PS-5, A NEW β-LACTAM ANTIBIOTIC FROM *STREPTOMYCES*

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(Received for publication February 27, 1978)

During the course of screening for antibiotics, *Streptomyces* sp. A271 ATCC 31358 was isolated from soil collected near Eiheiji Temple in the Yoshida District of Fukui Prefecture and was found to produce a new β-lactam antibiotic which we designate as PS-5. The antibiotic was found to have good inhibitory activity against a variety of Gram-positive and Gram-negative organisms and also inhibited β-lactamase produced by various bacteria.

In the note we wish to report the fermentation, isolation and structure of PS-5.

One ml of a spore suspension of *Streptomyces* sp. A271 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium (SE-4) having the following composition: beef extract, 0.3%; Bacto-tryptone, 0.5%; defatted soybean meal, 0.5%; glucose, 0.1%; soluble starch, 2.4%; yeast extract, 0.5% and $CaCO_3$, 0.4%. The pH was adjusted to 7.5 prior to sterilization. After 48 hours the growth of the seed flask was used to inoculate a 30-liter seed jar fermentor containing 15 liters of the same medium (SE-4). The jar fermentor was operated at 28°C using an agitation rate of 200 rpm and an air flow of 7.5 liters per minute for 24 hours. One liter of the growth in this seed culture was used to inoculate a 200-liter stainless steel fermentor containing 100 liters of production medium having the following composition: glycerol, 4.0%; peptone, 0.5%; glucose, 0.2%; potato starch, 0.2%; defatted soybean meal, 0.5%; dry yeast, 0.5%; NaCl, 0.5% and $CaCO_3$, 0.2% (pH 6.4). This fermentor was operated at 28°C using an agitation rate of 100 rpm and an air flow of 50 liters per minute for 72 hours.

The production of PS-5 was followed using an agar diffusion assay with *Comamonas terrigena* IFO 12685 as the assay organism. In the production medium the strain produced about 10 μg/ml of PS-5 in the culture filtrate.

For the isolation of the antibiotic the culture filtrate was passed through a Diaion PA306 column. The effluent was adsorbed on a Diaion HP-20 column and then eluted with 50% acetone. The active fractions were subjected to successive column chromatography using QAE-Sephadex A-25, Diaion HP-20 and Sephadex G-10. Finally, a colorless powder of the sodium salt of PS-5 was obtained by lyophilization of the active fractions from the last column. It had a purity of approximately 95%.

The physico-chemical properties of the purified PS-5 sodium salt are as follows: PS-5 sodium salt is highly soluble in water and substantially insoluble in ethyl acetate, acetone and benzene. Other characteristics are: Color, white; turns yellow around 148°C and gradually decomposes above 160°C; UV absorption, $\lambda_{max}^{H_2O}$ 301 nm, $E_{1cm}^{1\%}$ 267.5; PMR spectrum (100 MHz, D_2O), 1.06 (3H, t, $C\underline{H}_3$–CH_2–), 1.72~2.00 (2H, CH_3–$C\underline{H}_2$–), 2.05 (3H, s, CH_3–CO–), 2.88~3.58 (7H, –CH_2–, –$\underset{|}{C}H$–) and 3.9~4.20 ppm (1H, –$\underset{|}{\underset{N}{C}}H$–); $[\alpha]_D^{22}$ +1.23 (*c* 1.59, 0.01 M pH 8 sodium phosphate buffer); IR spectrum (KBr), characteristic absorptions attributable to β-lactam, amide and carboxylate (Fig. 1); Mass spectrum, 312.1131 (M^+ of the methyl ester), (calculated for $C_{14}H_{20}N_2O_4S$, 312.1143). Chromatographic characteristics are as follows: Thin-layer chromatography, Avicel/SF cellulose (*n*-butanol - ethanol - water; 7: 7: 6), Rf 0.94; Silica gel F_{254} (ethanol - water; 7: 3), Rf 0.82; Descending paper chromatography (acetonitrile - 0.1 M, pH 7.5, tris buffer - 0.1 M, pH 7.5, EDTA; 120: 30: 1), Rf 0.34; High voltage paper electrophoresis. PS-5 sodium salt moved 28 mm to the anode at a potential 42 V/cm in a veronal buffer (pH 8.6, I=0.05).

