FREDERICAMYCIN A, A NEW ANTITUMOR ANTIBIOTIC

I. PRODUCTION, ISOLATION AND PHYSICOCHEMICAL PROPERTIES*

RAMESH C. PANDEY, MARGARET W. TOUSSAINT, RONALD M. STROSHANE, CHABI C. KALITA, ADORJAN A. ASZALOS, ALINE L. GARRETSON, TENA T. WEI, KEVIN M. BYRNE, RICHARD F. GEOGHEGAN, Jr. and RICHARD J. WHITE

Chemotherapy Fermentation Program, Frederick Cancer Research Center
P.O. Box B, Frederick, Maryland 21701, U.S.A.

(Received for publication August 11, 1981)

A new antitumor antibiotic, fredericamycin A (FCRC-A48, NSC-305263), has been isolated from a strain of *Streptomyces griseus* (FCRC-48). Based on its unique ultraviolet-visible spectrum, infrared spectrum, proton and carbon-13 nuclear magnetic resonance spectra and mass spectra, it is judged to be a novel acid-base indicator type of compound. Its production, isolation and physicochemical properties are discussed. The isolation, ultraviolet-visible spectrum and some biological properties of two minor components, fredericamycin B and fredericamycin C, are also described.

During the course of a screening program for new antitumor antibiotics, we isolated a strain of *Streptomyces griseus* (FCRC-48) from a soil sample in Frederick, Maryland. Fermentation broths of *S. griseus* (FCRC-48) repeatedly showed high *in vitro* activity against KB and P388 tumor cell lines and *in vivo* activity against P388 tumor cells. A complex of several biologically active compounds was isolated from acidified (pH 2.0) filtered broth by cooling to 4°C for 96 hours or by extraction with ethyl acetate. Purification of the complex resulted in the isolation of one major and two minor components. Other components were present, but were not isolated. This paper deals with the production, isolation and physicochemical characterization of fredericamycin A (FCRC-A48, NSC-305263), the major component. The ultraviolet-visible spectrum and some biological properties of the two minor components fredericamycin B and fredericamycin C are also reported. The biological properties of fredericamycin A are presented in the accompanying publication[1].

Materials and Methods

General

Melting points, determined on a Kofler micro hot stage apparatus, are uncorrected. Ultraviolet-visible (UV-VIS) spectra were taken in methanol on a GCA/McPherson ultraviolet-visible spectrophotometer, Model EU-700-32. Infrared (IR) spectra (micro KBr pellet) were run by Mr. N. RISSER on a Perkin Elmer spectrophotometer, Model 180. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian HR-220 spectrometer equipped with a Nicolet Instrument Corp. TT220 Fourier transform accessory. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were run on a JEOL FX60 spectrometer. The electron impact mass spectra (EIMS) were run by Mr. S. HUANG on a Finnigan 3300 GC/MS with a 6000 MS data system at 70 eV (solid probe). Field desorption (FD) mass spectra were measured by Dr. G. MCCLUSKY on a Vg Micromass ZAB-2F mass spectrometer, equipped with a combination electron impact (EI)-chemical ionization (CI)-field ionization (FI)-FD ion source

* Presented in part at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, Abstr. 24, New Orleans, Louisiana, September 22~24, 1980.

and Vg 2035 data system. Craig-type countercurrent distribution (CCD) apparatus (H. O. Post Scientific Co.) with 30 tubes (manually operated) or 500 tubes (automatically operated) were used for countercurrent distribution studies.

Isolation of Organism

The culture was derived from a soil sample collected from a plowed vegetable field in Frederick, Maryland. The strain was designated as FCRC-48 and later identified as *Streptomyces griseus* (See Results and Discussions). The isolate was grown on medium number 36 described by PRIDHAM and GOTTLIEB[2,3], with 1% adamantane as the sole carbon source.

Fermentation

FCRC-48 complex was produced in 14-liter and 1,200-liter fermentors. Both small and large fermentors were equipped with 3 turbine impellers that measured 7.4 cm and 27.0 cm in diameter, respectively. The large fermentors had a 3: 1 height to diameter ratio. Agitation was accomplished through a bottom entering mechanically sealed shaft. Air sparging was conducted *via* a sparge ring located below the bottom-most impeller. The glucose concentration was determined using Worthington Statzyme Glucose Reagent[4,5] on a Bausch & Lomb Spectronic 21 spectrophotometer. Packed cell volume was determined using a DeLaval Gyro Centrifuge with 10 ml conical tubes. Dissolved oxygen levels were determined continuously using an in-place sterilizable galvanic probe.

Inoculum was produced from frozen seed stocks maintained at −76°C. Secondary (working) stocks were developed from the frozen stock and maintained on slants of ISP #2 medium (Difco Laboratories, Detroit, Michigan)[6].

Slants were incubated 7 days at 28°C. The well-sporulated slants of *S. griseus* (FCRC-48) were resuspended in 10 ml of sterile physiological saline, and 2 ml aliquots were introduced into 500 ml baffled Erlenmeyer shake flasks containing 100 ml of medium. The seed medium consisted of 1.0% soy flour, 0.5% Bactopeptone (Difco), 0.5% beef extract (ICN), 0.5% NaCl, 0.05% K_2HPO_4, 1.5% dextrose, and 0.4% (v/v) P-2000 antifoam (Dow Corning, used in the stirred vessels only). Seed flasks were incubated for 24 hours at 28°C and agitated at 250 rpm on a 1″-stroke rotary shaker. Production medium contained 1.3% soy flour, 0.6% Bactopeptone, 0.6% beef extract, 0.6% NaCl, 0.06% K_2HPO_4, 1.8% dextrose, 0.5% (v/v) P-2000 antifoam (used in stirred vessels only), and 1.0% dextrin (used in shake flasks only).

Production of fredericamycin A in shake flasks was accomplished using 500 ml baffled Erlenmeyer flasks containing 100 ml of medium. The flasks were inoculated with 2 ml of 24-hour seed and incubated for 72 hours at 28°C and 250 rpm on a 1″-stroke rotary shaker. Large-scale fermentation was performed by batching two 14-liter stir jars with 10 liters of seed medium and sterilizing for 45 minutes at 121°C. Each stir jar received a 2% (v/v) inoculum from the above shake flasks. The stir jars were cultivated for 24 hours at 0.75 vvm aeration, 500 rpm, and 28°C operating temperature. A 1,200-liter fermentor was batched with 850 liters of production medium and sterilized for 45 minutes at 121°C. The fermentor received a 2% (v/v) inoculum from the above stir jars. The fermentor was cultivated for 34 hours at 0.75 vvm aeration, 250 rpm, 28°C, and 0.28 Kg/cm^2 pressure.

