ALDGAMYCIN G, A NEW MACROLIDE ANTIBIOTIC

Sir:

During the course of screening for new antimicrobial antibiotics the cultured broth of the microorganism No. 1117 showed a marked antimicrobial activity against Gram-positive bacteria and was found to contain a new macrolide antibiotic, which we named aldgamycin G (**1**), together with aldgamycin F (**2**)[1,2]. In this communication, the isolation and characterization of aldgamycin G and F are reported.

Strain No. 1117 was isolated from a soil sample collected in Ikeda, Hokkaido, Japan. On the basis of taxonomic studies, it was identified as a strain of *Streptomyces avidinii*.

This organism was cultured at 27°C, for 48 hours in a 300-liter fermentation tank containing 150 liters of medium, composed of glycerol 3.0%, corn steep liquor 1.0%, dry yeast 0.3%, NaCl 0.5% and $CaCO_3$ 0.35% (pH 7.2).

The culture filtrate (150 liters) was applied to a column of Diaion HP-20. The column was washed successively with water, 80% methanol, and then the active material was eluted with

Table 1. Physico-chemical properties of aldgamycin G (**1**) and F (**2**).

	1	**2**
Appearance	Colorless crystals	Colorless crystals
MP (°C)	145.5~148.5	—
MW (ED-MS) (*m/z*)	740	756
$[\alpha]_D^{27}$	−29 (*c* 1.09, $CHCl_3$)	−24.1 (*c* 1.0, $CHCl_3$)
IR (KBr) λ_{max}	3580, 1800, 1705, 1650, 1630	3450, 1795, 1710, 1630
UV λ_{max}^{MeOH} nm (ε)	218 (22,500), 241 (12,300)	218 (21,100), 240 (12,400)
Rf $CHCl_3$ - MeOH (9:1)*	0.67	0.665
EtOAc	0.71	0.705
Solubility		
Soluble	MeOH, EtOH, $CHCl_3$, EtOAc, benzene	
Insoluble	Hexane, H_2O	

* TLC: Merck 5731.

Table 2. ^{13}C NMR chemical shifts for aldgamycin G (**1**), F (**2**) and chalcomycin.

Carbon No.	**1**	**2**	Chalcomycin	Carbon No.	**1**	**2**	Chalcomycin
C-1	165.4	165.5	164.7	Aldgarose			Chalcose
C-2	121.0	121.1	120.3	C-1′	101.1	101.1	102.9
C-3	150.7	151.2	151.0	C-2′	71.8	71.6	74.8
C-4	41.6	41.5	41.5	C-3′	84.8	85.0	80.3
C-5	86.7	87.3	87.5	C-4′	41.3	41.2	36.7
C-6	34.0	36.9	36.7	C-5′	67.2	67.0 (b)	67.5 (b)
C-7	32.1	33.9	33.9	C-6′	13.6	13.6	20.8
C-8	44.6	78.4	78.3	C-7′	81.4	81.5	C-3′OCH_3 56.8
C-9	200.9	200.2	199.4	C-8′	154.1	154.3	
C-10	125.7	124.9	124.5	C-5′CH_3	20.5	20.5	
C-11	144.7	147.0	145.8	Mycinose			
C-12	59.1	59.1 (a)	59.4 (a)	C-1″	101.2	101.7	100.5
C-13	59.1	59.7 (a)	58.8 (a)	C-2″	82.1	81.5	81.6
C-14	49.6	49.6	49.4	C-3″	79.8	79.7	79.4
C-15	68.9	68.9 (b)	68.5 (b)	C-4″	72.8	72.8	72.5
C-16	18.4	18.3 (c)	18.3 (c)	C-5″	70.8	70.8	70.4
C-17	16.9	18.9 (c)	18.5 (c)	C-6″	17.8	17.8	17.7
C-19	17.6	27.9	27.7	C-2″OCH_3	59.7 (a)	58.8	58.5
C-22	19.1	19.2 (c)	19.1 (c)	C-3″OCH_3	61.7 (a)	61.8	61.4
C-24	67.2	67.0	66.7				

(a)~(c): Assignment within any vertical column may be reversed.

Fig. 1. Aldgamycins G (**1**) and F (**2**).

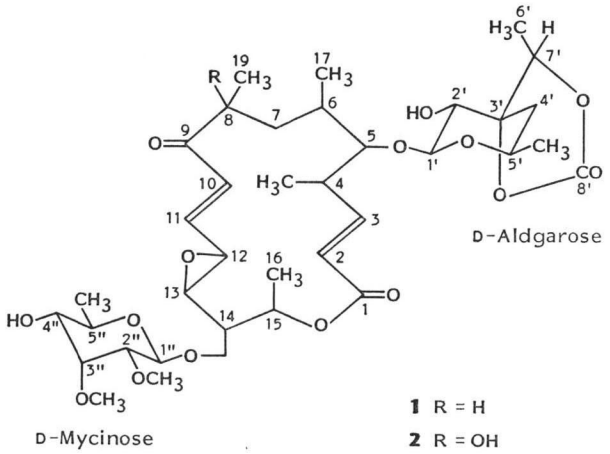

Table 3. Antimicrobial activity of aldgamycin G (**1**), F (**2**) and josamycin (JM) (MIC, μg/ml).

