THE PRODUCER AND BIOLOGICAL ACTIVITIES OF SO-075R1, A NEW MUTACTIMYCIN GROUP ANTIBIOTIC

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The producer of SO-075R1, a new anthracycline group antibiotic was identified as *Nocardia brasiliensis*. SO-075R1 was active against Gram-positive bacteria, but not active against Gram-negative bacteria or fungi. All tested *Nocardia brasiliensis* strains as well as the producer itself were resistant to SO-075R1, although four other pathogenic *Nocardia*, i.e. *N. asteroides*, *N. nova*, *N. farcinica* and *N. otitidiscaviarum* were sensitive.

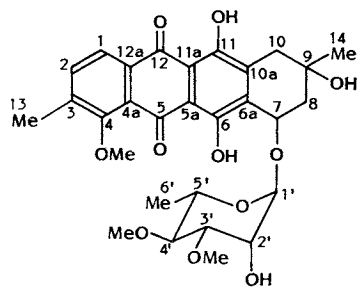
During our ongoing studies on bioactive substances from pathogenic microorganisms, we isolated a new anthracycline antibiotic (SO-075R1)[1] from IFM 075 strain which has been tentatively identified as *Nocardia* sp. As shown in Fig. 1, SO-075R1 is closely related to mutactimycin A produced by *Streptomyces* sp[2]. It was active against Gram-positive bacteria but not active against Gram-negative bacteria or fungi. In this paper, we report identification of the producing organism and biological activity of SO-075R1.

Materials and Methods

IFM 075 strain is a clinical isolate which is maintained in our laboratory. Determinations of diaminopimelic acid profile and cell wall sugar patterns, and physiological and biochemical characterization of the strain were done by method previously described[3]. Mycolic acids and menaquinone were analyzed using the procedure of SCHAAL[4]. SO-075R1 was prepared in our laboratory by the method described[1] earlier. The minimum inhibitory concentration (MIC), defined as the lowest drug concentration resulting in complete inhibition of visible growth, was determined by agar dilution method[5]. Sensitivity disk agar (STA, Eiken Chemical Co., Ltd., Tokyo, Japan) for bacteria and yeast morphology agar (Difco) for fungi were used.

Cell suspensions for inoculation were prepared by the methods described previously[5,6] and then diluted with sterile saline to give 10^7 colony forming units (cfu)/ml. Agar plates were spotted with a multipoint inoculator A400 (Denley Instruments, Ltd., Sussex, England) that delivered 0.005 ml of the diluted inoculum. *In vitro* antitumor activities were determined using a cultured cell line of L1210 mouse leukemia[7] and Vero cells[1]. Male ddY mice (Shizuoka Cooperative Society of Experimental Animals, Hamamatsu, Japan) were given a single intraperitoneal injection of SO-075R1 solution and

Fig. 1. Structure of SO-075R1.



LD_{50} values against the mice were determined.

Results and Discussion

Identification Studies

The substrate mycelium of IFM 075 strain was rudimentarily branched and fragmented into bacillary elements on most tested media such as Sabouraud Dextrose agar and brain heart infusion agar. In the culture primitive aerial mycelia similar to those of *Nocardia* were confirmed, however, any characteristic morphological features for *Streptomyces*, *Micromonospora* or *Actinoplanes* was not observed. An analysis of cell wall hydrolysates of IFM 075 strain revealed the presence of *meso*-diaminopimelic acid, and arabinose and galactose as major sugar constituents[8]. Therefore, the cell wall type was considered to belong to chemotype IV using the classification system of LECHEVALIER and LECHEVALIER[8]. MK-8 (H_4, cyclic) was detected as the major manaquinone (89.3%). Analyses of the strain by the method of SCHAAL[4] showed the presence of *Nocardia* or *Rhodococcus* type mycolic acid on TLC plate. On the basis of this typical nocardioform morphology with cell wall type 1V, coupled with the chemotypes of mycolic acids and menaquinone, IFM 075 strain was determined to be a member of the genus *Nocardia*. In BERGEY's Manual of Systematic Bacteriology, Vol. 4, 1989, nine species are described and they are differentiated primarily by physiological characteristics[8]. In 1988, KUDO *et al.* officially proposed a new species, *N. seriolae* as a pathogenic organism for cultured fish[9] and *N. nova* was also confirmed to be a species of *Nocardia* by YANO *et al.* in 1990[10].

A comparison of the physiological characteristics of IFM 075 strain (Table 1) with those of 11 species of *Nocardia* revealed that IFM 075 strain is closely related to those of *N. brasiliensis*. The differences observed in the physiological and biochemical characteristics between IFM 075 strain and

Table 1. Physiological characteristics of IFM 075 strain.

Characteristics	IFM 075	*N. brasiliensis*[a]	Characteristics	IFM 075	*N. brasiliensis*[a]
Acid fastness	±[b]	±	mannose	−	V[c]
Decomposition of			rhamnose	−	−
adenine	+	−	sorbitol	−	−
casein	+	+	Utilization of		
hypoxanthine	+	+	citrate	+	+
tyrosine	+	+	Growth at		
xanthine	−	−	45°C	−	−
Acid from			Production of		
adonitol	−	−	β-lactamase	+	+[d]
arabinose	−	−	Sensitivity to[e]		
erythritol	−		imipenem	+	−[d]
galactose	+	+	tobramycin	+	+[d]
glucose	+	+	5-fluorouracil	−	−[d]
inositol	+	+	kanamycin	+	+[d]
maltose	−	−			

[a] Data were obtained from ref 8.
[b] Partially acid fastness.
[c] Variable results.
[d] Tested in our laboratory.
[e] Sensitivity was determined by the method of ref 11.