Thin-Layer Chromatography (TLC) Studies

Several types of TLC plates and many solvents were used to check the purity of fredericamycin A (Table 1). Silica gel TLC plates and a solvent composed of $CHCl_3$ - MeOH - AcOH (87: 3: 3), hereafter designated as TLC solvent A, were used for separating the minor components from fredericamycin A. Analytical (0.25 mm thick) and preparative (1 mm thick) TLC studies were carried out on E. Merck precoated silica gel plates with fluorescent indicator. TLC plates were examined under short- and longwavelength ultraviolet light.

High-Performance Liquid Chromatography (HPLC) Studies

High-performance liquid chromatography was performed using a Waters Associates Model 6000A solvent delivery system, a U6K septumless injector (Waters Associates) and a Schoeffel Model SF 770 variable wavelength UV detector. The detector was set at 254 nm and 0.04 absorbance unit full scale, unless otherwise specified. Reverse-phase separations were carried out on a C_{18} μBondapak column (3.9 mm×30 cm, Waters Associates) and MeOH - H_2O - HOAc (70: 30: 1) as the solvent (flow 2 ml/

Table 1. Thin-layer chromatographic solvent systems for fredericamycin A.

Solvent No.	Solvent system	Ratio	Solvent front† (cm)	Rf
1	$CHCl_3$ - MeOH	95: 5	14.0	0.67
2	$CHCl_3$ - MeOH	90: 10	15.0	0.80
3	$CHCl_3$ - MeOH	80: 20	14.8	0.95
4	$CHCl_3$ - MeOH	50: 50	13.7	0.85
5	$CHCl_3$ - HOAc	95: 5	12.9	0.63
6	$CHCl_3$ - MeOH - HOAc	95: 5: 1	13.8	0.54
7	$CHCl_3$ - MeOH - HOAc	95: 4: 1	13.4	0.46
8	$CHCl_3$ - MeOH - HOAc	50: 40: 1	14.0	0.93
9	$CHCl_3$ - MeOH - HOAc	94: 4: 2	12.9	0.58
10	$CHCl_3$ - MeOH - HOAc	78: 20: 2	9.8	0.97
11	$CHCl_3$ - MeOH - HOAc (solvent A)	87: 3: 3	15.9	0.76
12	$CHCl_3$ - MeOH - HCOOH	85: 15: 2	9.8	0.98
13	$CHCl_3$ - MeOH - HCOOH	87: 3: 3	16.2	0.74
14	$CHCl_3$ - MeOH - H_2O	78: 20: 2	11.0	0.96
15	Cyclohexane - EtOAc - EtOH	5: 3: 2	9.0	0.61
16	Pyridine - 1-BuOH - H_2O	3: 6: 2	10.7	0.68
17	1-BuOH - Acetone - H_2O (upper)	4: 1: 5	10.6	0.78
18	*Iso*-octane - MeOH - $CHCl_3$	2: 1: 1	13.2	0.99
19	CH_2Cl_2 - MeOH - HOAc	87: 3: 3	14.0	0.70
20	$CHCl_3$ - MeOH - HOAc	80: 20: 3	7.3	0.85
21	Acetonitrile - H_2O - HOAc	93: 7: 0.1	11.2	0.0
22	MeOH - H_2O - HOAc*	70: 30: 1	11.7	0.0
23	$CHCl_3$ - MeOH**	90: 10	8.2	0.0
24	$CHCl_3$ - MeOH - HOAc**	78: 20: 2	6.5	0.69

* Reverse phase TLC plate
** Alumina TLC plate
† The Rf of the material is dependent on the solvent front.

minute). We used a μPorasil column (3.9 mm × 30 cm, Waters Associates) for normal-phase separation and $CHCl_3$ - MeOH - HOAc (87: 3: 3) as the solvent (flow, 1 ml/minute).

Assay of Fredericamycin A in Fermentation Broth

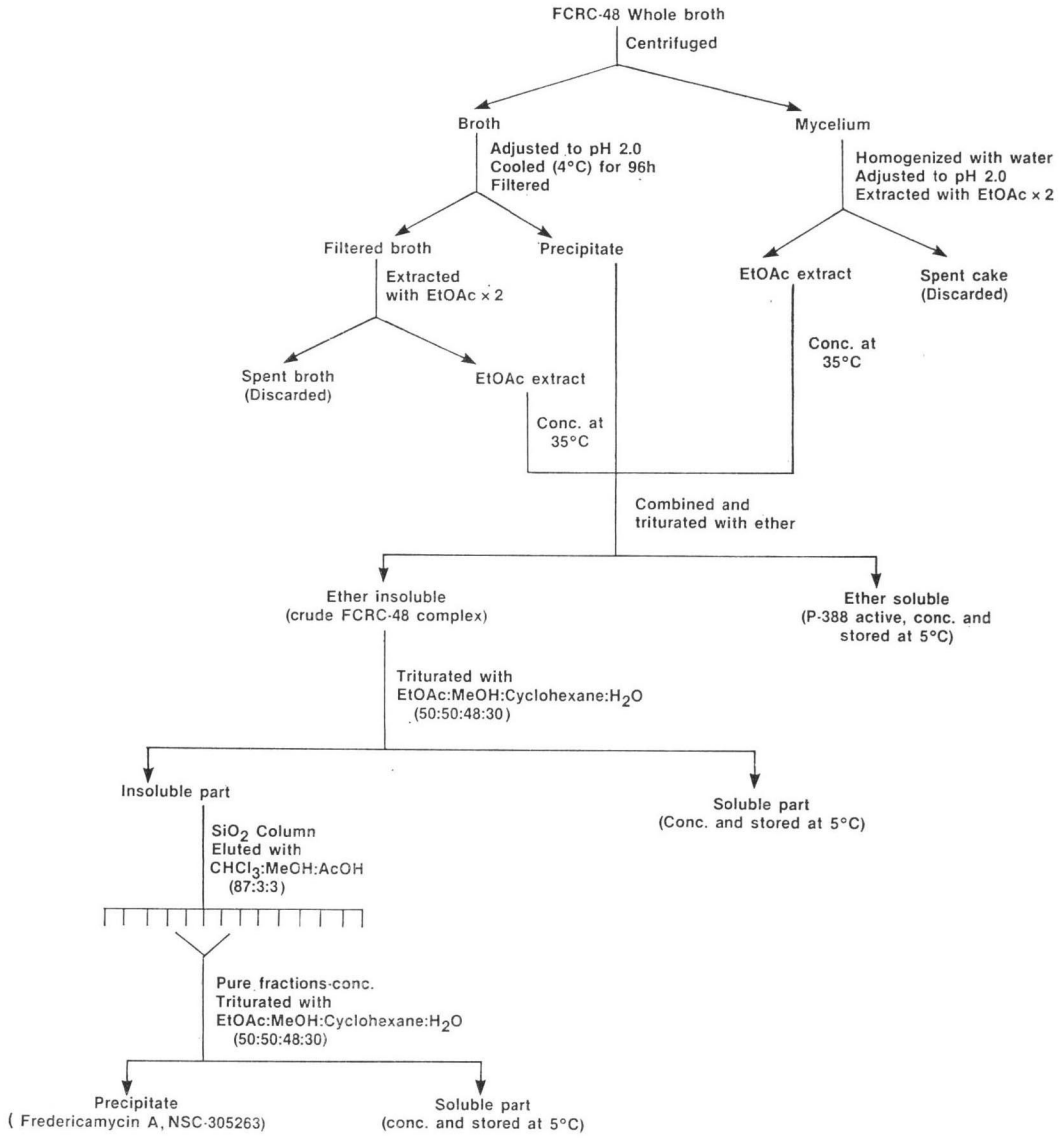
HPLC conditions were first established with pure fredericamycin A and then used for its assay in fermentation broths.

