Notes

IZENAMICINS: MACROLIDE ANTIBIOTICS

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In the course of screening for new antibiotics produced by *Micromonospora*, we discovered a series of 16-membered macrolide antibiotics, designated as izenamicins, produced by strain YS-02930K. The antibiotics were found to consist of at least seven components designated izenamicins A_1, A_2, A_3, B_1, B_2, B_3 and B_4. From the results of structure determination, izenamicins A_3, B_2 and B_3 are new natural products although they have been prepared previously by chemical transformations of tylosin[1~4]. This paper deals with the taxonomy of the producing strain, fermentation and purification of izenamicins.

Strain YS-02930K was isolated from a soil sample collected at Izena island, Okinawa Prefecture, Japan. The strain grew well on most media described by SHIRLING and GOTTLIEB[5]. Vegetative mycelia, measuring 0.5 to 0.8 μm in diameter, were branched well but no septum was observed. Aerial mycelia were not formed. A single spore was formed at a tip of the sporophore branching from the vegetative mycelium. The shape of the spore was spherical or ellipsoidal (0.8~1.2 μm in diameter). The vegetative mycelia were yellow orange to orange, becoming brown or blackish brown to black with a moistened to glossy surface. Whole cell analysis by hte method of LECHEVALIER[6] indicated the presence of hydroxy- and *meso*-diaminopimelic acid along with arabinose and xylose as characteristic sugars. Based on morphological and chemical characteristics, the strain was identified as a *Micromonospora* sp. YS-02930K. A culture of the strain has been deposited in the Fermentation Research Institute, Agency of Industrial Science and Technology, Japan, and assigned as *Micromonospora* sp. YS-02930K with the accession No. FERM P-7961.

A stock culture of *Micromonospora* sp. YS-02930K was inoculated into 60 ml of a seed medium in 500-ml Erlenmeyer flasks and incubated at 28°C on a rotary shaker. A 72-hour culture (0.75 liter) was transferred to 25 liters of a production medium in a 30-liter fermentor at 28°C for 72 hours with aeration of 25 liters per minute and agitation of 150 rpm. The composition of the seed medium was white-dextrin 2.0%, glucose 0.5%, Polypepton 0.5%, yeast extract 0.5%, corn steep liquor 0.5%, meat extract 0.3%, brain heart infusion 0.52% and $CaCO_3$ 0.2% (pH 8.0 before sterilization). The production medium consisted of potato starch 3.0%, soybean meal 1.5%, corn steep liquor 0.5%, yeast extract 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.3% and $CoCl_2 \cdot 6H_2O$ 0.002% (pH 7.1 before sterilization).

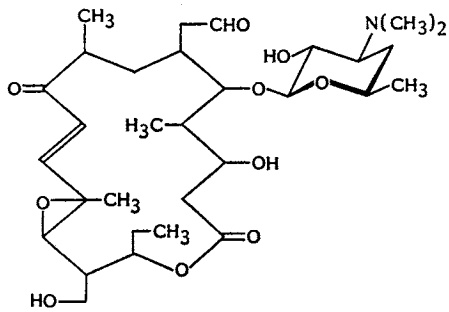
The broth filtrate (20 liters) was adjusted to pH 9 and extracted with EtOAc. The organic layer was extracted with a dilute hydrochloric acid solution (pH 3) and the aqueous layer was reextracted with EtOAc at pH 9. The organic

Table 1. HPLC behavior of izenamicins (IZM).

Antibiotics	Retention times (minutes)
IZM A_1	10.0
IZM A_2	7.3
IZM A_3	5.1
IZM B_1	8.6*
IZM B_2	7.4*
IZM B_3	5.3*
IZM B_4	4.6*

Column: YMC A-202, mobile phase: THF - CH_3CN - H_2O - 0.1 M phosphate buffer pH 5.0 (10 : 40 : 50 : 0.5), flow rate: 1 ml/minute, column temp: 33°C, detection: UV nm 240, 280*.

Fig. 1. Structures of izenamicins.