Interpretations of the above-mentioned physico-chemical properties and its chemical behaviors lead to the conclusion that PS-5 is a new β-lactam antibiotic having the 1-carbapenem nucleus seen in thienamycin.[4] The structure of PS-5 is shown in Fig. 2. A detailed account of the structure elucidation will be presented in a separate paper.

Fig. 1. 1R spectrum of PS-5 sodium salt.

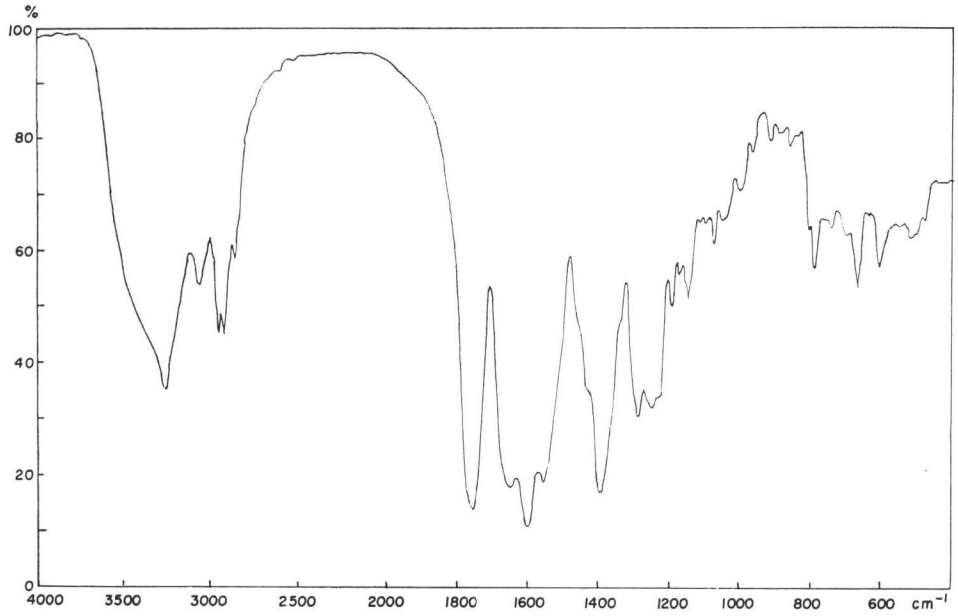

Fig. 2. Structure of PS-5.

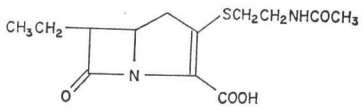

Table 1. Antimicrobial activity of PS-5 sodium salt.

Microorganisms	MIC (μg/ml)	
	PS-5 sodium salt	Cefoxitin
Staphylococcus aureus FDA 209P	0.16	1.25
Diplococcus pneumoniae Type III	0.02	1.25
Citrobacter freundii E-9*	3.13	>100
Enterobacter cloacae E-16*	12.5	>100
Klebsiella pneumoniae K-2*	3.13	6.25

Tests were conducted in Brain Heart Infusion broth (Eiken Chem. Co., Ltd.) inoculated with 10^5 cells per ml.

* β-Lactamase producing organism.

The minimum inhibitory concentration (MIC) of PS-5 against various microorganisms were determined using the broth dilution method. The results are shown in Table 1. When low levels of PS-5 were added to ampicillin or cephaloridine, the MIC values of the antibiotics against β-lactamase producing *Proteus vulgaris* P-5 were substantially lowered. The results are shown in Table 2.

Table 2. Enhancement of antimicrobial activity against the β-lactamase producing strain, *Proteus vulgaris* P-5.

Antibiotic	PS-5 sodium salt (2.5 μg/ml)	*Proteus vulgaris* P-5 MIC (μg/ml)
PS-5 alone		12.5
Ampicillin	−	1,250
Ampicillin	+	1.2
Cephaloridine	−	2,500
Cephaloridine	+	2.4

PS-5 is distinguishable from clavulanic acid[1], thienamycin[2~4], N-acetylthienamycin[5,6], epi-thienamycins[5,6], MM4550[7] (MC696-SY-A[8]), MM13902[7] and MM17880[9] on the basis of its physico-chemical properties, chromatographic behavior and antimicrobial activities.

Acknowledgement

The authors are greatly indebted to Prof. Y. YAMADA, Tokyo College of Pharmacy, for his helpful advice.

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