STUDIES ON ACTINOCARCIN, A NEW ANTITUMOR ANTIBIOTIC

Sir:

A *Streptomyces* resembling *Streptomyces cinnamomeus*, produced a new antitumor substance named actinocarcin. Since the active substance in the culture filtrate had no antimicrobial activity, its production and isolation were followed by determining activity against EHRLICH ascites carcinoma.

When the actinocarcin-producing strain, *Streptomyces* sp. 3654-JT$_1$, was shake-cultured in a medium containing 3.0 % glucose, 4.0 % Polypeptone and 0.5 % sodium chloride at 27°C for 7 days, the 20-fold diluted culture filtrate prolonged the survival time of mice inoculated with EHRLICH ascites carcinoma cells when injected daily for 6 days. Actinocarcin was adsorbed on a column of a cation-exchange resin, Amberlite IRC-50 (H-type). After the column was washed with distilled water and 0.5 N HCl, actinocarcin was eluted by 0.05 N HCl-acetone (1:1). After the active eluate was concentrated *in vacuo*, the active substance was precipitated by addition of 10 volumes of ethanol to the concentrate. The brownish powder obtained by drying the precipitate had a minimum active dose against EHRLICH carcinoma of 0.1 mg/mouse/day for 6 days. This crude powder of actinocarcin was dissolved in a mixture of 1-butanol-pyridine-water (1:1:2) and was adsorbed on a cellulose column. After the column was washed with the same solvent system followed by water, actinocarcin was eluted with 10 % pyridine - 5 % acetic acid. The eluate was lyophilyzed and a pale brownish powder was obtained. After the cellulose column chromatography had been repeated two times, the minimum active dose of the resulting powder was 5~3 mcg/mouse/day for 6 days.

Actinocarcin was further purified by CM-cellulose column chromatography. Gradient elution was carried out by increasing the concentration of pyridine-acetic acid from 5 % pyridine-2.5 % acetic acid to 10 % pyridine-5 % acetic acid. The eluate was collected in 10-ml fractions. The active substance was eluted in fractions 18~34 and each active fraction was subjected to cellulose acetate film electrophoresis using a pH 8.9 buffer (0.12 M tris buffer). Fractions 21~32 showed a single purple-red spot on the film after treatment with PONCEAU-3R, and were combined and concentrated *in vacuo*. The active powder obtained by lyophilization was dissolved in

Fig. 1. Ultraviolet spectrum of actinocarcin.

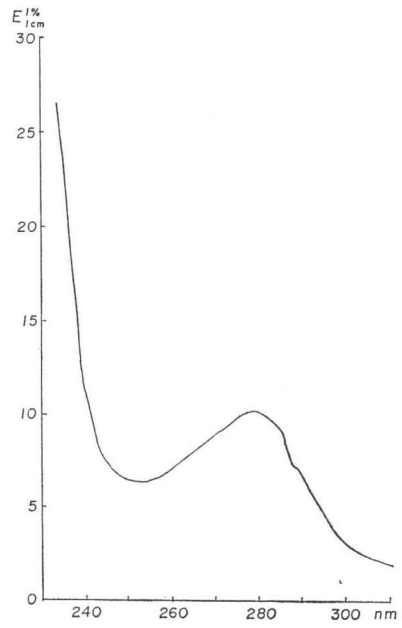
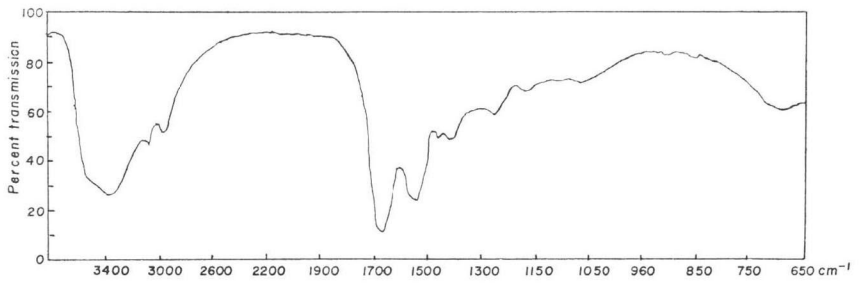


Fig. 2. Infrared spectrum of actinocarcin (KBr).



1 % acetic acid and purified by chromatography on a Sephadex G-25 column with the same solvent. After fractions showing activity against EHRLICH carcinoma were combined and lyophilized, pure actinocarcin was obtained as a colorless fluffy substance.

Actinocarcin is soluble in water but insoluble in most organic solvents. It shows a weak peak at 278 nm ($E^{1\%}_{1cm}$ 10.5 in H_2O) in the UV absorption spectrum as shown in Fig. 1. Its peptide nature is shown by the IR absorption spectrum shown in Fig. 2. Actinocarcin gives positive ninhydrin, FOLIN-phenol, SAKAGUCHI, and PONCEAU-3R reactions. MOLISCH, FEHLING and ferric chloride reactions are negative. Actinocarcin is optically active, $[\alpha]_D^{20}$ −23.4° (*c* 1, H_2O). It does not show clear melting point but changes to light brown at about 217°C. Actinocarcin is basic and is pure, as shown by cellulose acetate film electrophoresis using a pH 8.9 buffer, where moved to the cathode as a single entity.

The result of elemental analysis was as follows: C 52.42 %, H 7.63 %, N 16.92 %, O 22.62 %, S 0.73 %. After hydrolysis of actinocarcin in 5.7 N HCl at 110°C for 20 hours, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine were detected by STEIN-MOORE analysis.

Actinocarcin has no activity against microorganisms and thus is differentiated from A 280 substance[1], iyomycin[2], and neocarzinostatin[3] which were reported to have antimicrobial as well as antitumor activity. Carzinocidin[4] was reported to have only end absorption in the UV spectrum. Melanomycin[5] moved to the anode in the paper electrophoresis using a pH 6.0 buffer. Marinamycin[6] does not show any particular absorption maximum in the UV spectrum. Carzinostatin[7] must be an acidic polypeptide judging from its behavior in the paper electrophoresis. Peptimycin[8] gives a negative SAKAGUCHI reaction and has no optical rotation in an aqueous solution. Actinogan[9] is a glycopeptide and its nitrogen content is far less than that of ordinary polypeptides. Plurallin[10] is a glycoprotein having shoulders at 257 nm and 280 nm in the ultraviolet absorption.

Among known antitumor antibiotics, actinocarcin has similar properties to enomycin[11] and phenomycin[12]. However, amino acid analysis of enomycin showed no phenylalanine and this point differentiates actinocarcin from enomycin. Direct comparison by cellulose acetate film electrophoresis of a mixture of actinocarcin and authentic phenomycin showed two clearly separated purple-red spots on the film after treatment with PONCEAU-3R. Therefore, actinocarcin is not phenomycin. The authors found no known substance identical with actinocarcin.

Actinocarcin prolonged the survival period of mice inoculated with 2 million cells of EHRLICH carcinoma, at doses of 1 mcg/mouse/day for 6 days. Daily injections of 40~50 mcg/mouse for 6 days caused death of about half of the mice.

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