unsaturated carbonyl and epoxide functionalities (Table 2), which were confirmed by the presence of an ester carbonyl carbon at δ_C 165.6 and oxygenated carbons at δ_C 58.7 and 59.1 in the ¹³C NMR spectrum. Comparison of ¹³C NMR spectra of 1 and 3 was essentially identical except for C-3', C-4', C-7' and C-8' (1: δ 74.8, 37.1, 70.0, and 16.7, aldgamycin F: δ 85.0, 40.7, 81.5 and 13.0). Comprehensive NMR analysis, using data from HSQC, HMBC, ¹H–¹H COSY and ¹H–¹H TOCSY experiments allowed us to assign the structure of 1, which contained a decarboxylated D-aldgaroside, 4,6-dideoxy-3-C-(1-hydroxyethyl)-β-D-ribo-hexopyranose, instead of the D-aldgarose in earlier reported aldgamycin F (3). Aldgamycin I (1) was so named because aldgamycin H, an 8-dehydroxy derivative of 1, was described in an earlier French patent.¹⁰

The absolute configuration of 1 was determined mainly by comparison of the CD spectral data of aldgamycin I (1) and aldgamycin F (3). Treatment of 1 and 3 (each 0.5 mg) in dry pyridine (1 ml) with dimethylaminopyridine (0.5 mg) and 4-bromo benzoyl chloride (2 mg) at room temperature for 12 h, in separate experiments, yielded the reaction mixture including, 7',4''-di-O-*p*-bromobenzoyl-aldgamycin I (6; Figure 1). Compound 6 was purified by normal phase HPLC (Gilson 321 system; Luna 10 μ silica (2) 250×10 mm column, Phenomenex Inc.; 4 ml min^{−1}) using isocratic elution with hexane–EtOAc (2:1). Both products showed identical ¹H NMR and CD spectra (Figure 2), as well as similar quasi-molecular ion peaks and retention times in LC-MS data. These data suggested that the absolute configuration of 1 was the same as that of 3.

Compound 2 was also obtained as a white amorphous powder that analyzed for the molecular formula C₃₇H₅₈O₁₅ by HR-FAB-MS ((M+H)⁺=743.3860) in combination with ¹H and ¹³C NMR data. The IR absorption bands at 1800 and 1709 cm^{−1} suggested the presence of a ketone and an α,β-unsaturated carbonyl group. Analyses of the 2D NMR spectra of 2 allowed us to assign the structure of 2 as aldgamycin E, which was reported earlier.⁵ The ¹H NMR spectrum of 2 is very similar to that of aldgamycin E, although the reported structure of aldgamycin E was defined incompletely and its sugar linkage position was not determined. Two sugar units and an aglycone of 2, as well as the optical rotation (2 −50.4° (CHCl₃); aldgamycin E −56° (CH₃OH)) are the same as those of aldgamycin E. We, thus, proposed that the structure of 2 was identical to aldgamycin E. In addition to the ¹H NMR spectrum described earlier, the NMR data and the structure of compound 2 (Tables 1 and 2) need to be further described.

The absolute configurations of two sugar units of aldgamycin E were already described in an earlier literature.^{5,6} However, the aglycone of aldgamycin E was reported as a planar structure. In an earlier literature, the absolute configuration of GERI-155, a relative of chalcomyacin, was assigned to be identical to that of chalcomyacin on the similarities of their optical rotation values (GERI-155 [α]_D −75.5°; chalcomyacin [α]_D −43.5°) and NMR data.¹¹ Similarly, compound 2 has a very similar optical rotation value as that of 4 (2, −50.4°; 4, −29.0°). This comparison is further strengthened when biosynthetic origins are considered. As these compounds were isolated from the same microbial culture, it is likely that compound 2 is produced through the same biosynthetic pathway as aldgamycin G, and is then reduced by an enoylreductase.¹² On the basis of the spectroscopic data and biosynthetic considerations, the absolute configuration of 2 is proposed to be identical to that of aldgamycin G (4).

