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Identification and Antibacterial Activity of a New Oleandomycin Derivative from *Streptomyces antibioticus*

Beom Seok Kim, Hyuncheol Oh, Sun Young Kim, Jeong Ah Park, Yeo Joon Yoon, Sang Kil Lee, Bo Yeon Kim, Jong Seog Ahn

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Abstract During the study on the oleandomycin production, we purified a new oleandomycin derivative having a macrolactone of which biosynthesis does not follow the genetic architecture of the oleandomycin PKS. The molecular formula for the compound was suggested as $C_{35}H_{59}NO_{11}$ on the basis of the analysis of NMR and HRMS data (*m/z* 670.4185, $\Delta -1.9$ mmu, calcd for $C_{35}H_{60}NO_{11}$). ^{13}C NMR assignments and analysis of COSY, HMBC and HMQC data suggested that the compound differs from oleandomycin by formation of the olefinic functionality resulting from the dehydration of a hydroxy group in oleandomycin. The new oleandomycin derivative has antibacterial activities similar to those of oleandomycin agaisnt *Enterococcus faecalis*, *Bacillus subtilis* and *Staphylococcus aureus*.

Keywords new oleandomycin derivative, olefinic formation, dehydration of hydroxyl group, *Streptomyces antibioticus*

Macrolides are a large and structurally diverse class of natural products that possess a wide range of biological activities useful for medicinal and agricultural fields as antimicrobials, immunosuppressants, antiparasitics, and anticancer agents [1]. Despite their structural diversity, the macrolactone moieties are assembled by a common mechanism of decarboxylative condensations of simple malonate derivatives by polyketide synthases (PKSs) [1]. Type I PKSs are gigantic multifunctional modular proteins of which each module contains a set of acyl-chain elongation domains (ketosynthase, acyltransferase, and acyl carrier protein) and a variable set of β-keto reduction domains (ketoreductase, dehydratase, and enoyl reductase) [2, 3]. The structural variation of macrolactones mainly comes from the choice of malonate derivative precursors, the number of Claisen condensation, and the reduction level of the β-ketoacyl chain, as designated by the genetic architecture of the modules [4, 5]. The correspondence between the domain composition of consecutive modules and the structure of each newly added malonate derivatives is now accepted as a general property of type I PKSs [6]. Therefore, the structure of a macrolactone can be predicted through the examination of the genetic organization of domains and modules, or *vice versa*. In the current study on the oleandomycin production, we found a new oleandomycin derivative with a macrolactone which does not follow the genetic architecture of the oleandomycin PKS (olePKS). Here we report the chemical and biological characteristics of the oleandomycin derivative.

Oleandomycin (**1**) is an antibacterial macrolide antibiotics of which aglycone portion is generated by type I PKSs [7]. During the study on the antibiotic production of *Streptomyces antibioticus* ATCC 11891, one of the minor compounds produced along with oleandomycin was detected in HPLC analysis with a gradient elution from

J. S. Ahn (Corresponding author)**, B. S. Kim, H. Oh, S. Y. Kim, J. A. Park, B. Y. Kim:** Korea Research Institute of Bioscience and Biotechnology, 52 Oun-dong, Yuseong-gu, Daejeon 305-333, Korea, E-mail: jsahn@kribb.re.kr

Y. J. Yoon: Division of Nano Sciences and Department of Chemistry, Ewha Womans University, Seoul 120-750, Korea

S. K. Lee: School of Chemical Engineering, Seoul National University, Seoul 151-742, Korea

10% to 40% acetonitrile in 5 mM phosphate buffer (pH 8.0). We purified the minor component in small quantity, and it was suggested that the compound is an oleandomycin-type macrolide based on the similarity of the ^{1}H NMR data with that of oleandomycin. The presence of additional signal corresponding to an olefinic proton at 6.75 ppm inspired us to prepare the oleandomycin derivative in milligram quantities to elucidate its chemical structure and biological activity since there was no previous report on the oleandomycin derivative with olefinic functionality structure. A loopful of *S. antibioticus* was inoculated from a slant culture into 50ml of sterilized medium consisting of glucose 0.5%, soy bean meal 1%, soluble starch 1%, yeast extract 0.1%, and peptone 0.2% in 250 ml Erlenmeyer flask. The seed culture was carried out on a rotary shaker (220 rpm) at 30°C for 2 days and used as inoculums (0.5%) for the production culture. The production medium was composed of glucose 1.5%, soy bean meal 3%, soluble starch 1.5%, yeast extract 0.2%, peptone 0.5%, and $CaCO_3$ 0.3% and dispensed in 1 liter

Table 1 Comparison of NMR shift assignments for oleandomycin (**1**) and compound **2**

