Journal of Industrial Microbiology (1995) 15, 60–65

Korkormicins, novel depsipeptide antitumor antibiotics from *Micromonospora* sp C39500: fermentation, precursor directed biosynthesis and biological activities

KS Lam, DR Gustavson, GA Hesler, TT Dabrah[1], JA Matson, RL Berry, WC Rose and S Forenza

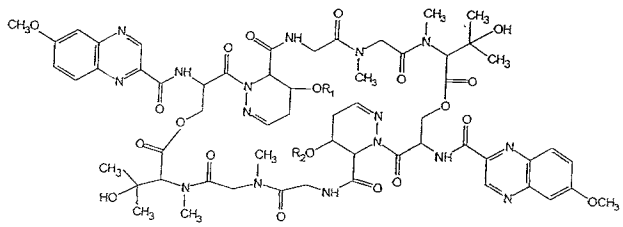
Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut 06492, USA

***Micromonospora* sp C39500, isolated in our laboratory from a soil sample, produced a complex of seven novel depsipeptide antitumor antibiotics, designated korkormicins. The major component of the complex, korkormicin A, has a MW of 1452 and a molecular formula of $C_{66}H_{84}N_{16}O_{22}$. Korkormicin A exhibits potent *in vivo* antitumor activity against P388 leukemia and M109 lung carcinoma implanted intraperitoneally (ip) in mice, with effective doses of 0.05–0.20 mg kg^{-1} injection^{-1}, for five or three ip injections, respectively. It is also active against Gram-positive bacteria but inactive against Gram-negative bacteria. The production of korkormicin A was enhanced by 3-fold when 0.1% L-valine was added to the production culture at 48 h. A titer of 401.0 μg ml^{-1} was achieved in the fermenter culture supplemented with 0.1% L-valine.**

Keywords: korkormicins; depsipeptide; antitumor antibiotic; directed biosynthesis

Introduction

In the course of our continuing search for novel antitumor antibiotics, a *Micromonospora* sp C39500 was found to produce a complex of novel cyclic depsipeptide antitumor antibiotics, designated korkormicins. The major component of the complex, korkormicin A, has a MW of 1452 and a molecular formula of $C_{66}H_{84}N_{16}O_{22}$. Korkormicin A has demonstrated *in vivo* activity against P388 leukemia and Madison M109 carcinoma in murine models. Korkormicins (Figure 1) are structurally related to the previously reported depsipeptide antitumor antibiotics luzopeptins [7,10,13,16,18], sandramycin [11,12] and echinomycins [2,19,20]. Korkormicins differ from each other only at the 4-substituted position on the tetrahydropyridazine moiety. The production of these analogs was controlled by the nitrogen sources in the fermentation. In this paper, we describe the taxonomy of the producing organism, fermentation and biological activities of the major component of the complex, korkormicin A. We also demonstrate the use of precursor-directed biosynthesis to specifically increase the production of korkormicin A by 3-fold and suppress the production of the other analogs in the fermentation.



Compound	R_1	R_2
Korkormicin A	$-COCH(CH_3)_2$	$-COCH(CH_3)_2$
Korkormicin B	$-COCH_2CH(CH_3)_2$	$-COCH(CH_3)_2$
Korkormicin C	$-COCH_2CH(CH_3)_2$	$-COCH_2CH(CH_3)_2$
Korkormicin D	$-COCH_2CH_3$	$-COCH_3$
Korkormicin E	$-COCH(CH_3)_2$	$-COCH_2CH_3$
Korkormicin F	$-COCH(CH_3)_2$	$-COCH_3$
Korkormicin G	$-COCH_2CH_3$	$-COCH_2CH_3$

Figure 1 Structures of korkormicins

Materials and methods

Microorganism

The korkormicin-producing microorganism was *Micromonospora* sp C39500. This strain has been deposited with the American Type Culture Collection with the accession number ATCC 55011. Frozen vegetative preparations were maintained in 10% glycerol/5% sucrose solution stored at −80° C for use as working stocks.