Table 1 ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **1** and **2** in CD_3CN

Position	Compound 1		Compound 2	
	δ_{H}^a , mult (J in Hz)	δ_{C}^a	δ_{H}^a , mult (J in Hz)	δ_{C}^a
1		165.6		165.7
2	6.01 d (15.5)	121.2	6.01 d (15.5)	121.2
3	6.65 dd (15.5, 10.5)	152.0	6.77 dd (15.5, 10.5)	151.6
4	2.75 ddq (10.5, 10.0, 7.0)	41.4	2.82 ddq (10.5, 10.0, 7.0)	40.6
5	3.26 d (10.0)	87.5	3.53 d (10.0)	85.4
6	1.17 m ^b	34.3	1.50 m	34.8
7	2.00 dd (15.0, 3.0)	36.8	1.80 m	32.2
	1.76 dd (15.0, 12.5)		1.33 m ^b	
8		78.6	2.52 m	45.5
9		201.1		208.1
10	6.92 d (15.0)	126.5	2.71 m	33.0
			2.05 m	
11	6.51 dd (15.0, 9.0)	145.4	1.97 m	26.5
			1.33 m	
12	3.42 dd (9.0, 2.0)	58.7	2.81 m	59.7
13	3.11 dd (9.0, 2.0)	59.1	2.67 m	57.5
14	1.43 br m	49.3	1.36 m	48.2
15	5.33 dq (10.5, 6.5)	68.4	5.28 dq (10.0, 6.0)	69.3
16 (15-CH ₃)	1.34 d (6.5)	17.6	1.33 d (6.0)	17.8
17 (6-CH ₃)	0.99 d (6.5)	18.7	0.96 d (6.5)	16.0
19 (8-CH ₃)	1.38 s	27.3	1.12 d (6.5)	17.3
22 (4-CH ₃)	1.20 d (7.0)	18.1	1.21 d (7.0)	17.7
24	4.08 dd (10.0, 2.5)	66.8	4.03 dd (10.0, 2.5)	67.0
	3.66 dd (10.0, 3.0)		3.60 dd (10.0, 3.0)	
1'	4.49 d (7.5)	102.6	4.58 d (8.0)	101.9
2'	3.52 m ^b	71.1	3.43 dd (8.0, 4.5)	70.8
3'		74.8		85.6
4'	1.51 dd (14.0, 2.0)	37.1	1.91 dd (14.5, 2.0)	40.6
	1.38 m ^b		1.60 dd (14.5, 11.0)	
5'	3.84 (11.0, 6.5, 2.0)	66.6	3.84 (11.0, 6.5, 2.0)	66.9
6' (5'-CH ₃)	1.16 d (6.5)	20.6	1.19 d (6.0)	20.3
7'	3.72 br m	70.0	4.47 q (6.0)	81.4
8' (7'-CH ₃)	1.15 d (6.5)	16.7	1.52 d (6.5)	12.8
O(C=O)O				154.3
1''	4.57 d (8.0)	101.1	4.53 d (8.0)	100.9
2''	3.06 dd (8.0, 2.5)	81.8	3.05 dd (8.0, 2.5)	81.7
3''	3.77 t (2.5)	80.1	3.76 t (2.5)	79.9
4''	3.15 td (9.5, 2.5)	73.1	3.13 td (9.5, 2.5)	73.0
5''	3.56 m ^b	70.3	3.55 m ^b	70.2
6'' (5''-CH ₃)	1.21 d (6.5)	17.4	1.20 d (6.5)	17.7
7'' (2''-OCH ₃)	3.52 s	58.6	3.51 s	58.4
8'' (3''-OCH ₃)	3.56 s	61.3	3.56 s	61.1
8-OH	3.75 br s		—	
2'-OH	2.22 ^b		ND ^c	
3'-OH	3.00 br s		—	
7'-OH	3.09 br s		—	
4''-OH	2.99 br d (9.5)		2.96 d (9.5)	

^aVarian unity: 500; reference standard: acetonitrile (δ_{H} : 1.96 p.p.m., δ_{C} : 1.79 p.p.m.).^bThe multiplicity of signal was unresolved by peak overlapping and the chemical shift was assigned by the analysis of HSQC and TOCSY spectra.^cThe signal was not detected.

