MEGALOMICIN, A NEW MACROLIDE ANTIBIOTIC COMPLEX PRODUCED BY *MICROMONOSPORA*

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A new macrolide antibiotic complex has been isolated from fermentation broths of two strains of a new species of *Micromonospora*, *M. megalomicea* sp. n. The antibiotic complex has been differentiated from other known macrolides and methods for its production, isolation and separation of components have been devised. Like other macrolides, it is primarily active against gram-positive bacteria and is more active at an alkaline pH. Of the four known components, megalomicin A has been studied most extensively. It has activity equal to or slightly less than that of erythromycin both *in vitro* and *in vivo*. In dogs dosed orally, it is better tolerated and gives higher peak serum levels with a much greater duration than erythromycin.

In a continuing search to examine the micromonospora as producers of antimicrobial substances, a new member of the genus was found which produces a novel macrolide antibiotic complex[1]. This new antibiotic complex has been named megalomicin and was isolated from fermentation broths of two strains of a new species of *Micromonospora*. This report presents initial data concerning the chemical and biological characteristics of the novel complex produced. Earlier studies have revealed the micromonospora to produce a variety of antibiotics.[2,3,4]

Materials and Methods

The organism producing the megalomicin complex has been named *Micromonospora megalomicea* sp. n.; taxonomic studies establishing the validity of this novel species on the basis of biochemical and morphological characteristics are in preparation. Two strains have been found which are natural color variants and are distinguished microscopically by the degree to which sporulation occurs. Cultures of the organisms have been deposited in the stock culture collection of the U. S. Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois, where they have been designated as NRRL 3274 and NRRL 3275.

For laboratory production of megalomicin, the growth from an agar slant is inoculated into flasks containing a germination medium with the composition as shown in Table 1. This is incubated at 35°C for 72 hours on a rotary shaker. A 5 % inoculum is transferred to a fermentation medium (Table 1) and incubated for 60~70 hours at 31°C in shake flasks or in fermentors.

Antibiotic potencies were determined by means of a cylinder cup agar diffusion assay similar to that described for erythromycin[5] for which *Sarcina lutea* ATCC 9341 was the

Table 1. Media for production of megalomicins

Germination medium		Fermentation medium	
Bacto beef extract (Difco)	3.0 g/liter	Bacto yeast extract (Difco)	5.0 g/liter
Bacto tryptone (Difco)	5.0	Dextrose	10.0
Dextrose	1.0	Starch	20.0
Potato starch	24.0	Casein hydrolysate	5.0
Bacto yeast extract (Difco)	5.0	$CaCO_3$	4.0
$CaCO_3$	2.0	Tap water	Q.S.
Tap water	Q.S.		

test organism. A unit of activity of the megalomicin complex is the amount of material which produces a zonal response of 17.8±0.5 mm under the conditions of this assay, and has been defined as one microgram.

For determinations of *in vitro* sensitivity, all test organisms were incubated in yeast-beef broth at 37°C for 18~20 hours, except where indicated.

Animal studies were carried out in CF-1 male albino mice weighing 18~20 g each and beagle type dogs of both sexes weighing approximately 10 kg each. Drug suspensions were prepared in 0.5 % aqueous carboxymethyl cellulose and ultrasonicated to reduce particle size. In therapeutic tests, animals were treated twice, shortly before and 4 hours after intraperitoneal challenge with approximately 10^7 organisms/mouse. Control infected mice died in 18~24 hours. Survivors in treated groups were determined 48 hours after infection. PD_{50} and LD_{50} values were determined by probit procedures.

Results and Discussion

Isolation and Characterization

The megalomicin complex can be isolated by adjusting the fermentation broth to pH 9.5, extracting with ethyl acetate, and concentrating to a small volume. Partial purification is achieved by chromatography on a column of LH 20 Sephadex using aqueous ethanol as the eluent. Active fractions are combined, concentrated and additional impurities removed by precipitation with petroleum ether (b.p. 30~60°C).