Media

The seed medium used was medium 53 (1% lactose, 3% soluble starch, 1% fish meal, 0.6% $CaSO_4$ and 0.3% $CaCO_3$). Two production media were used in this study. Medium 53A was prepared using 1% glucose, 3% soluble starch, 1% Pharmamedia (Traders Protein, Fort Worth, TX, USA), 0.5% Nutrex (Universal Foods Corp, Milwaukee, WI, USA), 0.6% $CaSO_4$ and 0.3% $CaCO_3$. Medium 53B

Correspondence: KS Lam, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA
[1]Present address: Central Research Division, Pfizer Inc, Eastern Point Road, Groton, CT 06340, USA
Received 9 November 1994; accepted 30 March 1995

consisted of 1% glucose, 3% soluble starch, 1% Bacto-liver (Difco, Detroit, MI, USA), 0.6% $CaSO_4$ and 0.3% $CaCO_3$.

Taxonomy

The cell wall content of strain C39500 was examined according to the method described by Becker *et al* [1].

Fermentation conditions

A seed culture of strain C39500 was prepared by transferring 4 ml of the stock culture to a 500-ml Erlenmeyer flask containing 100 ml of medium 53. The seed culture was incubated at 28° C on a rotary shaker set at 250 rpm. After 72 h, 4-ml aliquots of this seed culture were transferred to 500-ml Erlenmeyer flasks containing 100 ml of the production media (medium 53A or 53B). The production cultures were incubated at 28° C and 250 rpm on a rotary shaker for 6–7 days. For production in fermenters, 1.4 L of the seed culture was transferred to 35-L production media supplemented with 15 ml polypropylene glycol P-2000 as antifoam. The fermentations were incubated for 6–7 days under the following conditions: temperature, 28° C; agitation, 250 rpm; aeration, 0.7 vvm and back pressure, 0.35 kg cm^{-2}.

Analytical methods

Growth of the organism was determined by the % sediment measured after centrifugation of 10 ml of culture broth at 3000 × *g* for 10 min.

The production of the korkormicin complex in the fermentation was monitored by HPLC using a C-18 reverse-phase column (μBondapak, 3.9 × 300 mm, Waters Associates, Milford, MA, USA) and UV absorption at 258 nm. The solvent system was 0.1 M ammonium acetate/CH_3OH/CH_3CN (1/1/1) with a flow rate of 2 ml min^{-1}. The fermentation extracts for HPLC assay were prepared by extracting the fermentation broth with an equal volume of ethyl acetate. The ethyl acetate extracts were concentrated 10-fold. Twenty-five to fifty microliters of the extracts were used for HPLC analysis. The amounts of korkormicin A and its analogs in the extracts were determined by comparison with authentic standards isolated and characterized in our laboratories.

Antitumor assay

Tests of inhibition of P388 leukemia and Madison 109 lung carcinoma in mice were performed using the previously described procedures [6,15]. Specifically, in the tests described herein, female (BALB/c × DBA/2) F_1 hybrid mice were implanted intraperitoneally (ip) with 10^6 P388 or Madison 109 cells and the test compound was administered ip on Day 1 post-implant only. Five or three ip injections of the compound were given to the mice implanted with P388 or Madison M109 cells, respectively. There were four mice in each treatment group and 10 mice in the control groups. Efficacy was evaluated by determining the relative median lifespans of the treated (T) and control (C) mice and expressing the ratios (after multiplying by 100) in terms of %T/C values. A T/C value of ≥125% was considered active.

Results

Taxonomy of the producing organism

Micromorphological examinations of strain C39500 grown on various agar media were made *in situ* at magnifications ranging from 400× to 1250× with a Nikon Microphot light microscope. Open-web type sporulation, monopodially-borne monospores borne directly on the filamentous hyphae were all observed on Yeast extract–Malt extract agar (International *Streptomyces* Project Medium No 2). Other media on which hyphal-borne and/or terminal monospores were observed included Oatmeal–Nitrate agar, Modified Bennett's agar, Glucose–Asparagine agar, Casein–Starch agar and Inorganic salts–Starch agar (International *Streptomyces* Project Medium No 4). Clusters of monospores were also noted on Casein–Starch agar and Inorganic salts–Starch agar. Cellulose thin-layer chromatography was performed on dried cells hydrolyzed with 6 N HCl, and strain C39500 was determined to contain the *meso*-isomer of diaminopimelic acid. These characteristics of strain C39500 indicate that it is a species of *Micromonospora*.