The antimicrobial activities of aldgamycin I (**1**), aldgamycin E (**2**), aldgamycin F (**3**), aldgamycin G (**4**) and chalcomycin (**5**) were measured using a MIC assay against *Micrococcus luteus*, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhimurium*, *Aspergillus fumigatus*, *Trichophyton rubrum* and methicillin resistant *Staphylococcus aureus* (Table 3). Ampicillin and amphotericin B were used as positive controls in each assay. Interestingly, all compounds showed selective antibacterial activ-

ities against *M. luteus* and *S. typhimurium*. These compounds exhibited weak or no antimicrobial activities against *B. subtilis*, *P. vulgaris*, *A. fumigatus*, *T. rubrum* and MRSA as shown in Table 3.

7', 4''-DI-O-P-BROMOBENZOYL-ALDGAMYCIN I

^1H NMR (500 MHz, acetonitrile- d_3) δ 7.98 (4H, m, Bz^a 3-H and 3'-H), 7.74 (4H, m, Bz^a 4-H and 4'-H), 6.91 (1H, d, $J=15.5$ Hz, 10-H),

Table 2 Physicochemical properties of aldgamycins I (1) and E (2)

	1	2
Appearance	Colorless amorphous powder	Colorless amorphous powder
Molecular weight	730.8	742.8
Molecular formula	C ₃₆ H ₅₈ O ₁₅	C ₃₇ H ₅₈ O ₁₅
[α] _D	−19.1° (c 0.15, CHCl ₃)	−50.4° (c 0.28, CHCl ₃)
HR FAB MS (<i>m/z</i>)		
Found:	753.3668 (M+Na) ⁺	743.3860 (M+H) ⁺
Calculated:	753.3673	743.3854
UV $\lambda_{\max}$ nm (ϵ) (MeCN)	218 (22 500)	216 (12 900)
IR (KBr) $\nu_{\max}$	3467, 2975, 2933, 1709, 1655, 1629, 1235, 1083	3457, 2972, 2934, 1800, 1709, 1652, 1083
Solubility	Soluble: MeOH, MeCN, CDCl ₃ Insoluble: Hexane, H ₂ O	Soluble: MeOH, MeCN, CHCl ₃ Insoluble: Hexane, H ₂ O
HPLC, Rt (min) ^a	14.2 min	19.7 min

^aLuna C18 (2) (4.6×150 mm, 5 μ m, Phenomenex) 10–100% acetonitrile/water for 30 min, 1.0 ml min^{−1}. **Figure 2** CD spectra of 7',4''-di-O-p-bromobenzoates of 1 (a) and aldgamycin F (b).

