# ISOLATION AND CHARACTERIZATION OF A NEW ANTITUMOR AGENT PRODUCED BY *STREPTOMYCES GRISEUS**

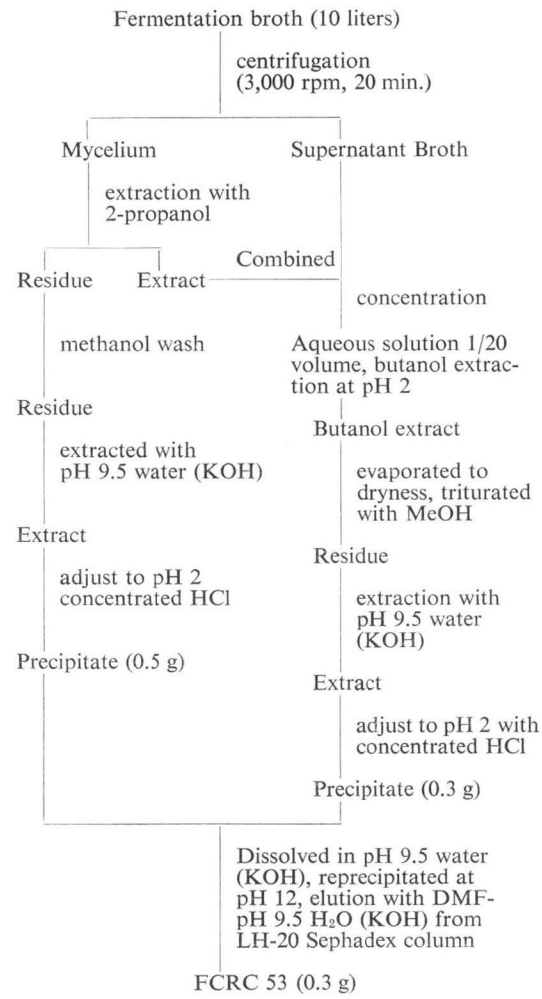
Sir:

A strain of *Streptomyces griseus* isolated from a bean field at Fort Detrick, Frederick, Maryland has been found to produce a substance cytotoxic to both KB and P388 cell lines and inhibitory to P388 ascites tumor. The producing strain was identified by Dr. RUTH E. GORDON (unpublished data) as having a pattern of physiological characteristics typical of the 46 reference strains of *S. griseus* as described by GORDON and HORAN[1].

The active principle was produced in a medium formulated as follows: Dextrose 15 g, soy flour 10 g, peptone 5 g, meat extract 5 g, NaCl 5 g, $K_2HPO_4$ 0.5 g, tap water 1,000 ml. The strain was cultured at 28°C for 30 hours at 200 rpm in 2-liter shake flasks containing 400 ml of the medium. Filtrates of the crude fermentation broth which were colored deep purple showed activity against *Bacillus subtilis* and *Staphylococcus aureus* and also produced a cytotoxic response from KB cells[2]. These filtrates were also active in the P388 ascites tumor system[2].

The active product was isolated from the fermentation broth using the procedure shown in Chart 1. The material, *ca* 80% pure after acid precipitation, was further purified into two bands on an LH-20 Sephadex column by elution with dimethylformamide - pH 9.5 water (2: 1). The faster moving major band was named FCRC 53. The homogeneity of FCRC 53 was established by obtaining a single band upon rechromatography in the following systems: Sephadex LH-20 with DMF-pH 9.5 water (2: 1) as eluant; upon chromatography on Dowex 1 column ($OH^-$ form) with water (pH 7) as eluant; and on cellulose column using water - pyridine (3: 1) as eluant. Furthermore, no increase in $E^{1\%}_{1cm}$ value at 255 nm was detected after any of these column chromatographs or after purification by dissolving in pH 9.5 water (KOH) and precipitating at pH 2 by addition of concentrated HCl. Thin-layer chromatography with cellulose plates using both water - pyridine (3: 1) and methanol - ammonia - water (4: 1: 5) as solvent systems revealed single spots having antimicrobial activity with Rf's at 0.9 and 0.45, respectively.

Chart 1.




Purified FCRC 53 is a brownish purple acidic compound with a melting point above 280°C. It is soluble in basic water (pH 9.5 $NH_4OH$) but insoluble in chloroform, methanol, and dimethylformamide. The antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* is retained by the compound after three weeks at room temperature in pH 9.5 water ($NH_4OH$). At pH levels above 11 ($NH_4OH$), the antimicrobial activity is lost rapidly.

Elemental analysis gave the following percents: C: 41.67, 42.28: H: 5.46, 5.16; N: 12.89, 13.29; ash: 4.84. The UV spectrum in pH 9.5 water ($NH_4OH$) showed maxima at 255 nm ($E^{1\%}_{1cm}$ 190),

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515 nm, 540 nm, and 580 nm. In 0.1 N NaOH, the UV spectrum showed maxima at 260 nm ($E^{1\%}_{1cm}$ 186), 515 nm, 540 nm, and 580 nm. IR (KBr) indicated the presence of amide linkages (1660 $cm^{-1}$). By atomic absorption spectroscopy, the sodium and potassium salts of FCRC 53 gave an equivalent weight of 570 and the barium salt gave an equivalent weight of 1144. Molecular weight estimation, using Sephadex G-75 superfine and Sephacryl S-200, was inconclusive because FCRC 53 was eluted with the void volume. Atomic absorption spectroscopy also showed a phosphorous content of 3.2%. A positive sodium molybdate—stannous chloride test[3,4] indicated the presence of organic phosphates. The organic phosphate groups and amino acid moieties were retained even after Dowex 1 and Sephadex G-75 column chromatography, indicating that the phosphate groups are covalently linked to the FCRC 53 molecule.