HPLC analysis of the fermentation extract of strain C39500

The production of korkormicins by strain C39500 can be monitored by HPLC analysis of the fermentation extract. An HPLC system has been developed for the separation of the analogs of korkormicins. Figure 2 shows a chromatogram of the HPLC of the extract from a 6-day-old culture of strain C39500 grown in medium 53A. Five analogs were produced by strain C39500 in this medium. These analogs were effectively separated by this HPLC system. Two other analogs which were produced by strain C39500 grown in medium 53B can also be detected by this HPLC system. The retention times of these cyclic depsipeptides are shown in Table 1.

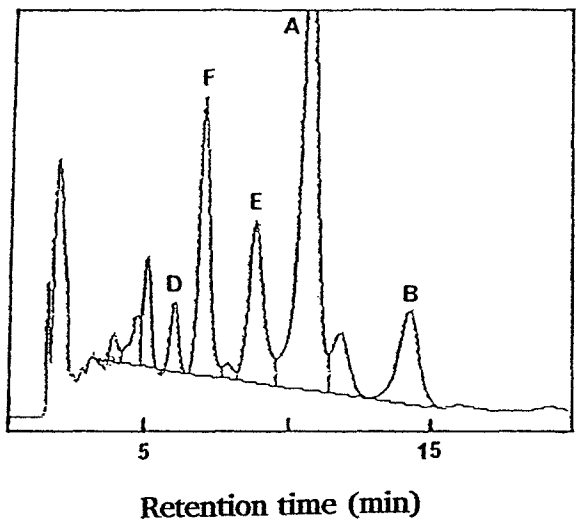


Figure 2 HPLC chromatogram of the extract of a 6-day-old shake flask culture of *Micromonospora* sp C39500 grown in medium 53A. A = korkormicin A; B = korkormicin B; D = korkormicin D; E = korkormicin E; F = korkormicin F

Table 1 Production of korkormicin complex by *Micromonospora* sp C39500 grown in medium 53A and 53B

Compound	Retention time[a] (min)	Medium 53A[b] (μg ml^{-1})	Medium 53B[b] (μg ml^{-1})
Korkormicin A	10.5	99.7	101.0
Korkormicin B	17.4	17.4	29.5
Korkormicin C	19.1	0	9.8
Korkormicin D	6.0	6.9	11.9
Korkormicin E	8.7	22.3	49.1
Korkormicin F	7.0	35.9	0
Korkormicin G	7.3	0	42.8

[a]The retention times of korkormicins were determined by HPLC using a C-18 reverse-phase column and solvent system of 0.1 M ammonium acetate/CH_3OH/CH_3CN (1/1/1) with a flow rate of 2 ml min^{-1} as described in the Materials and methods section
[b]The titers of korkormicins were determined at day 6 of the fermentation

Table 2 Production of the korkormicin complex by *Micromonospora* sp C39500 grown in medium 53A supplemented with 0.5% Bacto-liver[a] and 0.1% L-valine[b]

Compound	Korkormicin (μg ml^{-1}) produced in	
	Medium 53A + 0.5% Bacto-liver[c]	Medium 53A + 0.1% L-valine[c]
Korkormicin A	98.7	295.0
Korkormicin B	28.8	4.2
Korkormicin C	15.5	0
Korkormicin D	23.7	0
Korkormicin E	76.2	0
Korkormicin F	31.3	7.4
Korkormicin G	45.9	0

[a]0.5% Bacto-liver was added to the culture at the beginning of the production cycle
[b]0.1% L-valine was added to the culture at 48 h of the production cycle
[c]The titers of korkormicins were determined at day 6 of the fermentation

Effect of nitrogen sources on the production of korkormicin complex

Medium 53A differs from medium 53B only in the composition of nitrogen sources. Medium 53A contains Pharmamedia (cotton seed meal) and Nutrex (yeast extract) as the nitrogen sources while Bacto-liver is the sole nitrogen source in medium 53B. Table 1 summarizes the production of korkormicin complex by HPLC analyses of the extracts from a 6-day-old culture of strain C39500 grown in media 53A and 53B. The titers of korkormicin analogs reached a maximum level at day 6 of the fermentation and either levelled off or decreased with further incubation. The major metabolite produced in both media by strain C39500 was korkormicin A. The production of the other analogs of the complex was generally higher in medium 53B than in medium 53A with the exception of korkormicin F. The production of korkormicin F was observed in the culture grown in medium 53A but was absent in the culture grown in medium 53B. Korkormicin C and G were detected in the culture grown in medium 53B but were absent from the culture grown in medium 53A.