Whole broth samples (25 ml each) were adjusted to pH 2.0 and extracted with ethyl acetate (25 ml × 2) at different time intervals. The combined ethyl acetate extracts were dried with anhydrous sodium sulfate, filtered and evaporated to dryness under high vacuum at 35°C. The residue was dissolved in 2 ml of methanol, filtered and assayed by HPLC using the reverse-phase conditions described above.

Isolation of Crude FCRC-48 Complex

The whole broth from various fermentation runs (~125 liters) was centrifuged in a Sharples centrifuge, Model 16, to separate the mycelium from the broth. The pH of the filtered broth was adjusted to 2.0 with dilute sulfuric acid. It was left at 4°C for 96 hours and the precipitated FCRC-48 complex was filtered off. The filtrate was then extracted with ethyl acetate two times (64 liters × 1 and 32 liters × 1). The mycelium (~5 kg) was suspended in 15 liters of water and homogenized in a Waring blender. The pH of the mixture was adjusted to 2.0 with dilute sulfuric acid and extracted with ethyl acetate. The mixture was filtered through a Milton Press precoated with Celite 545 (Johns-Manville, Denver, Co). The ethyl acetate extract was separated, and the aqueous phase was discarded.

Fig. 1. General isolation and purification procedure for fredericamycin A (NSC-305263) from whole broth.



The precipitated complex was dissolved in the combined ethyl acetate extracts (~105 liters), and the solution was concentrated to *ca.* 4 liters and dried over anhydrous Na_2SO_4. Removal of ethyl acetate at 35°C afforded an oily residue that yielded 9.94 g of crude FCRC-48 complex as a dark red powder when it was triturated with ether. The isolation scheme is summarized in Fig. 1.

Purification of Fredericamycin A

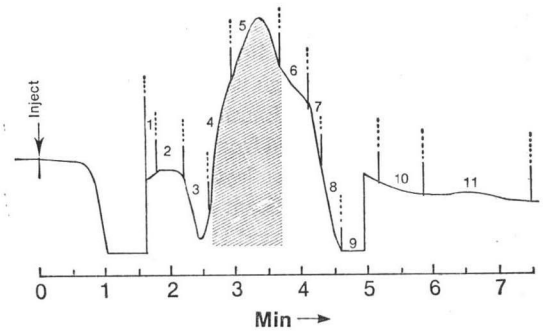
Three methods of purification are described here.

1. By Prep LC/500 Followed by Prep TLC: About 3.4 g of FCRC-48 complex was taken up in 40 ml of chloroform - methanol - formic acid (87: 3: 3). The insoluble material was filtered off (wt. 719 mg), and the soluble material was injected, in two injections, onto a Waters Associates Prep LC/System 500, fitted with a silica gel cartridge (Prep PAK Silica Cartridge). The solvent and the settings on the instrument were: solvent: $CHCl_3$ - MeOH - HCOOH (87: 3: 3); flow rate: 50 ml/minute; relative

response: 2; chart speed: 2 cm/minute. The Prep LC profile of the second injection is shown in Fig. 2. Eleven fractions were collected. These were combined into three fractions based on their TLC pattern: a fast moving material (Rf 0.94, solvent A), fredericamycin A (Rf 0.76, solvent A), and a slow-moving material (Rf 0.68, solvent A), which were the major components.

Fig. 2. Prep LC/500 profile of crude FCRC-48 complex.

Column: Prep PAK silica cartridge,
Solvent: $CHCl_3$ - MeOH - HCOOH (87: 3: 3),
Flow: 50 ml/minute.



All these fractions were concentrated and reinjected separately into Prep LC/500 using two silica gel cartridges in sequence. The three isolated compounds from the second injection were still contaminated with impurities. Further purification was achieved by preparative TLC on silica gel (20 cm×20 cm×1 mm) using solvent A. The final samples isolated were: the major component, fredericamycin A, 100 mg (Rf 0.76, solvent A); the minor component **I**, hereafter designated as fredericamycin B, *ca.* 20 mg (Rf 0.94, solvent A); and the minor component **II**, hereafter designated as fredericamycin C, 20 mg (Rf 0.68, solvent A).

