

PATENT SPECIFICATION

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(54) MEGALOMICIN COMPLEX AND METHODS FOR ITS PRODUCTION

(71) We, SCHERICO LTD., of Töpferstrasse 5, Lucerne, Switzerland, a body corporate constituted under the laws of Switzerland, do hereby declare the invention for which we pray that a Patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a new complex of antibiotics, and to methods for their production, isolation, and purification. More specifically, this invention relates to new and useful antibiotics obtainable by the cultivation of hitherto undescribed microorganisms of the genus *Micromonospora* of the order *Actinomycetales*.

THE MICROORGANISM

The hitherto undescribed microorganisms of the genus *Micromonospora* which were employed in the first production of the new complex of antibiotics of this invention were isolated originally from a soil sample obtained directly from the earth by one of the inventors named herein and has been designated *Micromonospora Megalomicea*. Two strains of this *Micromonospora* species have been isolated and found of particular use in the process of the invention. These two strains are natural color variants and in addition may be distinguished microscopically by the degree to which sporulation occurs. These strains have been named *Micromonospora megalomicea* var. *megalomicea* and *Micromonospora megalomicea* var. *nigra*. Cultures of living organisms of these two strains have been deposited and made a part of the stock culture collection in the United States Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Illinois, where they have been designated NRRL 3274 and NRRL 3275 respectively. Samples of these cultures are available from the collection agency upon request.

Macroscopic examination of 28 day old cultures (24—26° C.) of *Micromonospora megalomicea* var. *megalomicea* on Czapek's Sucrose

Agar is characterized by good growth exhibiting no aerial mycelium and by raised and plicate colonies of a soft, moist and non-waxy consistency producing no diffusible pigment. The color of the colony surface, described according to the "Descriptive Color Name Dictionary", Taylor, Knoche and Granville, published by the Container Corporation of America, 1950 (USA), together with the color chip number taken from the "Color Harmony Manual", 4th Edition, 1958, published by the same company, is orange m4-1a. This color corresponds to the synonym or near synonym, strong orange 50, as found in the National Bureau of Standards Circular No. 553, November 1, 1955, USA (all colony color designations employed hereafter are based upon the same color reference system).

Microscopic examination of the same culture shows a regular mycelium composed of long branched filaments approximately 0.6 μ in diameter. Spores are infrequently found.

Macroscopic examination of 28 day old cultures of *Micromonospora megalomicea* var. *nigra*, grown on the same medium and under the same conditions described above, is characterized by good growth exhibiting the same gross colony characteristics as the previous strain except for color. The color of the colony surface is black. Microscopically, this culture shows a regular mycelium composed of long branched filaments approximately 0.6 μ in diameter. In this strain, however, spores are very abundant and are randomly produced throughout the mycelium. The spores are ovoid to spherical in shape with a 0.7—1.0 μ diameter and are dark brown when mature.

Colonies of *Micromonospora megalomicea* may be further characterized by their growth characteristics under aerobic conditions on various nutrient media. Table 1 below gives the growth characteristics of *Micromonospora megalomicea* on some of the more widely used nutrient media. The colony observations were made on 14 day old (24—26° C.) cultures unless otherwise specified.

TABLE 1

COLONY CHARACTERISTICS OF MICROMONOSPORA
MEGALOMICEA ON VARIOUS MEDIA

Glucose-Asparagine Agar	Growth poor
Bennett's Agar	Growth poor
Emerson's Agar	Growth poor
Tomato Paste-Oatmeal Agar	Growth poor
Glucose-Yeast Extract Agar	Growth good, raised, finely plicate
Potato Slice	Growth poor
Sucrose Nitrate Agar (Czapek's Agar)	Growth good, raised, plicate
Tyrosine Agar	Growth fair, crystals poorly dissolved directly under colony, brown diffusible pigment produced after 3 weeks, diffusible pigment not black or typical of a tyrosine positive as produced by streptomyces
Observations after 2, 7 and 14 days (Gordon and Smith, J. Bac. 69,147)	

5 *Micromonospora megalomicea* shows particularly good growth at temperatures between 26 and 37° C. but no substantial growth at temperatures as high as 50° C. It is aerobic and does not reduce nitrate to nitrite. This species does not liquify gelatin; does not hydrolyze milk and does not decompose cellulose. No diffusible pigment is produced on peptone-iron agar.