The effect of Bacto-liver on the production of korkormicin complex by strain C39500 grown in medium 53A

Table 2 summarizes the production of korkormicin complex by HPLC analysis of the extract of a 6-day-old culture of *Micromonospora* sp C39500 grown in medium 53A supplemented with 0.5% Bacto-liver. All seven analogs were detected in the shake flask culture grown in this medium. Addition of 0.5% Bacto-liver had no effect on the production of korkormicin A. The production of korkormicin C and E in medium 53A supplemented with 0.5% Bacto-liver was 1.6-fold higher than that in medium 53B (1% Bacto-liver as sole nitrogen source).

The effect of L-valine on the production of korkormicin complex by strain C39500 grown in medium 53A

L-valine was identified as a structural component of korkormicin A (data not shown). Table 2 summarizes the production of korkormicin complex by HPLC analysis of the extract of a 6-day-old culture of *Micromonospora* sp C39500 grown in medium 53A with 0.1% L-valine added at 48 h of the production cycle. The production of korkormicin A in the culture supplemented with 0.1% L-valine was 3-fold higher than the production in the control culture with no L-valine addition (Table 1). The production of the other minor analogs was reduced significantly.

Production of korkormicin A in fermenter cultures

Figure 3 shows the production and fermentation profiles of *Micromonospora* sp C39500 in 50-L fermenters containing 35 L of medium 53A and medium 53A supplemented at 48 h of the production cycle with 0.1% L-valine. The growth (% sediment) of the organism and the pH of the fermentation were very similar in both fermenter cultures. However, the production of korkormicin A was 3.1-fold higher in the fermenter culture supplemented with 0.1% L-valine (401.0 μg ml^{-1}) than that in medium 53A (131.2 μg ml^{-1}). Addition of L-valine to the fermenter culture also suppressed production of the other minor analogs in the fermentation.

Biological activities of korkormicin A

The *in vivo* antitumor activity of korkormicin A was evaluated against lymphocytic leukemia P388 in CDF_1 mice and the results are summarized in Table 3. Against ip-implanted P388 leukemia, the mice were treated with either saline (control mice) or doses of korkormicin A once daily for 5 consecutive days beginning 1 day post-tumor inoculation. Korkormicin A showed a maximum effect (45% increase in life span) at dose levels ranging from 0.05 to 0.2 mg kg^{-1} injection^{-1}. Toxicity was observed at the dosage of 0.7 mg kg^{-1} injection^{-1} as indicated by a drop of the life span of the treated mice. Korkormicin A was also evaluated ip against ip-implanted Madison 109 lung carcinoma in CDF_1 mice and was found to be effective at a dose of 0.05 mg kg^{-1} injection^{-1}, given on days 2, 6 and 10 post-implant, with an increase of life span of 31%.

The antimicrobial spectrum of korkormicin A was determined by a serial broth dilution method using Nutrient