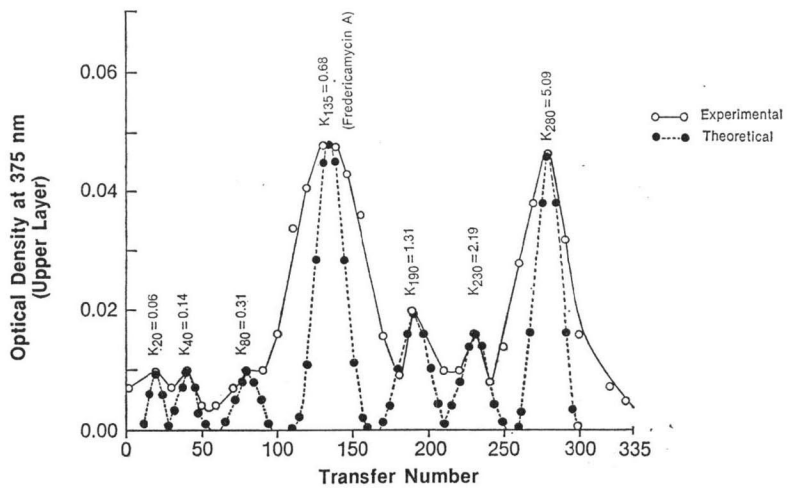
2. By Countercurrent Distribution: Fifteen liters of a two-phase solvent system (ethyl acetate - methanol - cyclohexane - water, 50: 50: 48: 30) was prepared and equilibrated for 12 hours at 23°C. The upper and lower layers were separated, and 10 ml of lower layer was placed in each tube from tube number 36 to 500. Ten ml of upper layer was also placed in every 30th tube for equilibration. About 5 liters of upper layer was placed in the solvent reservoir.

The crude FCRC-48 complex (5.0 g) was taken up in 350 ml each of upper and lower layers and vigorously stirred for 1 hour. The insoluble material (~3.0 g) was removed by filtration, and the filtrate was separated into upper and lower layers. Ten ml of each of these solutions was loaded into tube number 1 to 35, and the instrument was set for 335 transfers with 20 shakes and 10 minutes of settling time between each transfer.

After completion of the transfers, samples (100 μl) were removed from every 10th tube and diluted

Fig. 3. Countercurrent distribution profile of FCRC-48 complex.

Solvent system: ethyl acetate - methanol - cyclohexane - water, 50: 50: 48: 30, total number of transfers (n)=335.



to 3 ml with methanol. The optical density was measured at 375 nm. A plot of optical density *vs* tube number indicated two major and five minor peaks (Fig. 3). The distribution coefficient (K) was calculated[7] for these peaks from the formula $K=(N/n-N)$, where N is the tube number at the maximum concentration of compound and n is the total number of transfers. The distribution coefficient (K) for different peaks were $K_{20}=0.06$, $K_{40}=0.14$, $K_{80}=0.31$, $K_{135}=0.68$, $K_{190}=1.31$, $K_{230}=2.19$ and $K_{280}=5.09$. The major product, fredericamycin A, corresponds to K_{135}. Samples were removed and pooled from tube number 110 to 185, solvent was removed under vacuum, and the residue was precipitated from ether and dried under high vacuum (0.1 mm, 72 hours) to yield 85.5 mg of fredericamycin A.

3. CCD Solvent Extraction Followed by Silica Gel Chromatography: The crude FCRC-48 complex (44.8 g, obtained from various batches) was triturated with CCD solvent described above (100ml each of upper and lower layers $\times 5$), centrifuged and dried to afford 6.5 g of material. The filtrate, which contained a significant amount of fredericamycin A, was concentrated to dryness and triturated again with the CCD solvent (100 ml each layer $\times 5$), centrifuged and dried, yielding 1.6 g of additional material. The total amount of precipitate obtained was 8.1 g. The thin-layer chromatography (solvent A) indicated that it contained other impurities. Approximately 4.6 g of the above material ($E^{1\%}_{1cm}$ 247 at 784 nm) was taken in solvent A and loaded onto a silica gel column (70~230 mesh, 140 cm $\times$ 7.7 cm). The compound was eluted with the same solvent, and sixteen fractions (300 ml each) were collected. Thin-layer chromatographic analysis (solvent A) indicated fractions 3 and 4 to be pure fredericamycin A, which were therefore mixed, concentrated and triturated with CCD solvent (25 ml of each layer $\times 5$) to yield 2.2 g of pure material, Rf 0.78 (solvent A). The purification scheme is shown in Fig. 1.

Results and Discussion

The fredericamycin A producing strain, FCRC-48, was identified as *Streptomyces griseus* by Dr. RUTH E. GORDON, Waksman Institute of Microbiology, Rutgers, the State University, Piscataway, New Jersey, U.S.A. Initial fermentations were carried out in modified tubercidin medium[8] and were found active when tested *in vitro* against KB and *in vivo* against P388 tumor cell lines (Table 2). Subsequent fermentations were carried out in shake flasks and stirred vessels using media described in Materials and Methods.

Table 2. *In vitro* and *in vivo* activity of FCRC-48 fermentation broth.

I. *In vitro* activity (KB)

Broth #	Dilution for ED_{50}*
B001	1:170
B002	1:560

* The dose level of diluted broth at which 50% inhibition of growth *in vitro* is noted *vs.* untreated controls.

II. *In vivo* activity (P388)

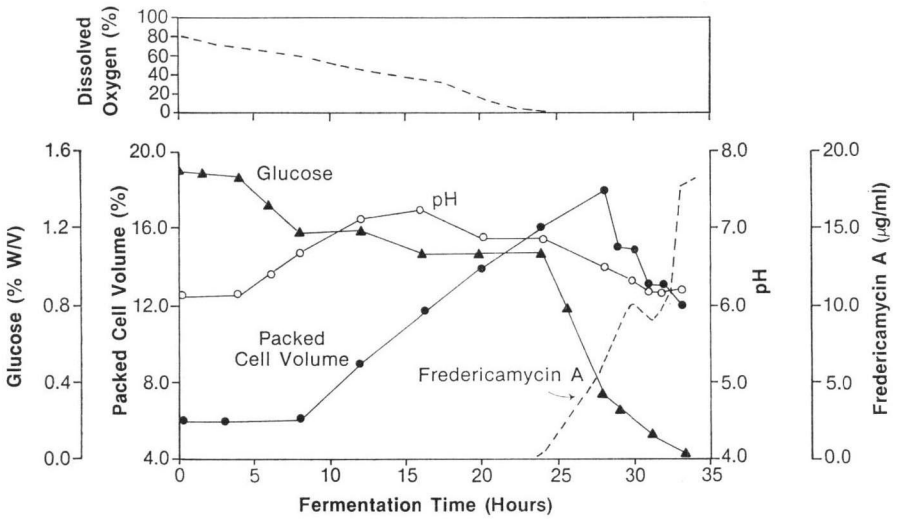
Broth #	Dilution	% T/C†
B003	1:16	132
D045	1:4	137
D052	1:8	137
D077	1:4	134
D078	1:2	142

† The ratio of average survival of treated animals to that of control, in days (T/C) $\times$ 100.

