16-Methyloxazolomycin, a New Antimicrobial and Cytotoxic Substance Produced by a *Streptomyces* sp.

GEONSEEK RYU, SEUNGJIN HWANG and SI-KWAN KIM*

Korea Ginseng & Tobacco Research Institute, 302 Shinseong-dong, Yuseong-ku, Taejon 305-345, Korea

(Received for publication August 11, 1997)

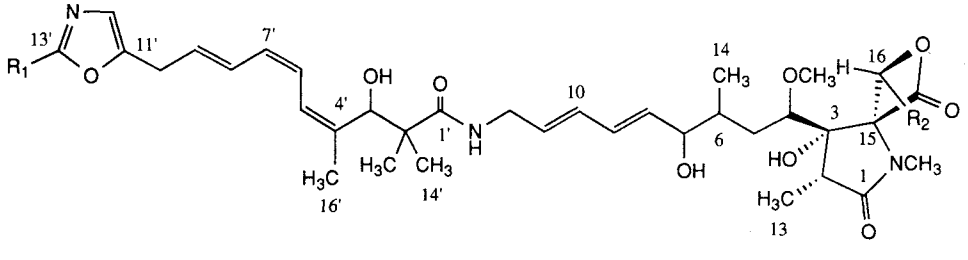
Triene antibiotics containing β-lactone-γ-lactam bicyclospiro and oxazole rings are unique metabolites produced by *Streptomyces* spp., such as oxazolomycins[1,2] and curromycins.[3,4] In the course of our continuing search for novel antimicrobial agents from microorganisms, an actinomycete strain *Streptomyces* sp. isolated from a soil sample collected in Taejon, Korea, was found to produce a new antibacterial 16-methyloxazolomycin (**1**). In this paper, we describe the production, isolation, structural elucidation and biological activities of **1**.

The strain was cultured in the seed medium consisting of glucose 2%, starch 1%, soybean flour 2.5%, yeast extract 0.4%, NaCl 0.2%, K_2HPO_4 0.005% and beef extract 0.1% (adjusted to pH 7.3 before sterilization). The seed culture was carried out on a rotary shaker (250 rpm) at 28°C for 24 hours in 500-ml Erlenmeyer flasks containing 100 ml of the seed medium. Then, the seed culture (100 ml) was inoculated to a 50-liter jar fermenter containing 10 liters of the production medium (antifoam 0.08%). Fermentation was carried out at 27°C for 4 days with aeration (10 liters/minute) under constant agitation (250 rpm).

The isolation of 16-methyloxazolomycin was guided by the use of *Bacillus subtilis* IAM 1069 as a test organism. The culture broth was centrifuged to separate the mycelial cake. The mycelial cake was stirred overnight in 70% aqueous acetone (5 liters) and filtered. The filtrate was concentrated *in vacuo* to remove the organic solvent, resulting in an aqueous solution. The combined filtrates were passed through a Diaion HP-20 (Mitsubishi Kasei, Japan) column, and washed with H_2O followed by MeOH. The methanol eluate was partitioned by the Kupchan's scheme.[5] The CH_2Cl_2 layer concentrated was fractionated by ODS flash chromatography with aqueous MeOH. The active 80% MeOH fraction was gel-filtered on Sephadex LH-20 with *n*-hexane/CH_2Cl_2/MeOH (4:2:1) to afford an active fraction. This fraction was finally purified by reversed-phase HPLC with 63% MeOH containing 0.5 mM $NaClO_4$ to yield 16-methyloxazolomycin (**1**, 6.0 mg) together with the known oxazolomycin (**2**, 15 mg).

Physico-chemical properties of **1** are as follows: pale yellow amorphous powder; $[\alpha]_D^{23}$ +3.6° (*c* 0.52, MeOH); UV λ_{max} (MeOH) nm (ε) 228 (23,000), 266 (27,200), 275 (32,000) and 285 (27,000); IR ν_{max} (film) 3350 (OH or NH), 2930, 1825 (β-lactone), 1690 (amide), 1645, 1240, 1040 cm^{-1}; ESI-MS *m/z* 724 $(M+MeOH+Na)^+$, 692 $(M+Na)^+$, 579, 507; HRESI-MS *m/z* 670.3706 cacld. for $C_{36}H_{52}N_3O_9$ (Δ −2.6 mmu); ^1H NMR and ^{13}C NMR data are shown in Table 1.

The ESI mass data of 16-methyloxazolomycin suggested that **1** was 14 units larger than oxazolomycin (**2**). The NMR spectra of **1** were similar to those of oxazolomycin except for the appearance of a doublet methyl (δ 1.69/17.9) and a quartet oxymethine (δ 4.82/78.4) instead of an oxymethylene in **2**, thus indicating that one hydrogen of the oxymethylene in **2** was displaced with the methyl as was the case with curromycin B (**3**). This was substantiated by interpretation of the HMBC spectrum[6] (Table 1); the methyl group was placed at C16 by HMBC cross peaks between H16 and C3 and C15, and between 16-CH_3 and C16 and C15, thus constructing gross structure **1** belonging to the oxazolomycin class. Because ^1H NMR spectrum measured in $CDCl_3$ revealed overlapped signals in the olefinic region, the geometries of the triene and diene systems



1 R_1=H, R_2=CH_3
2 R_1=H, R_2=H
3 R_1=CH_3, R_2=CH_3

Table 1. ^{1}H and ^{13}C NMR data of 16-methyloxazolomycin in DMSO-d_6.