The amino acid analysis of a hydrolysate prepared by refluxing FCRC 53 in 6 N HCl at 110°C for 16 hours is presented in Table 1. The equivalent weight based on the amino acid analysis is *ca* 5,000. There was no shift in the melting temperature of calf-thymus DNA in the presence of FCRC 53 in citrate-sodium chloride buffer indicating noninteraction between DNA and FCRC 53[5].

The *in vitro* antimicrobial activities (tube dilution method) of the purified product are summarized in Table 2. No activity was detected against *Escherichia coli, Saccharomyces cerevisiae* and *Penicillium notatum*. The $ED_{50}$ of FCRC 53 against KB is 13 mcg/ml. Its antitumor activity against P388 ascites tumor is shown in Table 3. It is nonmutagenic as determined by the AMES *Salmonella* mutagenecity test[6].

Based on a comparison of the known properties of FCRC 53 with 5,600 known natural products as described in our Natural Products Data Bank[7], FCRC 53 appears to be a new antitumor agent. Chemically it most closely resembles prunacetin A[8] but differs in amino acid composition, UV spectrum, elemental analysis, and presence of phosphate groups. It also differs from prunacetin A in its antimicrobial activity as *S. aureus* and *Proteus vulgaris* show a >5-fold lower sensitivity to FCRC 53.

Acknowledgment

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Table 1.

| Amino acid | Concentration (μmole/mg) | Molar ratio |
|---|---|---|
| Aspartic acid or asparagine | 0.109 | 3 |
| Threonine | 0.072 | 2 |
| Serine | 0.071 | 2 |
| Glutamic acid or glutamine | 0.137 | 4 |
| Proline | 0.068 | 2 |
| Glycine | 0.250 | 7 |
| Alanine | 0.172 | 5 |
| Valine | 0.096 | 3 |
| Methionine | 0.022 | 1 |
| Isoleucine | 0.065 | 2 |
| Leucine | 0.131 | 4 |
| Tyrosine | 0.039 | 1 |
| Pheylalanine | 0.061 | 2 |
| Histidine | 0.035 | 1 |
| Lysine | 0.060 | 2 |
| Arginine | 0.079 | 2 |

Table 2.

| Test microorganism | MIC (mcg/ml)* |
|---|---|
| *Staphylococcus aureus* 209P | 87.5 |
| *Bacillus subtilis* PCI 219 | 25.0 |
| *Sarcina lutea* | 25.0 |
| *Proteus vulgaris* OX19 | >125.0 |
| *Candida albicans* | >125.0 |

* The MIC values were determined at the following concentration in mcg/ml: 12.5, 25, 50, 87.5 and 125 for direct comparison with published values on prunacetin A[8]. All bacteria were assayed in Difco nutrient broth and *Candida albicans* in Difco fluid SABOURAUD broth.

Table 3. Antitumor activity of FCRC 53 on P388 ascites tumor*

| Total dose (mg/kg) | % T/C | Weight lost (g) | Survivors |
|---|---|---|---|
| 50 | 128 | 0.7 | 4/4 |
| 25 | 124 | 0.7 | 4/4 |
| 12 | 112 | 0.7 | 4/4 |

* P388 lymphocytic leukemia model system.
Schedule: daily single dose, for nine days.
Vehicle: Hydroxypropylcellulose.
Route: Interperitoneal.

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J. A. CHAN
T. T. WEI
C. C. KALITA
D. J. WARNICK
A. L. GARRETSON
A. A. ASZALOS

Chemotherapy Fermentation Laboratory, NCI Frederick Cancer Research Center
Frederick, Maryland 21701, U.S.A.

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## References

1) GORDON, R. E. & A. C. HORAN: A piecemeal description of *Streptomyces griseus* (KRAINSKY) WAKSMAN and HENRICI. J. Gen. Microbiol. 50: 223~233, 1968
2) Antileukemic activity was assayed under the auspices of the National Cancer Institute by the procedures described by GERAN, R. I.; N. H. GREENBERG, M. M. MACDONALD, A. M. SCHUMACHER & B. J. ABBOTT: Protocols for screening chemical agents and natural products against animal tumors and other biological systems. 3rd Ed., Part 3, 3: 9~11, 1972
3) CLARK, J. M., Jr. (Editor): Experimental Biochemistry, p. 36, W. H. Freeman and Co., San Francisco, 1964
4) MILTON, R. F. & W. A. WATERS (Editors): Methods of Quantitative Microanalysis. 2nd Ed. p. 332, Edward Arnold Ltd., London, 1955
5) ZUNINO, F.; R. GAMBETTA, A. DIMARCO & A. ZACCACA: Interaction of daunomycin and its derivatives with DNA. Biochim. Biophys. Acta 277: 489~498, 1972
6) AMES, N. B.; J. MCCANN & E. YAMASAKI: Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenecity test. Mutation Res. 31: 347~364, 1975
7) BOSTIAN, M.; K. MCNITT; A. ASZALOS & J. BERDY: Antibiotic identification: a computerized data base system. J. Antibiotics 30: 633~634, 1977
8) ARAI, T.; S. KUSHIKATA, K. TAKAMIYA, F. YANAGISAWA & T. KOYAMA: Prunacetin A, an antitumor antibiotic from *Str. griseus* var. *purpureus* (Syn. *Str. californicus*). J. Antibiotics, Ser. A 20: 334~343, 1967