Position	**1**		**2**	
	^{13}C	^{1}H[a]	^{13}C	^{1}H[b]
1	176.8		175.1	
2	44.6	2.78	44.5	2.63
3	79.5	3.76	78.7	3.71
4	44.1	1.84	43.3	1.88
5	83.1	3.49	85.1	3.46
6	32.9	1.87	31.0	1.85
7	31.7	2.50; 1.57	35.4	2.68; 1.31
8	63.2		61.6	
9	208.4		199.5	
10	44.9	3.15	136.5	
11	69.3	3.69	144.1	6.75
12	41.7	1.65	35.6	3.33
13	70.6	5.39	74.5	4.95
14	17.2	1.28	12.5	1.33
15	12.7	1.17	12.9	1.16
16	8.8	1.14	9.3	1.13
17	18.6	1.15	16.7	1.09
18	49.0	2.75	50.1	2.78; 2.88
19	6.9	0.99	10.5	1.85
20	8.4	0.97	13.9	1.01
1′	99.0	4.96	98.7	4.69
2′	34.2	2.38; 1.47	34.5	2.30; 1.46
3′	77.7	3.42	77.6	3.38
4′	75.9	3.04	76.1	3.02
5′	69.5	3.42	69.3	3.39
6′	17.3	1.25	17.2	1.17
1″	103.2	4.32	103.9	4.30
2″	65.5	3.41	65.6	3.34
3″	69.3	3.46	69.2	3.48
4″	30.7	2.06; 1.51	29.9	1.48; 1.98
5″	67.8	3.69	68.1	3.62
6″	19.9	1.27	19.9	1.26
O-CH_3	56.0	3.41	56.2	3.39
N-CH_3	39.0	2.84	38.5	2.76

[a] 500 MHz in CD_3OD

[b] 400 MHz in CD_3OD

baffled flasks. The flasks were placed at 30°C for 5 days on a rotary shaker (220 rpm). The culture broth was extracted with ethyl acetate after the adjustment to pH 8.0 with 1 M NaOH. The crude extract was fractionated on a silica gel column using dichloromethane/methanol (6 : 4). The fraction was further purified by using reverse phase HPLC with a gradient elution from 10% to 40% acetonitrile in 0.1 mM formic acid solution. The yield of the minor compound **2** (eluted after the major compound **1**) was 0.7 mg per 1 liter culture.

The structure of compound **1** was determined as oleandomycin by MS and NMR data comparison with those of authentic sample. The ^{1}H NMR spectrum of compound **2** revealed signals similar to those found in oleandomycin (Table 1). The only noticeable differences were the presence of signals corresponding to olefinic proton and allylic methyl protons and the absence of signal for one of the methyl protons coupled to methine proton. The ^{13}C and DEPT NMR data were again very similar with those for oleandomycin except for those near the newly-formed olefinic functionality (Table 1). The molecular formula for compound **2** was suggested as $C_{35}H_{59}NO_{11}$ on the basis of the analysis of NMR and HRMS data (*m*/*z* 670.4185, $\Delta -1.9$ mmu, calcd for $C_{35}H_{60}NO_{11}$).

Comparisons of molecular formula and ^{13}C NMR signals for oleandomycin and compound **2** (Table 1) suggested that compound **2** differs from oleandomycin by formation of the olefinic functionality resulting from the dehydration of an hydroxy group in oleandomycin. ^{1}H and ^{13}C NMR assignments for compound **2** were proposed by analysis of COSY and HMQC data, and by comparison to the assignments for oleandomycin. HMBC experiments were carried out to confirm the gross structure of compound **2** as shown (Table 2). For example, HMBC correlations of methyl protons H_3-19 to C-9, C-10, and C-11 together with correlations of H_3-20 to C-11, C-12, and C-13 confirmed the placement of the olefinic group at C-10. HMBC correlation of olefinic proton H-11 to C-9, C-13 and C-19 also supported this assignment.

Oleandomycin biosynthesis has long been studied by Salas and colleagues [8, 9] and major progress has been made therein. Recently the whole olePKS gene cluster producing 8,18-deoxyoleandolide (**3**, the macrolactone moiety of oleandomycin) was reported by McDaniel and his colleagues [7]. It revealed a genetic architecture similar to the domain composition and modular arrangement of the erythromycin PKS (eryPKS) which is one of the intensively studied PKS as a model system for polyketide biosynthetic study [10] (Fig. 2). This was not totally unexpected given the fact that the eryPKS produces the 14-membered macrolactone **4** (deoxyerythronolide, DEB) similar to **3** with the exception of a C-13 ethyl group compared to the C-13 methyl group of **3** [2]. Not only in the case of the olePKS and eryPKS, but various biosynthetic studies on other type I PKSs confirmed the correspondence between the genetic architecture of PKS and the structure of its product. Another 14-membered macrolactone similar to **3** and **4** is narbonolide (**5**) produced by the pikromycin PKS (pikPKS). Narbonolide has a C-3 keto group instead of a C-3 hydroxyl group of **3** and an olefinic structure at