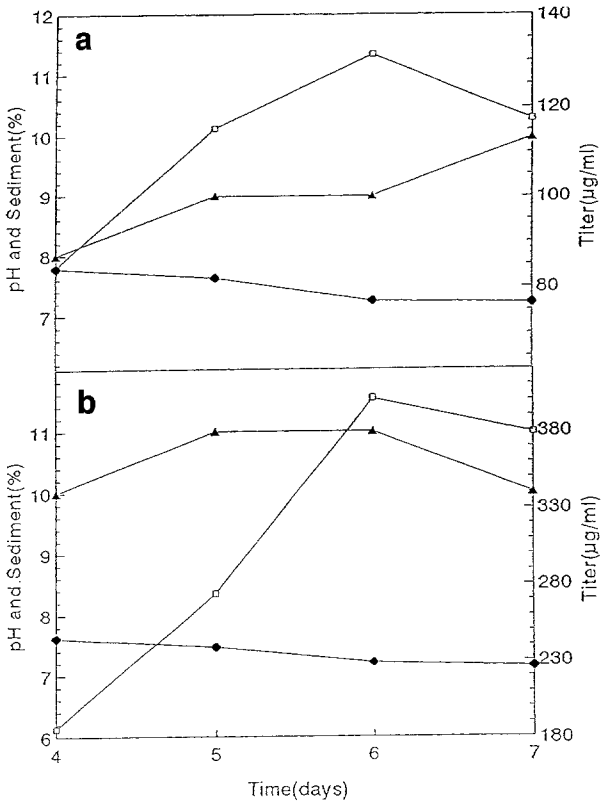


Figure 3 Time course of korkormicin A fermentation by *Micromonospora* sp C39500 grown in (**a**) medium 53A and (**b**) medium 53A supplemented with 0.1% L-valine in a 50-L fermenter. Symbols: ◆, pH; ▲, % sediment; and □, titer of korkormicin A

Table 3 Effect of korkormicin A on P388 leukemia

Treatment schedule	Dose, ip (mg kg^{-1} day^{-1})	Effect (% T/C)
QD 1–5 days	0.7	95
	0.2	145
	0.05	145
	0.02	115

Tumor inoculum: 10^6 cells, ip
Host: CDF_1 mice
Evaluation: MST = medium survival time
Effect: % T/C = (MST treated/MST control) × 100
Criteria: % T/C ≥ 125 considered significant antitumor activity
Vehicle: 10% DMSO (in saline)

Broth (Difco). The results are summarized in Table 4. Korkormicin A was effective against Gram-positive bacteria but inactive against the Gram-negative bacteria tested.

Discussion

Luzopeptins and echinomycins are cyclic depsipeptides with antitumor activity in several animal tumor systems [13,16]. The antitumor activity of these cyclic depsipeptides is due to their ability to bind DNA by the mechanism of bifunctional intercalation [3,8,9,19,20]. Luzopeptins, containing a quinoline chromophore, are produced by a strain of *Actinomadura luzonensis* [10,18]. Echinomycins, which contain a quinoxaline chromophore, are produced by a strain of *Streptomyces echinatus* [20]. In this paper, we describe the production of a complex of novel cyclic depsipeptides, designated korkormicins, structurally related to luzopeptins and echinomycins. They contain a quinoxaline chromophore like the echinomycins, and are produced by a strain of *Micromonospora* sp. However, these cyclic depsipeptides differ from echinomycins in that they have ten amino acid residues forming the cyclic peptide core as opposed to eight in echinomycins, two of which are cyclic imino acids incorporated into tetrahydropyridazine moieties. They also lack the sulfur-containing cross-bridge which is present in echinomycins. Korkormicins differ from luzopeptins in possessing quinoxaline instead of quinoline chromophores. They have however similar amino acid compositions in the core peptide.

Table 4 Antimicrobial spectrum of korkormicin A

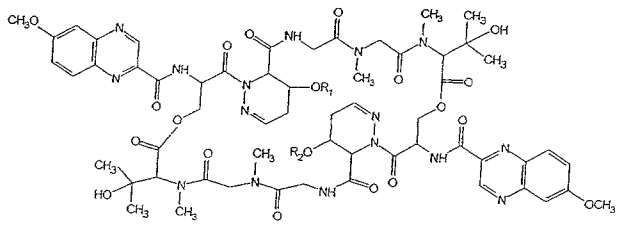
Test organisms	MIC (μg ml^{-1})
Enterococcus faecalis A20688	0.5
E. faecalis A25707 (ATCC 29212)	0.5
E. faecalis A25708 (ATCC 33186)	0.5
Staphylococcus aureus A9537	0.13
S. aureus A20698	0.25
S. aureus A24407 (ATCC 29213)	0.13
Escherichia coli A15119	>125
E. coli A20697	>125
E. coli A9751 (ATCC 33176)	>125
Klebsiella pneumoniae A9843	>125
K. pneumoniae A20468	>125
Pseudomonas aeruginosa A9843	>125
P. aeruginosa A20235 (ATCC 23389)	>125
P. aeruginosa A21508 (ATCC 27853)	>125