Production of fredericamycin A in shake flasks began at 36 hours and reached a maximum of 50~80 μg/ml at 60 hours. Utilization of glucose did not occur until 24 hours, when cell growth had reached a maximum. The pH normally decreased to ~6.5 during the first 24 hours and then rose during the remainder of the fermentation to a final pH of 8.0~8.5.

Fermentations carried out in stirred vessels gave time course profiles as shown in Fig. 4. Antibiotic production began at 24 hours during seed build-up and also at the production level. The pH rose to a maximum of 7.2 and gradually decreased to pH 6.2 as the antibiotic production peaked. At 24 hours the dissolved oxygen dropped to zero and the rate of glucose consumption became 0.1% per hour. As in shake flasks, fredericamycin A production closely followed glu-

Fig. 4. Time course of fredericamycin A production in a 1,200-liter fermentor.



cose utilization. The rate of fredericamycin A production decreased at 33 hours after achieving a final titer of 18.5 μg/ml.

Isolation and Properties of Fredericamycins A, B and C

Purification techniques involving Sephadex LH-20 were unsuccessful because of the retention of much of the material at the top of the chromatography column. Chromatography on ion exchange resins was hampered by the poor solubility of the material in aqueous solution. Silica gel chromatography appeared to be the best purification method, although there was some binding at the orgin and degradation as the material became purer. In spite of this it was possible to separate the FCRC-48 complex by Prep LC/500 followed by Prep TLC. Small quantities of fredericamycin A and two minor components, fredericamycin B and fredericamycin C, were thus isolated.

Fredericamycin B isolated in this way was brownish red and had the following constants: mp. >350°C (dec.), Rf 0.89 (solvent A), 0.93 (solvent system; $CHCl_3$ - MeOH - HCOOH, 87: 3: 3), 0.76 (solvent system; pyridine - 1-butanol - water, 3: 6: 2), UV (MeOH) λ_{max} ($E^{1\%}_{1cm}$) 781 (99), 584 (94), 544 (99), 505 (80), 384 (124), 371 (120), 346 (110), 262 nm (214). It showed weak activity against *Bacillus subtilis*.

Fredericamycin C was brownish red and moved more slowly than fredericamycin A in TLC and had the following constants: mp. >350°C (dec.), Rf 0.64 (solvent A), 0.53 (solvent system; $CHCl_3$ - MeOH - HCOOH, 87: 3: 3), 0.74 (solvent system; pyridine - 1-butanol - water, 3: 6: 2), UV (MeOH) λ_{max} ($E^{1\%}_{1cm}$) 784 (58), 750 (36), 565 (40), 555 (40), 535 (46), 509 (43), 390 (70), 372 (86), 350 (85), 330 (96), 316 (103), 295 (108), 255 nm (195). It showed weak activity against *Saccharomyces cerevisiae, Penicillium notatum, Candida albicans* and *B. subtilis*.

Neither component demonstrated detectable cytotoxicity against P388 cells when tested *in vitro*[9]. Because of their inactivity and the small quantities produced, no further work was done on these compounds at this time.

Based on the solubility and distribution of the crude extract in a number of two-phase solvent systems, ethyl acetate - methanol - cyclohexane - water (50: 50: 48: 30) was used for countercurrent distri-

bution studies.

The results of an experiment using a 500-tube apparatus is shown in Fig. 3. These studies indicated the presence of seven components in the complex. The minor components were not isolated at this time by this method.

The most effective purification scheme devised so far for isolation of fredericamycin A from fermentation broths is shown in Fig. 1. In this scheme, the crude complex is first triturated with the CCD solvent, then the CCD solvent-insoluble material is dissolved in chloroform - methanol - acetic acid (87: 3: 3) and chromatographed on a column of silica gel, eluting with the same solvent. The fredericamycin A-rich fractions are combined, the solvent is removed and the residue is triturated with CCD solvent to produce pure fredericamycin A as a red powdery material.

Physicochemical Properties of Fredericamycin A

The pure material behaves as an indicator which is red in acidic solution and green to blue in basic solution. It is soluble in acetic acid, dimethylsulfoxide, *N,N*-dimethylformamide and pyridine; partially soluble in acidic methanol, acidic chloroform and acidic ethyl acetate; and insoluble in water, petroleum ether and ether. It showed a single spot in various TLC solvent systems and plates (Table 1) and was homogeneous in HPLC under normal-phase and reverse-phase conditions(Fig. 5). Its melting point is >350°C (dec.). The ultraviolet-visible spectra at various pHs are shown in Fig. 6, which clearly show an isosbestic point at 554 nm and suggest the presence of one or more ionizable groups in fredericamycin A. The color change (red→green→blue) and spectral shift in base indicate the presence of phenolic hydroxyl groups. The absence of hydroxyl bands in the infrared spectrum (Fig. 7) suggests that the hydroxyl groups are hydrogen bonded. The strong band at 1720 cm^{-1} is probably due to a non-conjugated carbonyl group. The ^{1}H NMR spectrum (Fig. 8) shows the presence of some D_2O exchangeable and various unsaturated protons in the aromatic region. This is further corroborated by the ^{13}C NMR spectrum (Fig. 9) which shows only 14 carbon signals, of which 12 are in the olefinic region [signals at 166.5 (s), 155.8 (s), 153.9 (s), 139.7 (s), 137.7 (s), 133.6 (d), 132.7 (d), 131.1 (d), 122.1 (d), 111.7 (d), 108.6 (s), 105.9 (d), 32.1 (t), 18.1 (q)].

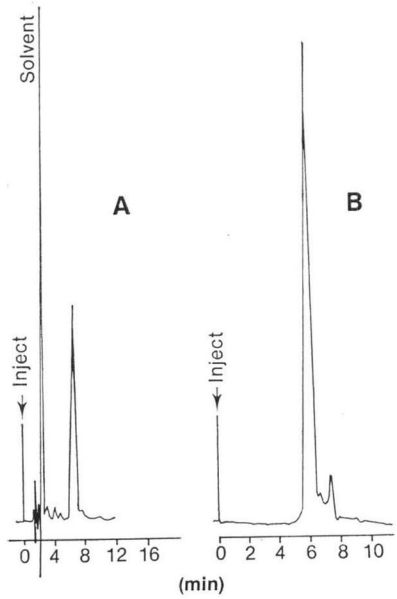


Fig. 5. High-performance liquid chromatograms of fredericamycin A.
Condition A;
Column: C_{18} μBondapak,
Solvent: methanol - water - acetic acid (70: 30: 1),
Flow rate: 2 ml/minute,
Detector: UV 254 nm.
Condition B;
Column: μPorasil,
Solvent: chloroform - methanol - acetic acid (87: 3: 3),
Flow rate: 1 ml/minute,
Detector: UV 254 nm.

