Communication to the editor

A NEW ANTITUMOR ANTIBIOTIC, PO-357*

Sir:

Recently we isolated a new antitumor antibiotic, PO-357, from the culture filtrate of a strain of *Streptosporangium*, strain No. PO-357. The present paper describes the production, isolation and preliminary characterizations of this antibiotic.

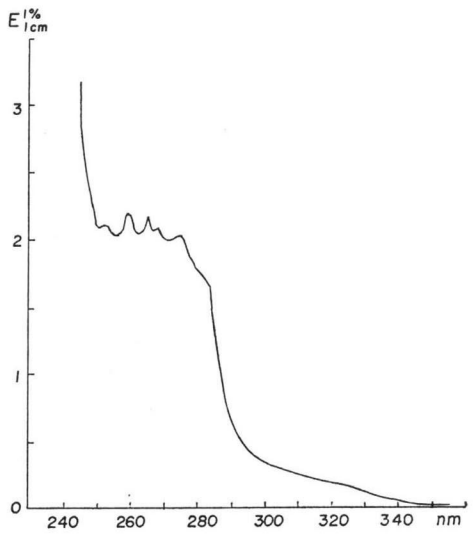
The taxonomic characteristics of strain No. PO-357 were very similar to those of *Streptosporangium pseudovulgare*. For example, aerial mycelium on oatmeal agar was coral red; the sizes of spores and sporangia were 0.9~1.4 μ and 5~10 μ, respectively; gelatin was liquified, starch was hydrolyzed, nitrate was reduced to nitrite; it grew at 43°C, no melanoid pigment was produced. *meso*-Diaminopimelic acid was found in the cell wall and madurose in whole cells; galactose and arabinose were not detected.

Antibiotic PO-357 was produced at 27°C for 50 hours in a jar fermentor. The fermentation medium contained 2.0% glucose, 0.5% peptone, 0.3% dried yeast, 0.5% meat extract, 0.5% NaCl and 0.3% $CaCO_3$. To the culture filtrate (36 liters) was added 25 kg of ammonium sulfate and the mixture was allowed to stand overnight at 5°C. The precipitate containing PO-357 was collected by centrifuging and dissolved in ice-cold distilled water. The resulting solution in a cellophane tube was dialysed against ice-cold water for 3 days. The non-dialysable solution was subjected to DEAE cellulose column chromatography. The antibiotic was eluted with a linear gradient of 0~1.0 M sodium chloride in 0.002 M phosphate buffer at pH 7.4. Active fractions were precipitated with ammonium sulfate, and the collected precipitate was dissolved in water. The solution was placed in a cellophane tube and dialysed against ice-cold water for 3 days. The non-dialysable solution was again passed through a DEAE cellulose column. The antibiotic was eluted with the same linear gradient described above except that the pH of the solution was adjusted to 5.4. Active substance was precipitated with ammonium sulfate and was dialysed for 2 days. After lyophilization, it was applied to a Sephadex G-75 column and eluted with water; the eluant was lyophilized to yield 530 mg of a white powder.

The UV spectrum of PO-357 (Fig. 1) exhibited absorption maxima [nm ($E^{1\%}_{1cm}$)] at 252 (2.16), 258 (2.22), 265 (2.19), 267 (2.09), 275 (2.05) and 285 nm (sh.) in neutral and acidic water, and 292 (2.76) in alkaline water. Fig. 2 shows the IR spectrum in KBr. PO-357 became brown at 220~225°C but had not melted at 280°C. It was optically active, $[\alpha]_D^{20}-56°$ (c 0.5, H_2O), and soluble in water but insoluble in organic solvents such as methanol, acetone *etc*. It gave positive reactions with FOLIN-LOWRY, EHRLICH and biuret reagents. The ninhydrin reaction was weakly positive, but the MOLISCH, ferric chloride, anthrone, FEHLING and TOLLENS reactions were negative. As shown in Plate 1, PO-357 gave a single Schlieren peak during sedimentation in a Spinco model E ultracentrifuge. The molecular weight was calculated to be 8,500~9,000. In analytical polyacrylamide gel (7.5%, pH 4.0) electrophoresis, a well defined single band staining with amide black was observed. Elementary analysis: C 44.8, H 6.4, N 14.0.

Analysis of PO-357 after hydrolysis with 6 N HCl at 110°C for 15 hours indicated the following amino acids; lysine, asparatic acid, threonine, serine, glutamic acid, glycine, alanine, valine,

Fig. 1. UV Spectrum of PO-357.



