RENASTACARCIN, A NOVEL ANTITUMOR SUBSTANCE PRODUCED BY *STREPTOMYCES*

Sir:

During the course of our screening program for new antitumor substances of microbial origin by measurement of labeled substrates incorporated into tumor cells,[1] we found that a strain of *Streptomyces* sp.* newly isolated from soil produced a substance having marked antitumor activity against both EHRLICH and Sarcoma 180 ascites carcinoma in mice. After isolation and characterization of the active principle, it was shown that this substance belongs to a new class of macromolecular antitumor compounds. The name renastacarcin (RNC) was given to the active substance.

We report herein the production, isolation and preliminary characterization of RNC.

Production and Isolation Procedures for RNC

Fermentation of RNC was conducted under aerobic conditions at 27°C for 72 hours in a jar fermentor (20 liters) containing a medium (12 liters) comprising 3% starch, 1% meat extract and 1% Polypepton.

The culture filtrate (10 liters) was adjusted to pH 4.0 with hydrochloric acid followed by the addition of ammonium sulfate to give 40% saturation. The whole mixture was allowed to stand overnight at 4°C to achieve complete precipitation of the active principle. The brownish precipitate (10 g, weighed wet) collected by centrifugation was dissolved in 0.02 M Tris buffer at pH 8.5 (100 ml). An insoluble solid was removed by filtration and discarded. After re-precipitation of the active principle from the buffer solution by ammonium sulfate, the harvested precipitate was dissolved in a small volume of the buffer solution and dialyzed overnight at 4°C (against the same buffer solution).

The dialysate was then subjected to column chromatography using DEAE Sephadex A25 (2×100 cm) previously saturated with the buffer.

Elution of RNC was effected with the same buffer containing 0.3 M ammonium chloride and active fractions were collected. The combined solution was concentrated directly with Ficol (Pharmacia Fine Chemicals) in a cellophane tube and the concentrate was subsequently purified by gel filtration on a column (3×120 cm) of Sephadex G-100 using the same Tris buffer. Active fractions were combined and then dialyzed overnight against redistilled water at 4°C. Subsequent lyophilization of the dialysate afforded an amorphous friable powder of RNC (500 mg).

Physicochemical Properties of RNC

The proteinaceous nature of RNC was presumed from the isolation procedures. The homogeneity of the isolated powder was first investigated, and confirmed by two criteria, *i.e.*, disc electrophoresis and ultracentrifugal analysis.

Fig. 1. Polyacrylamide disk electrophoresis of renastacarcin (Tris buffer pH 8.9)

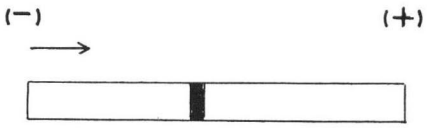
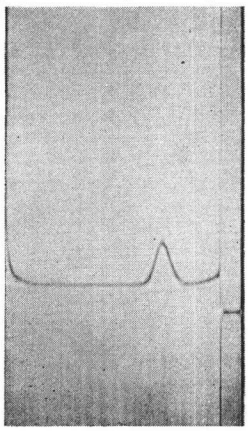


Fig. 1 shows a well defined single band of RNC when subjected to disk electrophoresis on polyacryamide gel at pH 8.9 in Tris buffer. Fig. 2 shows a fine single peak of RNC in the sedimentation pattern of the ultracentrifugation experiments using a Spinco model E ultracentrifuge. In these studies, RNC sedimented at 55,430 r.p.m. as a homogeneous substance with a sedimentation coefficient;

Fig. 2. Sedimentation diagram of renastacarcin (55.430 rpm, 30 minutes, 22.5°C)



* The characteristics of the strain will be published in the following paper.

$S_{20,w}$=2.12S. The molecular weight of RNC was calculated to be 34,500 according to the ARCHIBALD formula.[2]

This latter result is in good agreement with the value (35,000) calculated according to the procedure of MORRIS[3] based on the Rf value of RNC on a thin layer plate of Sephadex G-100 in comparison with the reference compounds of known molecular weight, ovalbumin and chymotripsin.

The UV spectrum of RNC in 0.1 N hydrochloric acid showed a peak at 282 nm ($E^{1\%}_{1cm}$ 11.2) with a shoulder at 290 nm, which collapsed at 282 nm in alkaline solution. The IR spectrum of RNC in a KBr tablet showed the typical characteristics of proteinaceous compounds, *i.e.*, the bands at 3260, 2940, 1650 and 1550 cm^{-1} corresponded to amide absorption.

RNC showed positive color reactions of MILLON, SAKAGUCHI and EHRLICH, but negative ninhydrin, MOLLISCH and ammoniacal silver nitrate tests.

On exhaustive hydrolysis with 5.7 N hydrochloric acid, RNC gave sixteen of the usual amino acids as detected by an automated amino acid analyser. Neither ether extractable organic acids nor carbohydrates could be detected in the hydrolysate. The amino acids characterized were lysine, arginine, histidine, aspartic acid, glutamic acid, threonine, serine, proline, cystine, glycine, alanine, valine, leucine, isoleucine, tyrosine and phenylalanine.

RNC was very unstable to heat. The antitumor activity was depressed gradually despite storage in a deep freezer at −20°C and complete loss of activity was observed when RNC was incubated at 60°C for 30 minutes in Tris buffer.

Biological Properties of RNC

A notable characteristic feature of RNC is that it did not exhibit antimicrobial activity against most Gram-positive and negative bacteria, fungi and yeast even at the concentration of 1,000 mcg/ml.

However, it showed a marked antitumor activity at quite a low concentration against EHRLICH ascites and Sarcoma 180 in *dd* mice. It was observed that RNC was less active against solid type tumors. The intraperitoneal administration of 6 mcg/mouse/day for 5 days of RNC to mice inoculated with 1×10^7 tumor cells before the experiments showed a marked curative effect both in EHRLICH and Sarcoma 180 ascites tumors.

The LD_{50} of RNC in mice was in the range of 35~40 mg/kg intraperitoneally, and 20~23 mg/kg intravenously.

On the basis of the foregoing results, RNC can be discriminated from the known macromolecular antitumor antibiotics such as melanomycin,[4] peptimycin,[5] actinogan[6], phenomycin,[7] macromomycin,[8] lymphomycin,[9] neocarzinostatin[10] and actinocarcin[11] by comparison of physicochemical and biological properties.

Some observations on the mode of action of RNC will be reported in the following paper.

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