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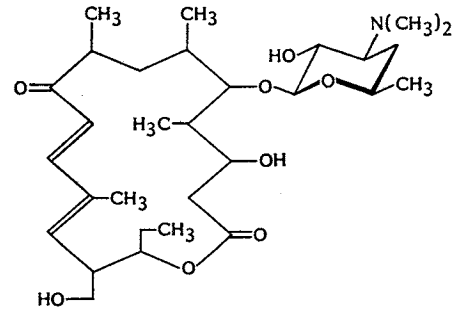

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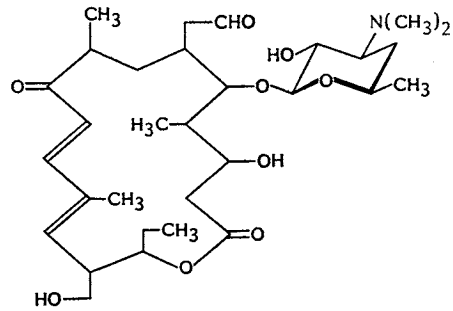

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Table 2. Antimicrobial spectra of izenamicins (IZM) A_3, B_3 and mycaminosyl tylonolide (MT).

Test organisms	MICs (μg/ml)		
	IZM A_3	IZM B_3	MT
Bacillus subtilis ATCC 6633	1.56	3.13	3.13
Staphylococcus aureus ATCC 6538P	1.56	0.78	1.56
S. aureus Smith	0.78	0.78	1.56
S. epidermidis IID 866	0.78	0.39	0.78
Streptococcus pyogenes Cook	0.39	0.39	0.2
Enterococcus faecalis IID 682	1.56	0.78	0.78
Escherichia coli NIHJ	25	6.25	12.5
E. coli Ebara	6.25	3.13	50
Klebsiella pneumoniae ATCC 10031	3.13	1.56	1.56
Shigella boydii IID 627	12.5	6.25	25
Salmonella enteritidis 1891	3.13	6.25	1.56
Proteus vulgaris OXK US	>100	50	100
Pseudomonas aeruginosa NCTC 10490	>100	50	50

Inoculum size: 10^6 cfu/ml, medium: mueller-Hinton agar.

layer was concentrated *in vacuo* to afford a crude powder of izenamicins (1.2 g). The initial EtOAc extract on concentration gave crude everninomicins[7]. The crude izenamicin complex was dissolved in a small amount of $CHCl_3$ and chromatographed on a column of silica gel using a solvent system of $CHCl_3$ - MeOH - 28% NH_4OH, 50:1:0.1. Each eluate was monitored by TLC on Silica gel 60 F_{254} plates with a mixture of $CHCl_3$ - MeOH - 28% NH_4OH, 160:40:0.5 and HPLC system (Table 1). Thus, seven components could be obtained respectively

as a white amorphous powder.

From the results of physico-chemical analysis and NMR studies, izenamicins A_3(**1**), B_2(**2**) and B_3(**3**) were found to be antibiotics belonging to the basic 16-membered macrolide antibiotic group (structures shown in Fig. 1). Synthesis of 12,13-epoxy-4′-deoxy mycaminosyl tylonolide (izenamicin A_3), 19-decarbonyl-4′-deoxy mycaminosyl tylonolide (izenamicin B_2) and 4′-deoxy mycaminosyl tylonolide (izenamicin B_3) from tylosin have been reported. Direct comparison of physico-chemical properties and NMR spectra indicated that izenamicins A_3, B_2, B_3 and authentic antibiotics (Institute of Bio-organic Chemistry, Japan) are identical in all respects. Izenamicins A_1, A_2, B_1 and B_4 were found to be identical with rosamicin[8], juvenimicin A_4[9], juvenimicin B_1 and juvenimicin B_3, respectively.

Among the izenamicin components, izenamicin B_3 showed the highest antimicrobial activity against Gram-positive and Gram-negative bacteria (Table 2). An approximate ED_{50} of 89 mg/kg was observed when izenamicin B_3 was administered orally to fasting mice challenged with *Staphylococcus aureus* Smith. The antibiotic injected intravenously into mice showed an approximate LD_{50} value of 248 mg/kg.

We consider that izenamicin B_3 (4′-deoxy mycaminosyl tylonolide) is an important intermediate for the synthesis of clinically useful 16-membered macrolide antibiotics. To our knowledge, this is the first report that izenamicins A_3, B_2 and B_3 are produced by a microorganism.

Acknowledgments

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