Megalomicin has been shown to be different from other antibiotics except the macrolide group by paper and thin-layer chromatography. Studies to be reported in this paper and additional chemical data[6] clearly indicated the antibiotic to be a novel desosamine-containing macrolide. By thin-layer chromatography on silica gel G plates, megalomicin can be readily differentiated from other macrolide antibiotics as seen in Table 2. Although the Rf's of the

Table 2. Comparative thin-layer chromatography of megalomicin and other macrolides

System	Antibiotic	Rf-Spot color*	Rf Inhibition zone
I	Megalomicin	0.98 blue-black	0.98
	Oleandomycin	0.98 green	0.98
	Erythromycin	0.98 yellow-tan	0.98
	Magnamycin	0.98 blue-purple	0.98
II	Megalomicin	0.13 red-purple	0.13
	Erythromycin	0.26 green-brown	0.26
	Spiramycin	0.06, 0.30 purple, green	0.06
	Oleandomycin	0.19 green	0.19
	Magnamycin	0.40, 0.47 purple, red-purple	0.40

* H_2SO_4 spray, heat at 100°C for 3~5 minutes.
Plated against *S. aureus* ATCC 6538P.
Solvent system: I; $CHCl_3$ - methanol - 17 % NH_4OH (2 : 1 : 1).
II; Butanol - acetic acid - water (3 : 1 : 1).

macrolides in the chloroform-methanol-ammonia system are identical, distinct differences in the colors of the spots are produced by use of a sulfuric acid spray and subsequent heating. In the butanol-acetic acid-water system, differences in Rf values and colors are seen. Thin-layer chromatography in a solvent system consisting of 60 parts chloroform to 40 parts methanol, indicates that megalomicin is composed of four major active components (Fig. 1). These are identified as A, B, C_1, and C_2 respectively. The complex was assigned a potency of 1,000 units/mg by the previously described assay. Separation of these four megalomicin components has been presented.[6,7] In the assay described, the components exhibit the following potencies: A=625 units/mg; B=305 units/mg; C_1=3,800 units/mg; C_2=4,800 units/mg. Each pure component base has been assigned a potency of 1,000 mcg/mg and all subsequent assays are based on their respective base component standards.

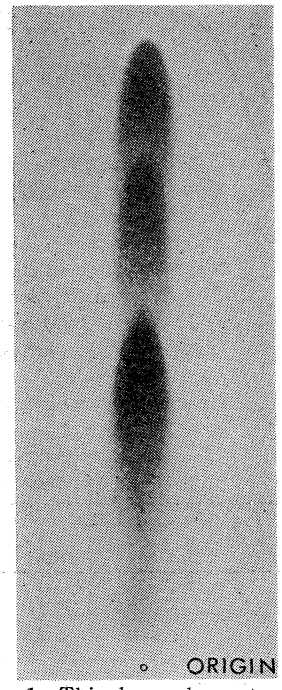

Fig. 1. Thin-layer chromatogram of megalomicin complex, silica gel G, chloroform-methanol (3 : 2) solvent system. Visualized by sulfuric acid spray. Components are, from origin, A, B, C_1 and C_2 respectively.

The megalomicin components are generally soluble in polar organic solvents such as ethanol, acetone and methanol with only slight water solubility.

Megalomicins A and B are stable from pH 6 to 10 and the C_1 and C_2 components are stable from pH 4 to 10 at temperatures up to 100°C for 30 minutes when tested against *B. subtilis*.

Due to the development of a simple method of conversion, as described elsewhere[7], megalomicin A has been studied most extensively. Much of the remainder of this paper will deal primarily with megalomicin A as the base.

In Vitro Activity

The megalomicin complex has *in vitro* activity against a variety of gram-positive bacteria with minimal activity against gram-negative bacteria (Table 3). The megalomicin complex has activity approximately equal to or 1/2 that of erythromycin depending upon the organism.

As shown in Table 4, the 4 megalomicin components have a similar spectrum of activity. The C_2 component is most active and the B component is least active. The A component has the highest activity against gram-negative bacteria.

Clinical isolates of *Staphylococcus* and *Streptococcus* strains which are resistant to erythromycin were, with only one exception (strain 386), cross-resistant to megalomicin (Table 5).

As with other macrolides, megalomicin had greatly enhanced activity at higher pH levels.