Fig. 6. Ultraviolet-visible spectra of fredericamycin A in methanol at various pHs.

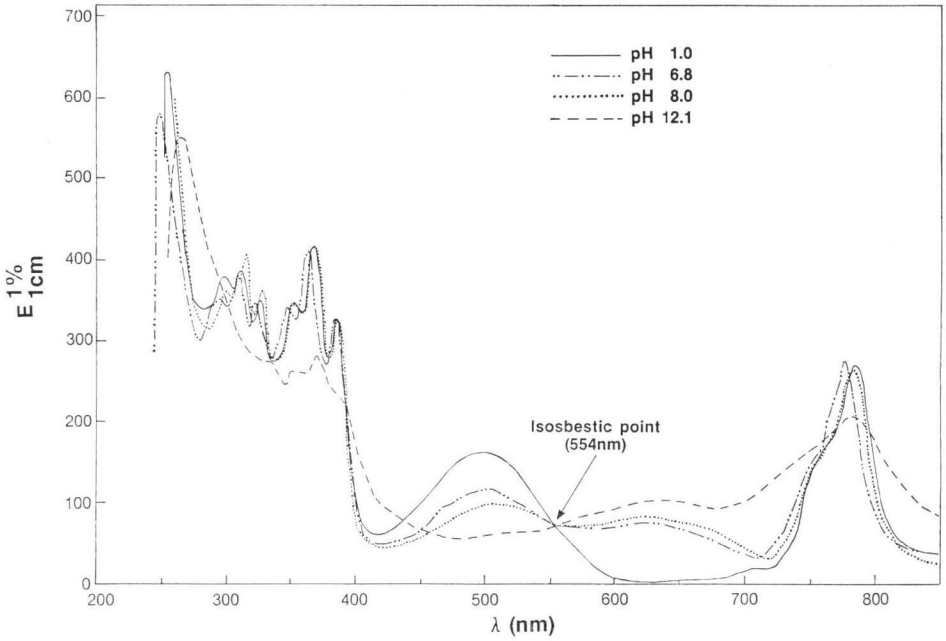
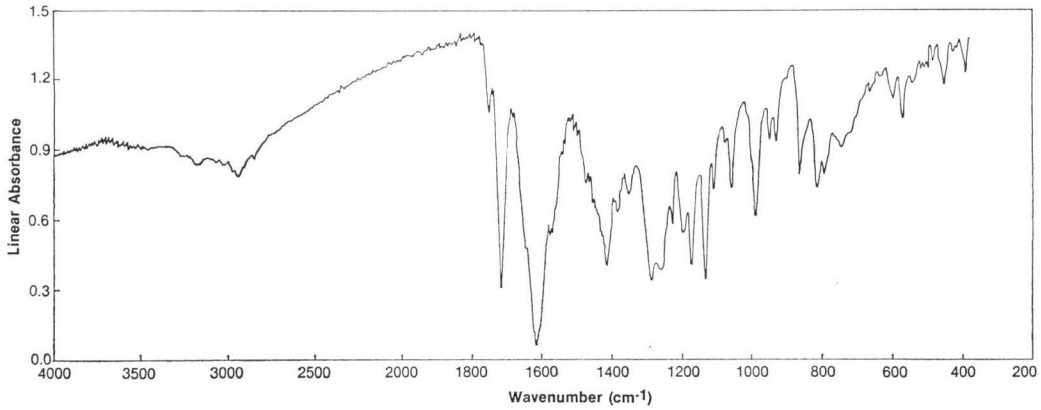


Fig. 7. Infrared spectrum of fredericamycin A (KBr).



The highest ion observed by both the electron impact mass spectrum (Fig. 10) and by the FDMS (Fig. 11) was at *m/z* 554. Fredericamycin A has two *pKa* values, determined in DMF to be 6.80 and 8.88, and analyzes for C, 66.77, 67.02; H, 5.43, 4.18; N, 2.40, 2.42%. The physicochemical properties are summarized in Table 3.

A literature search using BERDY's data bank[10] revealed that fredericamycin A is a new compound. Studies on the structure and biosynthesis of fredericamycin A are in progress.

Fig. 8. Proton nuclear magnetic resonance (^{1}H NMR) spectrum (220 MHz) of fredericamycin A in DMSO-d_6. (Shaded peaks disappear on D_2O shake.)

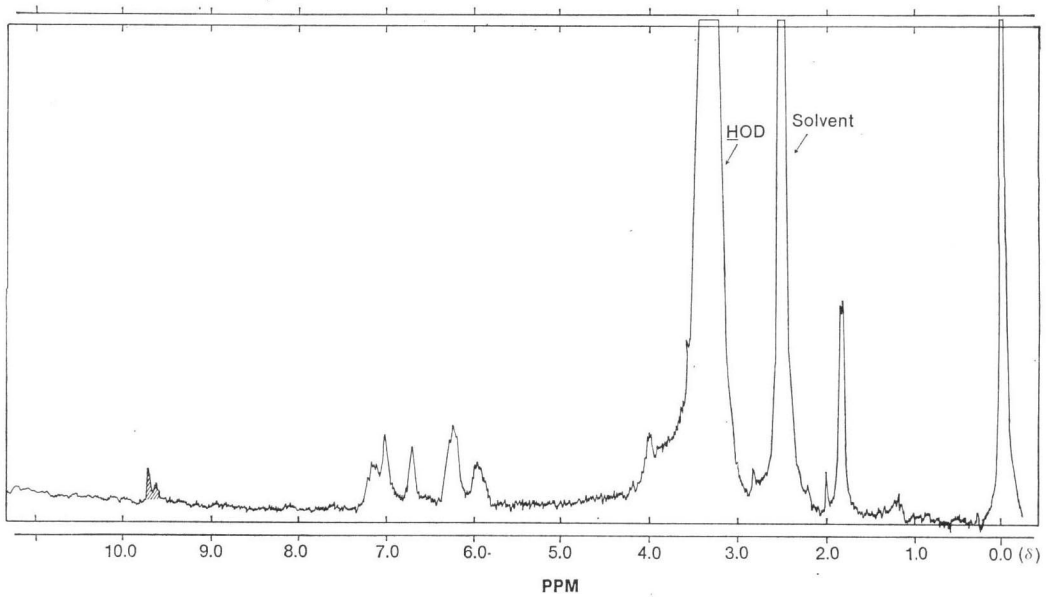


Fig. 9. ^{1}H Decoupled carbon-13 nuclear magnetic resonance spectrum (^{13}C NMR, FX-60, DMSO-d_6) of fredericamycin A.