* PO-357 was named as sporamycin.

Plate. 1. Ultracentrifuge pattern of PO-357 at 52,000 rpm (synthetic boundary cell).

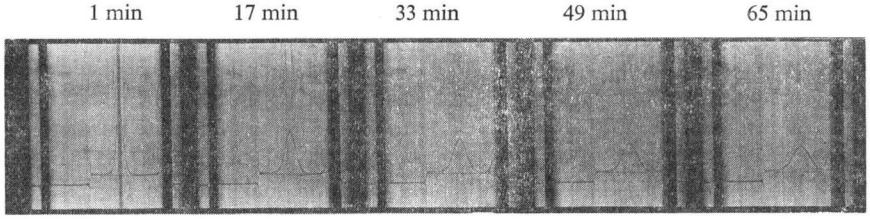


Fig. 2. IR Spectrum of PO-357 (KBr).

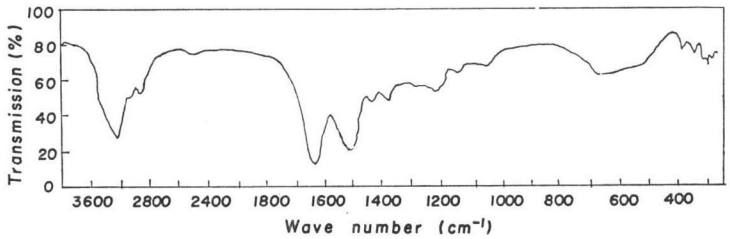


Table 1. Antimicrobial spectrum of PO-357

Test organism		Minimum inhibitory concentration (mcg/ml)	Test organism	Minimum inhibitory concentration (mcg/ml)
Staphylococcus aureus	FDA 209P	0.1	*Aerobacter aerogenes* ATCC 9621	>100
" "	JC-1	0.05	*Pseudomonas aeruginosa* P-3	>100
" "	FS 1277	2.1	*Candida albicans*	>500
Bacillus subtilis	PCI 219	2.1	*Saccharomyces sake*	>500
Bacillus cereus	IFO 3001	10.3	*Aspergillus niger*	>500
Sarcina lutea	PCI 1001	50.0	*Trichophyton interdigitale*	>500
Mycobacterium smegmatis	ATCC 607	>100	*Trichophyton mentagrophytes*	>500
Escherichia coli	NIHJ	>100	*Piricularia oryzae*	>500
Shigella sonnei	E 33	>100	*Cryptococcus neoformans*	>500
Salmonella typhimurium		>100	*Alternaria kikuchiana*	>500
Klebsiella pneumoniae	PCI 602	>100	*Sclerotinia cinerea*	>500
			Penicillium notatum	>500

isoleucine, leucine, tyrosine and phenylalanine.

As shown in Table 1, the antibiotic was effective against most Gram-positive bacteria, but inactive against *Mycobacterium*, Gram-negative bacteria, fungi and yeasts. Its antitumor activity against EHRLICH ascites carcinoma is shown in Table 2. It was also effective against mouse leukemia P-388 and L-1210, S-180 solid tumor and B-16 melanoma. Acute toxicities of PO-357 in mice were 13 mg/kg and 15 mg/kg upon injection intravenously and intraperitoneally, respectively.

Many macromolecular antitumor antibiotics have been reported. Neocarzinostatin[1] and

Table 2. Antitumor activity of PO-357 on EHRLICH ascites carcinoma.

Total dose (mg/kg)	Schedule	% Increased life span	No. of survivors on day 60 / No. of animals treated
10	Days 1~9	>75	4/7
7.5		>110	5/7
5.0		>114	5/7
2.5		>125	6/7
1.9		>81	3/7
1.3		>44	2/7

EHRLICH ascites cells (2×10^6) were inoculated ip (day 0) and mice were treated from day 1.

macromomycin[2] were reported to be acidic polypeptides, whereas PO-357 was identified as a basic substance by paper electrophoresis. Actinocarcin[3] and phenomycin[4] belong to the group of basic antitumor substances, but it has been reported that these have no antibacterial activity. To our knowledge, therefore, there is no known substance identical with PO-357.

Acknowledgements

We wish to thank Mr. KEN'ICHI SUGIMOTO, Mr. ROKUROU MASUMA and Miss FUJIKO KIMURA for their technical assistance.

This work was partially supported by a Grant-in-aid for Cancer Research from the Ministry of Health and Welfare, the Ministry of Education, Culture and Science, and by a fund from Japan Keirin Association.

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(Received August 23, 1976)

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