Serum has a variable effect on the *in vitro* activity of megalomicin; occasionally minimum inhibitory concentration (MIC) values are increased in the presence of

Table 3. *In vitro* activity of megalomicin complex and erythromycin base*

Organism	MIC (mcg/ml)		
	No. Strains	Megalomicin complex	Erythromycin base
Bacillus subtilis	1	0.03	0.03
Bacillus megaterium	1	0.3	0.08
Diplococcus pneumoniae	2	0.05～0.5	0.05～0.2
Enterococcus sp.	3	0.3	0.3
Sarcina lutea	1	0.008	0.006
Staphylococcus aureus	10	0.03～0.3	0.01～0.75
Streptococcus faecalis	1	0.3	0.005
Streptococcus pyogenes	3	0.3 ～0.75	0.08～0.3
Mycobacterium smegmatis	1	0.7	5.0
Escherichia coli	1	6.0	7.5
Klebsiella pneumoniae	1	12.0	7.5
Proteus vulgaris	1	6.0	17.5
Pseudomonas aeruginosa	1	6.0	12.5
Salmonella schottmuelleri	1	6.0	7.5

* Medium: Yeast-beef broth, pH 7.8.

Table 4. *In vitro* activity of megalomicin components*

Organism	No. Strains	MIC (mcg/ml)			
		A	B	C_1	C_2
Bacillus subtilis	1	0.3	0.05	0.3	0.005
Bacillus megaterium	1	0.75	1.2	0.75	0.6
Diplococcus pneumoniae	2	0.5 ～1.2	0.5～1.2	0.5 ～1.2	0.05～0.6
Enterococcus sp.	3	0.3	0.2～0.6	0.3	0.03～0.6
Sarcina lutea	1	0.02	0.005	0.003	0.0005
Staphylococcus aureus	10	0.075～0.75	0.2～0.6	0.01～0.75	0.03～0.6
Streptococcus faecalis	1	0.075	0.1	0.075	0.005
Streptococcus pyogenes	3	0.75	0.2～5.0	0.3 ～0.75	0.03～0.6
Mycobacterium smegmatis	1	0.3	5.0	0.3	0.05
Escherichia coli	1	3.0	12.0	7.5	12.0
Klebsiella pneumoniae	1	6.0	24.0	12.5	24.0
Proteus vulgaris	1	6.0	24.0	12.5	24.0
Pseudomonas aeruginosa	1	3.0	12.0	7.5	6.0
Salmonella schottmuelleri	1	3.0	12.0	7.5	12.0

* Medium: Yeast beef broth, pH 7.8.

Table 5. *In vitro* activity of megalomicin against erythromycin resistant clinical isolates*

Organism	MIC (mcg/ml)		
	Megalomicin A	Megalomicin C_1	Erythromycin base
Staphylococcus aureus DA 2033	>32.0	> 32.0	> 32.0
Staphylococcus aureus DA 303	18.0	38.0	38.0
Staphylococcus aureus DA 309	38.0	38.0	38.0
Staphylococcus aureus DA 383	>75.0	18.0	> 75.0
Staphylococcus aureus DA 386	>75.0	3.0	63.0
Staphylococcus aureus DA 388	>75.0	63.0	63.0
Staphylococcus aureus DA 426	>75.0	> 75.0	7.5
Streptococcus pyogenes DC 9	>75.0	> 75.0	> 75.0
Streptococcus pyogenes DC 31	>75.0	63.0	> 75.0
Streptococcus pyogenes DC 33	>75.0	63.0	> 75.0
Streptococcus pyogenes DC 76	18.0	18.0	8.0
Streptococcus pyogenes DC 77	18.0	38.0	18.0
Streptococcus pyogenes DC 80	18.0	18.0	8.0

* Medium: Yeast beef broth, pH 7.8.

serum. Serum binding is of the same order as erythromycin and when determined by dialysis ranged from 20 to 30% for the megalomicin components. As with erythromycin, megalomicin is bactericidal against some organims and only bacteristatic against others.

In Vivo Activity

The acute toxicity of megalomicin A base in male Carworth CF-1 mice is shown in Table 6 in comparison with erythromycin base. Both antibiotics were found to have similar toxicities. Mice tolerated daily subcutaneous doses of 500 mg/kg/day for 21 days with only slight initial weight loss.

As shown in Table 7, megalomicin A base was tested for protective activity against a variety of acute lethal infections in mice. As with erythromycin, megalomicin A was more active parenterally than orally. Megalomicin has the same or 1/2 the activity of erythromycin against gram-positive infections, depending on the organism, and is slightly more active than erythromycin against gram-negative infections.

The absorption of megalomicin A base was compared with erythromycin base in dogs after a single oral dose of 500 mg/dog which approximated 50 mg/kg (Table 8).