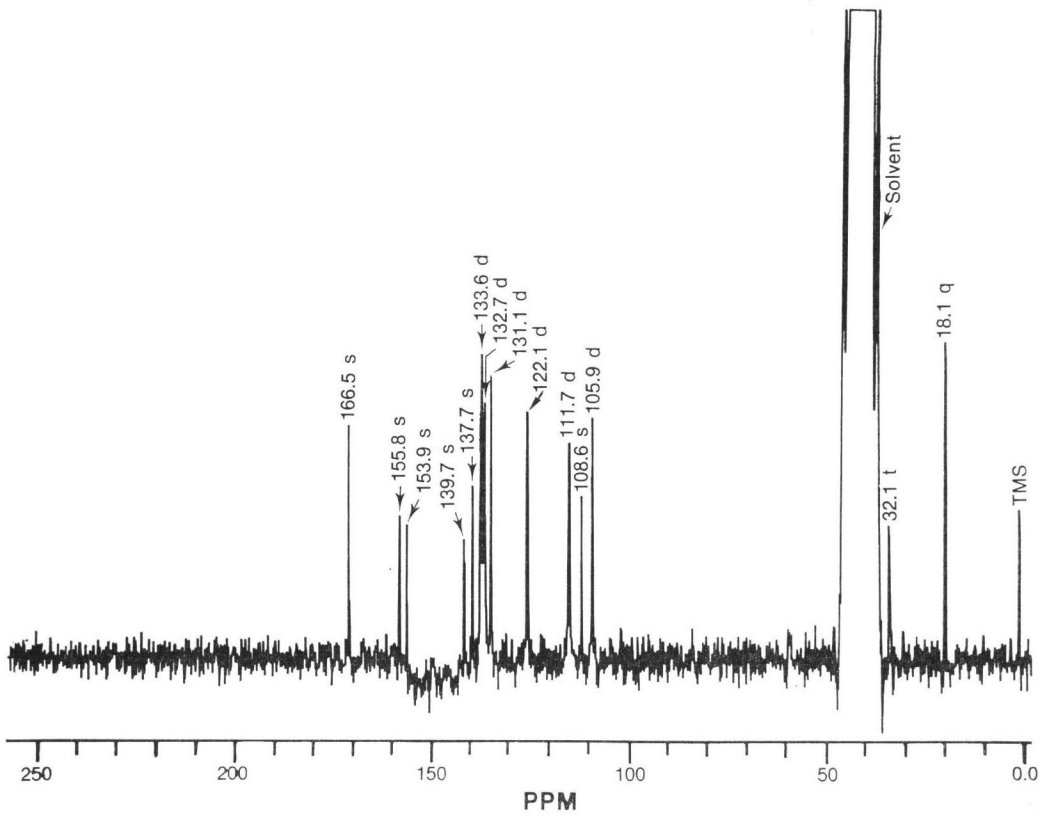


Fig. 10. Electron impact mass spectrum of fredericamycin A at 70 eV.

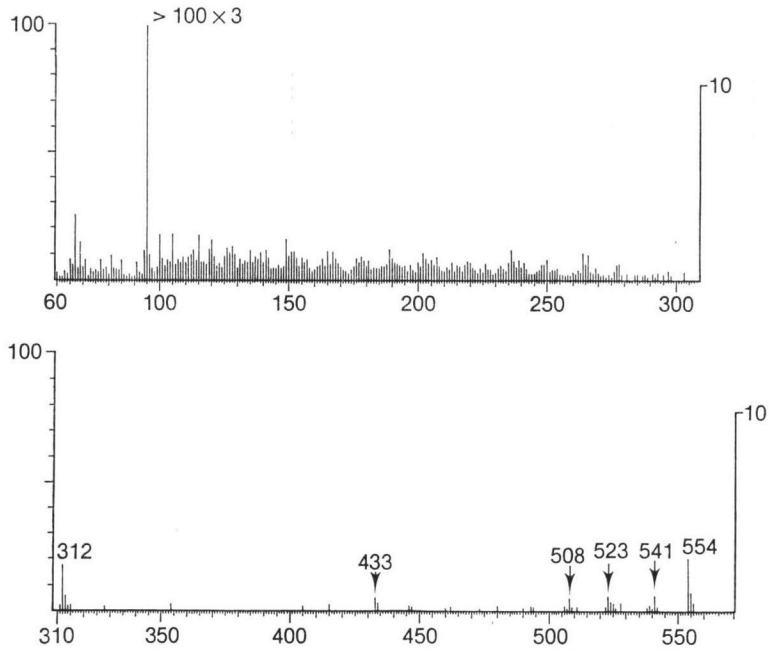
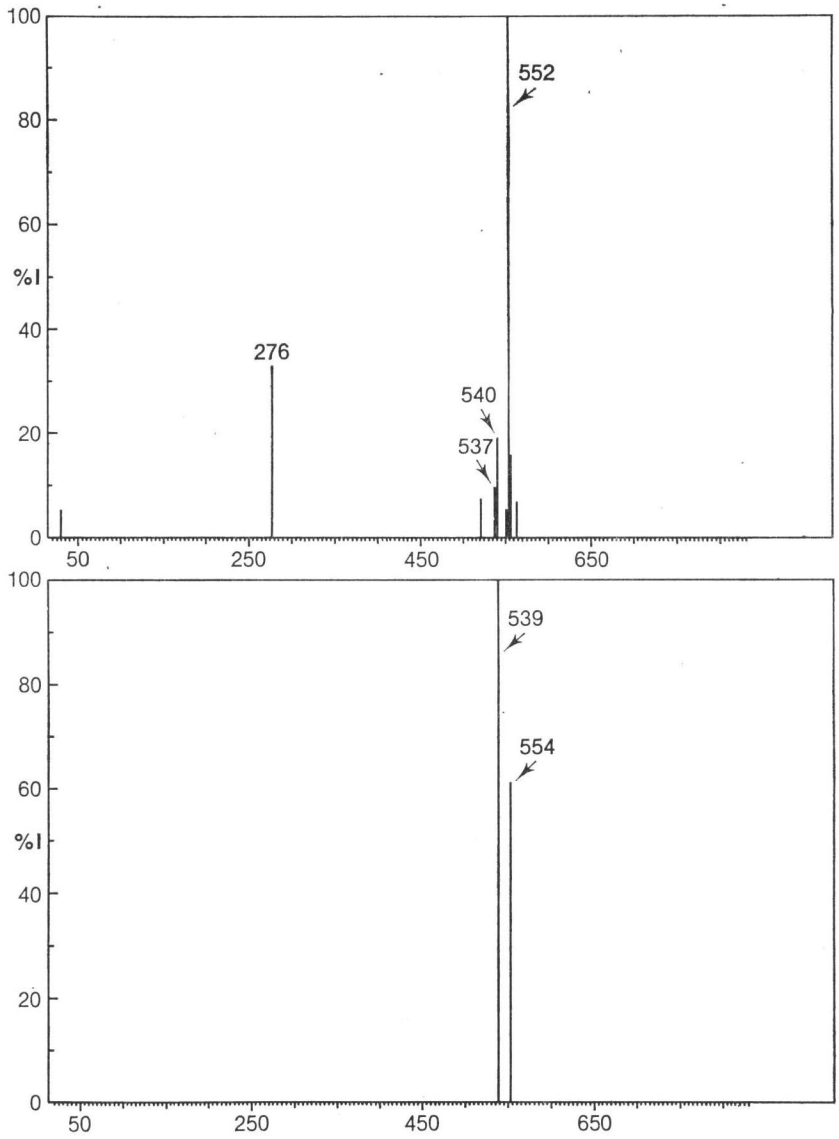