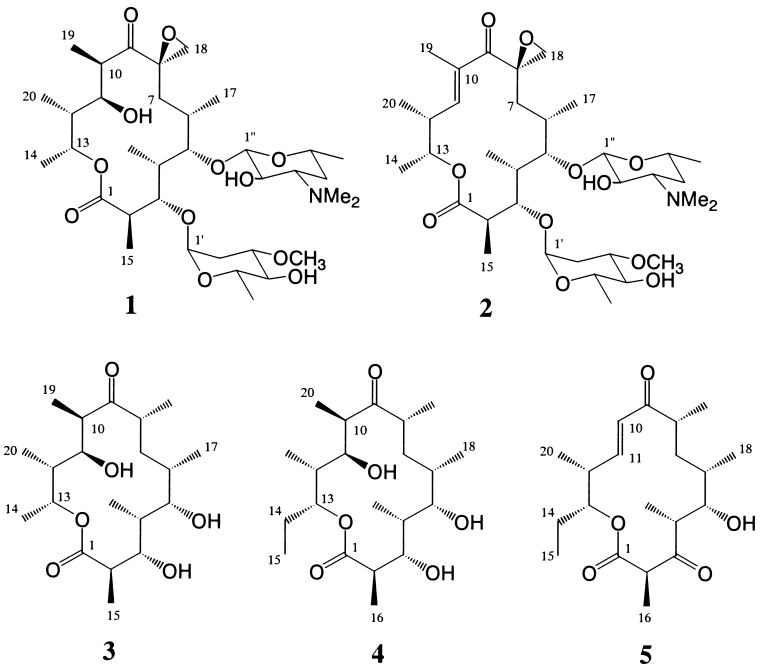

Fig. 1 The structures of oleandomycin (**1**) and its derivative (**2**) produced by *Streptomyces antibioticus* ATCC11891 and the 14-membered macrolactones synthesized by the olePKS (**3**), the eryPKS (**4**), and the pikPKS (**5**).

Table 2 NMR assignment for compound **2** in CD_3OD[a]

Position	^{13}C	^{1}H (int.; mult; *J* in Hz)	HMBC
1	175.1		
2	44.5	2.63 (1H; dd; 7.2, 5.6)	
3	78.7	3.71 (1H; d; 3.4)	C-1, C-4, C-5, C-15, C-16, C-1′
4	43.3	1.88 (1H; m)	
5	85.1	3.46 (1H; m)	C-4, C-6, C-7, C-16, C-17
6	31.0	1.85 (1H; m)	
7	35.4	1.31 (1H; m)	C-9
		2.68 (1H; dd; 15.1, 1.5)	
8	61.6		
9	199.5		
10	136.5		
11	144.1	6.75 (1H; d; 9.6)	C-9, C-13, C-19
12	35.6	3.33 (1H; m)	
13	74.5	4.95 (1H; m)	C-11
14	12.5	1.33 (3H; d; 6.4)	C-12, C-13
15	12.9	1.16 (3H; d; 7.2)	C-1, C-2, C-3
16	9.3	1.13 (3H; d; 6.4)	C-3, C-4, C-5
17	16.7	1.09 (3H; d; 6.4)	C-5, C-6, C-7
18	50.1	2.78 (1H; d; 5.2)	
		2.88 (1H; d; 5.2)	C-7, C-8
19	10.5	1.85 (3H; s)	C-9, C-10, C-11
20	13.9	1.01 (3H; d; 6.8)	C-11, C-12, C-13
1′	98.7	4.69 (d; 2.4)	C-3, C-3′, C-5′
2′	34.5	1.46 (1H; m)	
		2.30 (1H; dd; 4.8, 2.3)	C-1′, C-3′, C-4′
3′	77.6	3.38 (1H; m)	
4′	76.1	3.02 (1H; dd; 9.2, 9.2)	C-3′, C-5′, C-6′
5′	69.3	3.39 (1H; m)	
6′	17.2	1.17 (3H; d; 6.0)	C-4′, C-5′
1″	103.9	4.30 (d; 6.8)	C-5, C-2″
2″	65.6	3.34 (1H; m)	
3″	69.2	3.48 (1H; m)	
4‴	29.9	1.48 (1H; m)	
		1.98 (m)	C-2″, C-3″
5″	68.1	3.62 (1H; m)	
6″	19.9	1.26 (3H; d; 6.4)	C-4″, C-5″
O-CH_3	56.2	3.39 (3H; s)	C-3′
N-CH_3	38.5	2.76 (6H; s)	C-3″