6.64 (1H, dd, $J=15.5, 10.5$ Hz, 3-H), 6.52 (1H, dd, $J=15.5, 9.0$ Hz, 11-H), 6.01 (1H, d, $J=15.5$ Hz, 2-H), 5.33 (1H, dq, $J=10.5, 6.5$ Hz, 15-H), 5.20 (1H, m, 7'-H), 4.73 (1H, d, $J=10.0, 2.5$ Hz, 4''-H), 4.69 (1H, d, $J=8.0$ Hz, 1''-H), 4.52 (1H, d, $J=8.0$ Hz, 1'-H), 4.11 (1H, dd, $J=10.0, 3.0$ Hz, 24a-H), 4.07 (2H, m, 3''-H and 5''-H), 3.91 (1H, m, 5'-H), 3.75 (1H, s, 8-OH), 3.71 (1H, dd, $J=10.0, 3.0$ Hz, 24b-H), 3.52 (3H, s, 3''-OCH₃), 3.48 (3H, s, 2''-OCH₃), 3.42 (1H, dd, $J=9.0, 2.0$ Hz, 12-H), 3.30 (1H, m, 2'-H), 3.26 (1H, d, $J=10.0$ Hz, 5-H), 3.22 (1H, dd, $J=8.0, 3.0$ Hz, 2''-H), 3.12 (1H, dd, $J=9.0, 2.0$ Hz, 13-H), 3.08 (1H, br s, 3'-OH), 2.71 (1H, m, H-4), 2.22 (1H, br s, 2'-OH), 1.93 (1H, m, 7a-H), 1.82 (1H, dd, $J=14.0, 2.0$ Hz, 4'a-H), 1.71 (1H, dd, $J=15.0, 12.0$ Hz, 7b-H), 1.59 (1H, dd, $J=14.0, 10.0$ Hz, 4'b-H), 1.44 (1H, m, 14-H), 1.34 (3H, d, $J=6.5$ Hz, 15-CH₃), 1.33 (3H, s, 8-CH₃), 1.31 (3H, d,

Table 3 Antimicrobial activities of compounds 1–5 (MIC, μ g ml^{−1})

	<i>Micrococcus luteus</i> IFC12708	<i>Bacillus subtilis</i> ATCC6633	<i>Proteus vulgaris</i> ATCC3851	<i>Salmonella typhimurium</i> ATCC14028	<i>Aspergillus fumigatus</i> HIC 6094	<i>Trichophyton rubrum</i> IFO 9185	<i>MRSA</i> ATCC 43300
1	3.13	> 50	> 50	6.25	> 100	> 100	> 100
2	0.78	12.50	50.00	1.56	> 100	> 100	> 100
3	0.78	25.00	> 50	1.56	> 100	> 100	> 100
4	1.56	50.00	> 50	1.56	> 100	> 100	> 100
5	0.78	12.50	12.50	1.56	> 100	> 100	> 100
A	0.78	3.13	1.56	12.50	—	—	12.5
B	—	—	—	—	1.56	1.56	—

Abbreviations: A, ampicillin; B, amphotericin B.

$J=6.0$ Hz, 7'-CH₃), 1.22 (3H, d, $J=6.0$ Hz, 5'-CH₃), 1.21 (3H, d, $J=6.5$ Hz, 5''-CH₃), 1.18 (3H, d, $J=7.0$ Hz, 4-CH₃), 1.17 (1H, m, 6-H), 0.99 (3H, d, $J=6.5$ Hz, 6-CH₃). Carbon chemical shift in HSQC and HMBC spectra (125 MHz, acetonitrile-*d*₃) δ 200.8 (C-9), 165.3 (C-1), 164.8 (Bz^a 1 and 1'), 151.7 (C-3), 145.3 (C-11), 132 (Bz^a 4 and 4'), 128.0 (Bz^a 2 and 2'), 127.7 (Bz^a 5 and 5'), 131.5 (Bz^a 3 and 3'), 126.2 (C-10), 121.0 (C-2), 102.4 (C-1'), 100.9 (C-1''), 87.8 (C-5), 80.4 (C-2''), 78.2 (C-8), 77.5 (C-3''), 75.3 (C-4''), 74.1 (C-3'), 71.4 (C-7'), 70.5 (C-2'), 68.2 (C-15), 67.8 (C-5''), 66.9 (C-24), 66.5 (C-5'), 60.9 (C-7''), 58.7 (C-13), 58.5 (C-12), 58.3 (C-8''), 49.0 (C-14), 41.1 (C-4), 36.7 (C-4'), 36. (C-7), 27.2 (C-18'), 20.5 (C-6'), 18.6 (C-17), 17.8 (C-22), 17.6 (C-16), 17.1 (C-6), 17.1 (C-6''), 12.8 (C-19). ^aBz: *p*-bromobenzoyl group. ESI-MS m/z 1095 (M+H)⁺.

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