While developing media for improving the production of the major component of the complex, korkormicin A, we observed that the analog production profiles were very different in medium 53A and medium 53B even though the production of korkormicin A is the same in both media (Table 1). Medium 53A differs from medium 53B only in the composition of nitrogen sources. Different nitrogen sources may provide different concentrations of precursors in the fermentation and therefore yield different metabolite production profiles by this organism in different media. Several analogs were produced at higher concentration in medium 53B than in medium 53A (Table 1). The most significant difference in production was that korkormicin G, which was produced at a relatively high concentration (42.8 μg ml^{-1}) in medium 53B, was not produced in medium 53A. Bacto-liver is the sole nitrogen source in medium 53B. Supplementing the culture grown in medium 53A with 0.5% Bacto-liver also resulted in good production (45.9 μg ml^{-1}) of korkormicin G (Table 2).

Examining the structures of korkormicins (Figure 1), the cyclic depsipeptides differ from each other only at the 4-substituted position on the tetrahydropyridazine moiety. For korkormicin G, the substitutent group is a propionyl moiety. Bacterial metabolism of L-methionine produces α-

ketobutyric acid [17] and oxidative decarboxylation of α-ketobutyric acid yields propionyl-CoA. Therefore, L-methionine could be the precursor for the propionyl group on the tetrahydropyridazine moiety of korkormicin G. Liver is a rich source of vitamin B_{12} [14] and vitamin B_{12} functions as a methyl group carrier for N^5-methyltetrahydrofolate, which can methylate homocysteine to form L-methionine. We could therefore expect the L-methionine concentration in the liver-containing medium (medium 53B) to be higher than that in medium 53A. Therefore medium 53B or medium 53A supplemented with Bacto-liver is capable of supporting higher production of korkormicin G than that of medium 53A by supplying the precursor L-methionine to the fermentation.

The substituted group on the tetrahydropyridazine moiety of korkormicin A is an isobutyryl group. Transamination of L-valine produces α-ketoisovaleric acid and further oxidative decarboxylation yields isobutyryl-CoA which may serve as the precursor for the substituted group of korkormicin A. Addition of L-valine to the fermentation (Table 2 and Figure 3) significantly enhanced the production of korkormicin A by 3-fold which supports the hypothesis that L-valine is the precursor for the substituted group of korkormicin A. By similar reasoning, the other substituents on the tetrahydropyridazine moiety of korkormicin complex could be derived from L-leucine and L-alanine. The presumptive precursors for the 4-substituted group on the tetrahydropyridazine moiety of the korkormicin complex are summarized in Figure 4. Addition of L-valine to the fermentation suppressed the production of korkormicins B, E and F (Table 2) even though one of the side chains on the tetrahydropyridazine moiety of these analogs is derived from L-valine (Table 4). With such a high concentration of L-valine (0.1%) in the fermentation, it is difficult for the other amino acids (ie L-leucine, L-methionine and L-alanine) to compete with L-valine for attachment to the other side chain on the tetrahydropyridazine moiety, forming the corresponding korkormicin analog.



Compound	R_1	R_2
Korkormicin A	**Valine**	**Valine**
Korkormicin B	**Leucine**	**Valine**
Korkormicin C	**Leucine**	**Leucine**
Korkormicin D	**Methionine**	**Alanine**
Korkormicin E	**Valine**	**Methionine**
Korkormicin F	**Valine**	**Alanine**
Korkormicin G	**Methionine**	**Methionine**

Figure 4 The presumptive precursors for the 4-substituted groups of the korkormicin complex

Novel echinomycin analogs with different quinoxaline chromophores can be obtained by directed biosynthesis [4,5]. If our working hypothesis is correct, it will be possible to produce a variety of analogs of this class of cyclic depsipeptides with different chromophores and substituted groups on the tetrahydropyridazine moiety by directed biosynthesis in the microbial fermentation. SAR studies can then be carried out to select the best analog for clinical evaluation.