[a] 400 MHz

C-11, 12 instead of the methyl group at C-11 and hydroxyl group at C-12 [11, 12]. The olefinic structure is generated by the DH domain in module 2 of the pikPKS (PikAI) which is deficient in the module of the olePKS and the eryPKS. As is the case of the pikPKS, the β-keto group originated from malonate derivative precursor is expected to be reduced to hydroxyl, methylene, or acyl group as designated by the array of reductive domains in the module [11, 13]. Without a reductive domain in the module, the corresponding reduction of β-keto group might not be possible. Considering the general properties of type I PKS, therefore, the oleandomycin derivative with olefinic functionality was an unexpected product, since the modules of the olePKS do not have a set of KR-DH which can generate a double bond by sequential reduction of the β-keto group. Although the mechanism of the unexpected

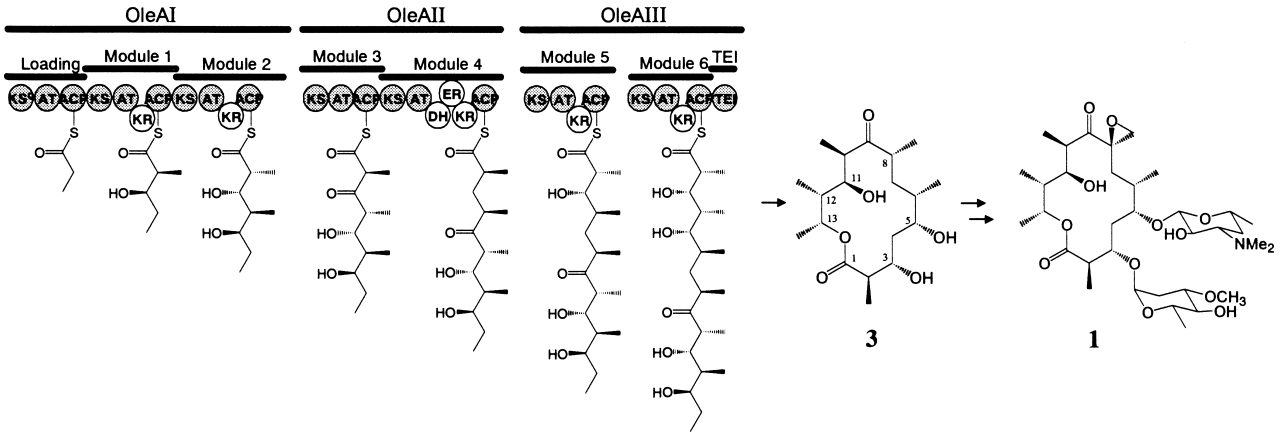

Fig. 2 The biosynthesis of 8,18-deoxyoleandolide (**3**), the macrolactone precursor of oleandomycin (**1**) [7]. KS, ketosynthase; AT, acyltransferase; ACP, acyl carrier protein; KR, ketoreductase; DH, dehydratase; ER, enoylreductase; KSq, KS like domain having active-site cysteine substitution by glutamine.

Table 3 Antibacterial activity of oleandomycin (**1**) and oleandomycin derivative (**2**)

Microorganisms	MIC (μg/ml)	
	1	**2**
Enterococcus faecalis ATCC29212	16	16
Enterococcus faecium ATCC8043	16	32
Staphylococcus aureus ATCC6538	8	8
Streptococcus pneumoniae ATCC4919	2	4
Bacillus subtilis ATCC21394	4	4
Pseudomonas aeruginosa ATCC27853	>128	>128

dehydration at C-11 of **2** is yet to be elucidated, an accidental β-elimination by chemical reaction might be a possible process to yield **2**. However, the possibility of involvement of biosynthetic apparatus can not be excluded.

The MICs of **1** and **2** were determined by the two fold agar dilution method as suggested by the NCCLS [14]. The oleandomycin derivative (**2**) has an antibacterial spectrum similar to that of oleandomycin (**1**), and exhibited antibacterial activity against Gram-positive bacteria but not against *Pseudomonas aeruginosa* up to 128 μg/ml. The MICs of **2** against *Enterococcus faecalis*, *Bacillus subtilis* and *Staphylococcus aureus* are similar to those of oleandomycin. However, the compound **2** was two fold less effective than oleandomycin against *Enterococcus faecium* and *Streptococcus pneumoniae*.

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