Megalomicin A base was well tolerated while erythromycin base was emetic in approximately 1/2 of the dogs studied. The data in Table 8 are averages from 9 dogs dosed with megalomicin A base and 7 dogs dosed with erythromycin base. Only dogs in which emesis did not occur are included for erythromycin.

Table 6. Acute toxicity of megalomicin A and erythromycin base in mice

	Route	Megalomicin A base	Erythromycin base
Acute LD_{50} (mg/kg)	Oral	7,500	7,500
	S. C.	7,000	8,000
	I. P.	350	500
Subacute LD_{50} (mg/kg/day)	S. C. 21 days	>500	> 500

Table 7. Protective activity of megalomicin A and erythromycin in mice*

Infecting organism	PD_{50} (mg/kg)			
	Megalomicin A		Erythromycin	
	Oral	S. C.	Oral	S. C.
Streptococcus pyogenes No. 22	180	180	100	50
Streptococcus pyogenes No. 9	250	167	120	140
Staphylococcus aureus W	117	20	53	20
Staphylococcus aureus No. 41	150	20	75	20
Diplococcus pneumoniae No. 2	250	70	134	75
Escherichia coli ATCC 10536	200	100	>250	160
Klebsiella pneumoniae DA 20	250	150	>250	>250
Pseudomonas aeruginosa ATCC 8689	>250	161	>250	130

* Each preparation was tested as a suspension in 0.5% carboxymethyl cellulose. Treatment was divided into two doses and given 30 minutes before and 4 hours after intraperitoneal infection. Survivors were determined 48 hours after infection.

Table 8. Oral absorption in dogs after a single dose (50 mg/kg)*

	No. Dogs	Serum levels (mcg/ml) at time (hrs.)								
		0	1	2	4	6	24	48	72	96
Megalomicin A base	9	0	9.3	7.8	3.5	3.0	0.8	0.3	0.3	0
Erythromycin base	7	0	4.9	3.6	1.9	1.1	0	0	0	—

	No. Dogs	Urine levels total mg excreted		Percent of dose excreted	
		0~24 hrs.	25~48 hrs.	0~24 hrs.	48 hrs.
Megalomicin A base	9	65.2	15.4	13.0	3.0
Erythromycin base	7	59.7	0.5	11.9	0.1

* Drug was administered in gelatin capsules.

Megalomicin A base was absorbed considerably better than erythromycin in dogs, resulting in higher peak levels and serum levels of greater duration. The prolonged blood levels are also reflected by urine levels, in particular the considerable amount excreted in the 25~48 hour period in dogs given megalomicin A base.

Literature Cited

1) Weinstein, M. J.; G. H. Wagman, J. Marquez, G. Luedemann, E. Oden & J. A. Waitz: Megalomicin. I. New *Micromonospora*-produced macrolide antibiotic complex. Abstracts of Papers, Eighth Interscience Conference on Antimicrobial Agents and Chemotherapy p. 4, 1968 (Oct. 21~23, 1968, New York)
2) Weinstein, M. J.; G. M. Luedemann, E. M. Oden & G. H. Wagman: Gentamicin, a new broad-spectrum antibiotic complex. Antimicr. Agents & Chemoth. -1963 : 1~7, 1964.
3) Weinstein, M. J.; G. M. Luedemann, E. M. Oden & G. H. Wagman: Everninomicin, a new antibiotic complex from *Micromonospora carbonacea*. Antimicr. Agents & Chemoth. -1964 : 24~32, 1965.
4) Weinstein, M. J.; G. M. Luedemann, E. M. Oden & G. H. Wagman: Halomicin, a new *Micromonospora*-produced antibiotic. Antimicr. Agents & Chemoth. -1967 : 435~441, 1968.
5) Grove, D. C. & W. A. Randall: Assay methods of antibiotics, a laboratory manual. Medical Encyclopedia Inc., New York, 1955.
6) Marquez, J.; G. H. Wagman, R. T. Testa & M. J. Weinstein: Megalomicin. II. Fermentation and isolation of the antibiotic. Abstracts of Papers, Eighth Interscience Conference on Antimicrobial Agents and Chemotherapy p. 4, 1968 (Oct. 21~23, 1968, New York)
7) Reimann, H.; R. S. Jaret & A. K. Mallams: Megalomicin. III. Purification and chemical studies. Abstracts of Papers, Eighth Interscience Conference on Antimicrobial Agents and Chemotherapy p. 4, 1968 (Oct. 21~23, 1968, New York)