References

1 Becker B, MP Lechevalier and HA Lechevalier. 1965. Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl Microbiol 13: 236–243.
2 Dell A, DH Williams, HR Morris, GA Smith, J Feeney and GCK Roberts. 1975. Structure revision of antibiotic echinomycin. J Am Chem Soc 97: 2497–2502.
3 Fox KR, H Davies, GR Adams, J Portugal and MJ Waring. 1988. Sequence-specific binding of luzopeptin to DNA. Nucleic Acid Res 16: 2489–2507.
4 Gauvreau D and MJ Waring. 1984. Directed biosynthesis of novel derivatives of echinomycin by *Streptomyces echinatus*. I. Effect of exogenous analogues of quinoxaline-2-carboxylic acid on the fermentation. Can J Microbiol 30: 439–450.
5 Gauvreau D and MJ Waring. 1984. Directed biosynthesis of novel derivatives of echinomycin. II. Purification and structure elucidation. Can J Microbiol 30: 730–738.
6 Geran RI, NH Greenberg, MM MacDonald, AM Schumacher and BJ Abbott. 1972. Protocols for screening chemical agents and natural products against tumors and other biological systems. Cancer Chemother Rep (Part 3) 3: 1–103.
7 Huang C-H and ST Crooke. 1986. Effects of structural modifications of anti-tumor antibiotics luzopeptins on cell growth and macromolecule biosynthesis. Anti-Cancer Drug Design 1: 87–94.
8 Huang C-H, S Mong and ST Crooke. 1980. Interactions of a new antitumor antibiotic BBM-928A with deoxyribonucleic acid. Bifunctional intercalative binding studied by fluorometry and viscometry. Biochemistry 19: 5537–5542.
9 Huang C-H, AW Prestayko and ST Crooke. 1982. Bifunctional intercalation of antitumor antibiotics BBM-928A and echinomycin with deoxyribonucleic acid. Effects of intercalation on deoxyribonucleic acid degradative activity of bleomycin and phleomycin. Biochemistry 21: 3704–3710.
10 Konishi M, H Ohkuma, F Sakai, T Tsuno, H Koshiyama, T Naito and H Kawaguchi. 1981. BBM-928, a new antitumor antibiotic complex III. Structure determination of BBM-928 A, B and C. J Antibiot 34: 148–159.
11 Matson JA and JA Bush. 1989. Sandramycin, a novel antitumor antibiotic produced by a *Nocardioides* sp. Production, isolation, characterization and biological properties. J Antibiot 42: 1763–1767.
12 Matson JA, KL Colson, GN Belofsky and BB Bleiberg. 1993. Sandramycin, a novel antitumor antibiotic produced by a *Nocardioides* sp. II. Structure determination. J Antibiot 46: 162–166.
13 Ohkuma H, F Sakai, Y Nishiyama, M Ohbayashi, H Imanishi, M Konishi, T Miyaki, H Koshiyama and H Kawaguchi. 1980. BBM-928, a new antitumor antibiotic complex. I. Production, isolation, characterization and antitumor activity. J Antibiot 33: 1087–1097.
14 Rhodes A and DL Fletcher. 1966. Principles of Industrial Microbiology. Ch 6, Pergamon, New York.
15 Rose WC. 1981. Evaluation of Madison 109 lung carcinoma as a model for screening antitumor drugs. Cancer Treatment Rep 65: 299–312.
16 Rose WC, JE Schurig, JB Hoftalen and WT Bradner. 1983. Experimental antitumor activity and toxicity of a new chemotherapeutic agent, BBM-928A. Cancer Research 43: 1504–1510.
17 Sokatch JR. 1969. Bacterial Physiology and Metabolism. Ch 11, Academic Press, New York.

18 Tomita K, Y Hoshino, T Sasahira and H Kawaguchi. 1980. BBM-928, a new antitumor antibiotic complex. II. Taxonomic studies on the producing organism. J Antibiot 33: 1098–1102.
19 Waring MJ and KR Fox. 1983. Molecular aspects of the interaction between quinoxaline antibiotics and nucleic acids. In: Molecular Aspects of Anti-cancer Drug Action (S Neidle and MJ Waring, eds), pp 127–156, Macmillan Press, Houndmills, UK.
20 Waring MJ and LPG Wakelin. 1974. Echinomycin: a bifunctional intercalating antibiotic. Nature 252: 653–657.