each description are decomposition of adenine and sensitivity to the carbapenem antibiotic imipenem. We had reported that pathogenic *Nocardia* shows species-specific drug sensitivity patterns[11], and had especially indicated that imipenem was a good choice to differentiate the *N. asteroides* group from *N. brasiliensis* and *N. otitidiscaviarum*. According to the definition, the *N. brasiliensis* strain should be resistant to imipenem; however, the IFM 075 strain was different from other *N. brasiliensis* and sensitive to imipenem. Interestingly, our recent studies also indicated that *N. brasiliensis* SF 2457 which has been reported as a new amicetin group antibiotic producer by MIYADOH *et al.*[12], was also imipenem-sensitive (unpublished data). Therefore, there may be some correlation between imipenem-

Table 2. Antimicrobial activity of SO-075RI.

Microorganisms	MIC (μg/ml)
Micrococcus luteus IFM 2066	≦0.2
Staphylococcus aureus 209P IFM 2014	3.13
S. albus IFM 2013	50.0
S. citreus IFM 2075	1.56
Bacillus cereus IFM 5058	0.78
B. subtilis PCI 219	6.25
Corynebacterium xerosis IFM 2075	0.78
Mycobacterium sp. 607	3.13
Escherichia coli NIHJ JC2	>100.0
Klebsiella pneumoniae IFM 3008	6.25
Pseudomonas aeruginosa IFM 1045	>100.0
Aspergillus niger IFM 40606	>100.0
Penicillium chrysogenum Q176	>100.0
Candida albicans 1001	>100.0
Cryptococcus neoformans IFM 40038	>100.0

MICs were determined by the agar dilution method using nutrient agar and Sabouraud Dextrose agar for bacteria and fungi, respectively.

Table 3. Antimicrobial activity of SO-075R1 against five species of pathogenic *Nocardia*.

Microorganisms	Strain No. (IFM No.)	Drugs (MIC (μg/ml))	
		SO-075R1	Daunomycin
Nocardia asteroides	0280	12.5	25
	0229	6.25	12.5
	0319*	12.5	12.5
	0342	6.25	3.13
	0349	6.25	3.13
Nocardia farcinica	0275	6.25	3.13
	0284*	12.5	12.5
	0294	12.5	25
	0320	12.5	12.5
	0348	6.25	3.13
Nocardia nova	0253	6.25	1.56
	0274	6.25	1.56
	0290*	6.25	0.78
	0279	6.25	1.56
	0356	12.5	6.25
Nocardia brasiliensis	0234	>100	25
	0236*	>100	25
	0256	100	25
	0279	100	3.13
	0281	100	12.5
	075[a]	>100	6.25
Nocardia otitidiscaviarum	0239*	6.25	6.25
	0273	6.25	6.25
	0301	6.25	25
	0337	6.25	25
	0354	6.25	6.25
	0362	6.25	25

MIC was determined by the agar dilution method using Mueller-Hinton agar.
* Denotes type strain of each species.
[a] Producer of SO-075RI.

sensitivity and the antibiotic production of *N. brasiliensis*. Although the inability to decompose adenine is another exceptional observation with the present strain, this was considered to occur at the strain level, and we finally identified the strain as *N. brasiliensis* (Lindenberg) Pinoy. The following antibiotics have been reported to date to be produced by *N. brasiliensis*; an amicetin group antibiotic[12], cyclodepsipeptides and siderochromes[13]. This is thus the first report that *N. brasiliensis* produces an anthracycline group antibiotic.

Biological Activities

As shown in Table 2, SO-075R1 was active against Gram-positive bacteria and most species were inhibited at concentrations between 0.2 and 6.25 μg/ml. *Micrococcus luteus* was the most sensitive and *Staphylococcus albus* IFM 2013 was relatively resistant. Gram-negative bacteria except for *Klebsiella pneumoniae* and fungi were resistant. Since SO-075R1 is produced by *N. brasiliensis*, we were interested in its antinocardial activity, and the results are shown in Table 2 in comparison with those of a reference antibiotic, daunomycin. Among the five tested species of pathogenic *Nocardia*, *N. asteroides*, *N. farcinica*, *N. nova* and *N. otitidiscaviarum* were sensitive to SO-075R1 and inhibited at concentrations ranging from 6.25 to 12.5 μg/ml. The remaining *N. brasiliensis* (6 strains) were resistant and their mean MIC values against SO-075R1 were more than 100 μg/ml. On the other hand, all *Nocardia* species were sensitive to daunomycin[14] and inhibited at concentrations from 3.13 to 25.0 μg/ml. Detailed structure activity relationship among these anthracycline antibiotics against pathogenic *Nocardia* are of interest. Furthermore, SO-075R1 can be used for the selective isolation of *N. brasiliensis* from a clinical specimen, since clinical specimens are frequently contaminated with unfavorable Gram-positive bacteria.

Cytotoxic activity of SO-075R1 against L1210 cultured cells and Vero cells was studied, and the ED_{50} values were 7.4 μg/ml and 50.0 μg/ml, respectively. The LD_{50} value of SO-075R1 for ddY mice by single intraperitoneal injection was above 130 mg/kg, which is considerably higher than those of adriamycin and daunomycin[14]. Originally, mutactimycin A was reported to be an anthracycline antibiotic with antiviral activity[2]. Our preliminary *in vitro* studies confirmed such antiviral activity against herpes simplex virus (HSV). Although antiviral activities of anthracycline group antibiotics have been reported[14], further detailed experiments on the antiviral activity of SO-075R1 are of interest, because of its relatively lower toxicity.

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