Table 3. Physicochemical and biological properties of fredericamycin A.

Type of compound	Indicator type, red in acidic solutions and green to blue in basic solutions.
Solubility	Soluble in acetic acid, dimethylsulfoxide, *N,N*-dimethylformamide, pyridine. Partly soluble in acidic methanol, acidic chloroform, acidic ethyl acetate. Insoluble in water, petroleum ether, ether.
Rf on analytical TLC plates	(See Table 1)
HPLC	On a C_{18} μBondapak column and a μPorasil column: See Fig. 5.
Distribution coefficient (K)	0.68 (solvent system; ethyl acetate - methanol - cyclohexane - water, 50: 50: 48: 30).
Melting point	>350°C (dec.)
UV (MeOH, pH 8.0)	λ_{max} ($E^{1\%}_{1cm}$) 784 (350), 756 sh (247), 500 (145), 391 (428), 371 (541), 356 (434), 330 (421), 316 (454), 302 (415), 256 nm (785). At various pHs see Fig. 6.
IR (KBr)	2940, 1720, 1615, 1570, 1415, 1285, 1260, 1225, 1195, 1170, 1130, 1110, 1055, 990, 950, 930, 865, 810 cm^{-1} (Fig. 7).
^1H NMR (220 MHz, DMSO-d_6)	Fig. 8.
^{13}C NMR (FX-60, DMSO-d_6)	Signals at δ 166.5, 155.8, 153.9, 139.7, 137.7, 133.6, 132.7, 131.1, 122.1, 111.7, 108.6, 105.9, 32.1, 18.1 ppm (Fig. 9).
EIMS (70 eV)	Intense ions at *m/z* 554, 541, 524, 523, 508, 494, 480, 433, 312, 292, 266, 264, 250, 248, 236, 220, 219, 207, 189, 178, 165, 152, 149, 100, 95, 91, 85, 81, 77, 69, 62, 56, 43 (100%). (Fig. 10)
FDMS	Intense ions at *m/z* 554, 552, 540, 539, 537, 276. See Fig. 11
Molecular wt. (osmometry)	675
pKa (solvent DMF)	6.80, 8.88
Elemental analysis	C, 66.77, 67.02; H, 5.43, 4.18; N, 2.40, 2.42.
Antitumor activity*	ED_{50}: 1.2×10^{-1} μg/ml against KB cell lines. ED_{50}: 1.0×10^{-5} μg/ml against L1210 cell lines. ED_{50}: 1.0×10^{-5} μg/ml against P388 cell lines. %T/C: 190 at 0.5 mg/kg dose level against P388 tumor cells.

* Determined according to the protocols of National Cancer Institute, U.S.A.[9]

Fig. 11. Field desorption mass spectrum of fredericamycin A at two wire currents.



Acknowledgements

We thank Dr. JOHN DOUROS of the National Cancer Institute for his interest and encouragement of this work, Dr. RUTH E. GORDON, Waksman Institute of Microbiology, Rutgers, the State University, Piscataway, NJ, for the identification of the strain, Mr. BRENT HAASE, University of Wisconsin, Madison, WI, for KB, P388 and L1210 *in vitro* assay, Ms. PEG SHERIDAN, Battelle Laboratories, Columbus, OH, for *in vivo* assay, and Dr. STEPHEN E. ULRICH, University of Illinois, Urbana, IL, for ^{1}H NMR spectra.

This research was sponsored by the National Cancer Institute, U.S.A. Contract No. NO-1-CO-75380.

References

1) WARNICK-PICKLE, D. J.; K. M. BYRNE, R. C. PANDEY & R. J. WHITE: Fredericamycin A, a new antitumor antibiotic. II. Biological properties. J. Antibiotics 34: 1402~1407, 1981
2) PRIDHAM, T. G. & D. GOTTLIEB: The utilization of carbon compounds by some actinomycetales as an aid for species determination. J. Bacteriol. 56: 107~114, 1948
3) WAKSMAN, S. A.: The Actinomycetes. II. Classification, Identification and Descriptions of Genera and Species, p. 332, Williams and Wilkins Co., Baltimore, 1961
4) SLEIN, M. W.: Methods of Enzymatic Analysis (H. U. BERGMEYER, *Ed.*), p. 117, Academic Press, New York, 1963
5) BONDAR, R. J. L. & D. MEAD: Evaluation of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* in the hexokinase method for determining glucose in serum. Clin. Chem. 20: 586~590, 1974
6) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
7) CRAIG, L. C. & D. CRAIG: Laboratory extraction and countercurrent distribution. *in* "Techniques of Organic Chemistry. III", 2nd ed., p. 149~332, New York Interscience, 1966
8) ANZAI, K.; G. NAKAMURA & S. SUZUKI: A new antibiotic, tubercidin. J. Antibiotics, Ser. A 10: 201~204, 1957
9) 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. Cancer Chemother. Rep. Part 3, 3: 7~61, 1972
10) BOSTIAN, M.; K. MCNITT, A. ASZALOS & J. BERDY: Antibiotic identification: a computerized data base system. J. Antibiotics 30: 633~634, 1977