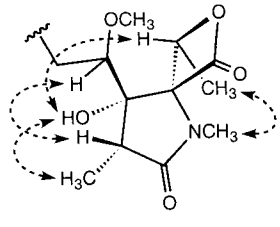
Position	δ ^{13}C	δ ^{1}H (mult., JHz)	HMBC
1	174.2		H2, NCH_3, H13
2	44.7	2.34 (H, q, 6.6)	H13
3	81.8		H2, H13, H16, 3-OH
4	83.7	3.38 (H, t, 6.9)[a]	OCH_3, 3-OH
5	33.1	1.95 (H, m)	H7, H14
		1.15 (H, m)	
6	37.9	1.58 (H, m)	H8, H14, 7-OH
7	76.4	3.81 (H, m)	H8, H9, 7-OH
8	129.5	5.57 (H, dd, 16.8, 11.2)	H9, H10, 7-OH
9	130.0	6.14 (H, dd, 11.2, 16.8)	H7, H10, H11
10	131.5	6.09 (H, dd, 14.3, 10.8)	H8, H11, H12
11	134.0	5.59 (H, m)	H10, H12
12	42.2	3.71 (2H, m)	H10, H11, NH
13	20.8	1.03 (3H, d, 6.6)	H2
14	17.2	0.86 (3H, d, 6.2)	
15	84.7		H4, H16, 16-CH_3, NCH_3
16	78.4	4.82 (H, q, 6.3)	16-CH_3
17	170.2		H16
1′	176.2		H12, H3′, H14′, H15′, NH
2′	46.7		H14′, H15′, 3′-OH
3′	74.4	4.59 (H, s)	H5′, H14′, H15′, H16′
4′	141.0		H3′, H6′, H16′
5′	124.6	6.35 (H, d, 11.3)	H3′, H16′
6′	125.5	6.27 (H, dd, 10.9, 11.3)	H5′, H7′
7′	128.2	5.90 (H, dd, 11.5, 10.9)	H6′, H9′
8′	129.1	6.70 (H, dd, 11.5, 14.5)	H6′, H7′, H9′, H10′
9′	129.8	5.75 (H, dt, 14.5, 4.5)	H7′, H10′
10′	29.4	3.52 (2H, br d, 7.0)[b]	H8′, H9′
11′	151.1		H9′, H10′, H12′, H13′
12′	122.9	6.84 (H, s)	H10′, H13′
13′	152.1	8.14 (H, s)	H12′
14′	22.6	0.95 (3H, s)	H3′, H15′
15′	26.1	1.10 (3H, s)	H3′, H14′
16′	20.9	1.70 (3H, s)	H3′, H5′
16-CH_3	17.9	1.69 (3H, d, 6.3)	H3
NCH_3	27.1	2.77 (3H, s)	
OCH_3	57.1	3.15 (3H, s)	H4
3-OH		5.31 (H, s)	
7-OH		4.82 (H, s)	
3′-OH		5.49 (H, s)	
NH		7.63 (H, br t, 5.9)	

[a,b] Proton chemical shifts and coupling constants were deduced from the ^{1}H NMR spectrum in $CDCl_3$.

were determined by the spin coupling constants measured in DMSO-d_6 (Table 1). Furthermore, stereochemistry of β-lactone-γ-lactam spiro ring except for C16 was deduced to be identical with that of oxazolomycin by NOESY experiments[7] (Fig. 1). NOESY cross peaks H16/3-OH and 16-CH_3/NCH_3 exhibited *S* configuration of C16.

16-Methyloxazolomycin (**1**) showed antibacterial and antialgal activities against *Bacillus subtilis* 1069 (MIC, 5.0 μg/ml) and *Chlorella vulgaris* IFO 15941 (MIC, 10 μg/ml), respectively and cytotoxicities (IC_{50}, 0.23 μg/ml against P388 leukemia cells; 4.6 μg/ml against A-549 human lung adenocarcinoma cells).

Fig. 1. Partial correlations obtained from NOESY experiment for **1**.



References

1) MORI, T.; K. TAKAHASHI, M. KASHIWABARA, D. UEMURA, C. KATAYAMA, S. IWADARE, Y. SHIZURI, R. MITOMO, F. NAKANO & A. MATSUZAKI: Structure of oxazolomycin, a novel β-lactone antibiotic. Tetrahedron Lett. 26: 1073~1076, 1985
2) TAKAHASHI, K.; M. KAWABARA, D. UEMURA, S. WADARE, R. MITOMO, F. NAKANO & A. MATSUZAKI: Structure of neooxazolomycin, an antitumor antibiotic. Tetrahedron Lett. 26: 1077~1078, 1985
3) OGURA, M.; H. NAKAYAMA, K. FURIHATA, H. SETO & N. OTAKE: Structure of a new antibiotic curromycin A produced by a genetically modified strain of *Streptomyces hygroscopicus*, a polyether antibiotic producing organism. J. Antibiotics 38: 669~673, 1985
4) OGURA, M.; H. NAKAYAMA, K. FURIHATA, A. SHIMAZU, H. SETO & N. OTAKE: Isolation and structural determination of a new antibiotic curromycin B. Agric. Biol. Chem. 49: 1909~1910, 1985
5) KUPCHAN, S. M.; R. W. BRITTON, M. F. ZIEGLER & C. W. SIGEL: Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica*. J. Org. Chem. 38: 178~179, 1973
6) BAX, A.; A. AZOLOS, Z. DINYA & K. SUDO: Structure elucidation of the antibiotic desertomycin through the use of new two-dimensional NMR techniques. J. Am. Chem. Soc. 108: 8056~8063, 1986
7) BODENHAUSEN, G.; H. KOGLER & R. R. ERNST: Selection of coherence-transfer pathways in NMR pulse experiments. J. Magn. Reson. 58: 370~388, 1984