Organisms	Compounds		
	1	**2**	JM
Gram-positive bacteria			
Staphylococcus aureus FDA 209P	1.56	1.56	0.2
S. aureus Smith*	0.39	3.12	0.78
S. aureus MS 15009**	>100	>100	>100
S. aureus MS 353	1.56	6.25	1.56
Streptococcus pyogenes 1099*	1.56	12.5	0.78
S. pyogenes DP type 2*	100	>100	0.78
Bacillus subtilis ATCC 6633	1.56	6.25	0.39
B. cereus IAM 1729	0.1	0.39	0.78
Micrococcus luteus ATCC 9341	0.2	0.39	0.1
Gram-negative bacteria			
Escherichia coli NIHJ-JC2	>100	>100	>100
Klebsiella pneumoniae PCI-602	>100	>100	>100
Salmonella typhimurium IID 971	>100	>100	>100
Serratia marcescens IAM 1184	>100	>100	>100
Pseudomonas aeruginosa NCTC	>100	>100	>100

* Clinically isolated strains. ** Macrolide resistant strains.

MICs were determined by the agar dilution method using Mueller - Hinton agar (Difco) after incubation at 37°C for 20 hours with an inoculum size of about 10^6 cfu/ml.

methanol. The dried residue (13 g) was dissolved in a small amount of chloroform and subjected to silica gel column chromatography. After washing with chloroform, the two active fractions were eluted with chloroform - methanol (9: 1) and each fraction was concentrated into a small volume *in vacuo*. Then each fraction was passed through a column of Toyoparl HW 40F with methanol. The purified active fractions were collected and concentrated *in vacuo* to give oily solids which were crystallized from chloroform-hexane to give aldgamycin G (**1**, 77 mg) and aldgamycin F (**2**, 43 mg), respectively in pure form. Physico-chemical properties of **1** and **2** are shown in Table 1.

The ^{13}C NMR spectra of **1** and **2** are shown in Table 2. Chemical shift assignments for **2** were based on the reported data of chalcomycin[3], and those for **1** were based on the spectra of **2** and COSY and NOESY spectra of **1**. As shown in Table 2, chemical shifts of all carbon signals except the C-8 and C-19 signals for **1** were in agreement with those of **2**. The C-8 and C-19 carbons of **2** appeared at 78.4 and

Table 4. Chemotherapeutic activity of aldgamycin G (**1**), josamycin (JM) and erythromycin (EM).

Microorganisms	Compounds	Challenge dose (cells/mouse)	ED_{50} (mg/mouse)	MIC (μg/ml)
Staphylococcus aureus Smith	**1**	2.0×10^5	0.6	0.39
	JM	2.0×10^5	1.2	0.78
	EM	2.0×10^5	1.0	0.39
Streptococcus pyogenes	**1**	8.0×10^5	>10	1.56
	JM	8.0×10^5	5.0	0.78
	EM	8.0×10^5	2.1	0.78
S. pyogenes DP type 2	**1**	2.6×10^5	>10	>100
	JM	2.6×10^5	6.0	0.78
	EM	2.6×10^5	NT	0.39

Mice (ICR, 4w, ♂, 20±1 g, 6 animals/group) were infected intraperitoneally with three kinds of microorganisms (mentioned in Table 4) in 5% mucin. Compounds suspended in 1% Tween 80 were administered orally immediately post infection. ED_{50} were determined by the Behrens-Karber method according to the mortality of mice at 7 days after infection.

LD_{50} of **1**: >1,000 mg/kg (ip).

NT: Not tested.

27.9 ppm, respectively, whereas the C-8 and C-19 carbons of **1** appeared at 44.6 and 17.6 ppm, respectively, that is, the C-8 and C-19 carbons of **1** show approximately 35 and 10 ppm upfield shift respectively. This suggested that the OH-group at C-8 of **1** is replaced by hydrogen atom, that is, **1** is a 8-dehydroxy derivative of **2**. This was confirmed by mass spectroscopy, *m/z* 740 (M^+), and ^1H NMR spectroscopy in $CDCl_3$; 2.60 (H-8, m), 1.14 (H-19, d, J=6.6 Hz). Irradiation at 1.14 ppm changed 2.60 ppm signal into a double doublet (J=12.5 and 4.0 Hz). Thus the structure of **1** is shown by Fig. 1.

Table 3 shows the antimicrobial activity of **1** and **2** as determined by the agar dilution method in comparison with josamycin. **1** exhibited antibacterial activity against Gram-positive bacteria, and this activity is higher than that of **2**. This result indicates that the 8-OH group of **2** is unfavorable to its antimicrobial activity. Moreover, **1** was as active as, or more active than josamycin against *Staphylococcus aureus* MS 353, *Bacillus cereus* IAM 1729 and *Staphylococcus aureus* Smith.

In vivo antimicrobial activity of **1** is shown in Table 4. **1** showed a good therapeutic effect in mice infected with *S. aureus* Smith, which was the same in extent as josamycin and erythromycin, but less effective in mice infected with other clinically isolated strains.

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