10 *Micromonospora megalomicea* shows good growth on carbohydrate media containing 0.5 percent yeast extract base and 1 percent of l-arabinose, glucose, starch, and sucrose but

only fair growth where the carbohydrate source is 1 percent xylose. It grows poorly on such media in which the sole carbohydrate source is 1 percent d-arabinose, cellulose, dulcitol, galactose, glycerol, lactose, laevulose, melibiose, melizitose, raffinose, rhamnose, ribose, inositol or mannitol. 15 20

Further characteristic of *Micromonospora megalomicea* is its ability to utilize nitrogen sources. Colony growth characteristics on typical nitrogen sources, plus 1% glucose, observed after 14 days (24—26° C.) growth, are set forth in Table 2 below. 25

TABLE 2
UTILIZATION OF NITROGEN SOURCES

Nitrogen Source	Growth Characteristics
0.5% Difco** Yeast Extract	Growth good, raised, finely plicate
1.0% NZ Amine Type A*	Growth fair
1% Asparagine	Growth poor
1% Glutamic Acid	Growth poor
1% Ammonium Nitrate	Growth poor

* Product of Sheffield Chemical Company, Norwich, New York

** "Difco" is a Registered Trade Mark.

The novel antibiotic complex hereinafter to be described and which has been named "megalomicin complex", or a constituent antibiotic thereof, may be produced by incubating a megalomicin complex-producing micro-organism (or strain) of the species *Micromonospora megalomicea*, in an aqueous nutrient medium, under aerobic conditions. The micro-organism utilised may be one of the variants *Micromonospora megalomicea* var. *megalomicea* or *Micromonospora megalomicea* var. *nigra*, for example *Micromonospora megalomicea* var. *megalomicea* NRRL 3724, *Micromonospora megalomicea* var. *nigra* NRRL 3725 or a descendant thereof or a mutant thereof produced from these organisms by mutating agents such as, for example, high frequency radiation including x-ray and ultraviolet, actinophages and nitrogen mustard. In the following description, wherein the process for antibiotic production is elaborated, it is to be understood that the use of the name *Micromonospora megalomicea* is intended to include the color variants specifically described above as well as all megalomicin complex-producing variants of this species such as megalomicin complex-producing mutants and descendants of the described microorganisms.

THE ANTIBIOTIC

(1) Preparation of Megalomicin Complex

The complex mixture of antibiotic substance hereinafter referred to as Megalomicin Complex may be produced from a suitable micro-organism by the controlled fermentation techniques described hereafter.

In order to prepare a suitable inoculum for subsequent fermentation, the microorganism is

preferably grown under submerged aerobic conditions with continual agitation at temperatures ranging from about 22° to about 37° C. for a period of from about 2 to about 4 days. Generally, a temperature of 35° C. over a 3 day period is found most satisfactory. The medium normally used in this first germination stage is aqueous and contains quantities of carbon and nitrogen sources assimilable by the organism. The alkalinity of the media desirable is adjusted to about pH 7.5 before autoclaving by the addition of base such as sodium hydroxide.

Exemplary of media employed in the germination stage, are the following: *Medium I*: Bacto-Beef Extract (Difco), 3.0 g; Bacto-Tryptone (Difco), 5.0 g; dextrose, 1.0 g; starch (potato), 24.0 g; Bacto-Yeast Extract (Difco), 5.0 g; CaCO₃, 2.0 g; tap water, 1000 ml; *Medium II*: Bacto-Yeast Extract (Difco), 5.0 g fish solubles, 1.0 g; corn steep liquor, 1.0 g; dextrose, 20.0 g, CaCO₃, 1.0 g; tap water, 1000 ml.

Desirably, a second germination stage (e.g. inoculum stage) is employed prior to fermentation and harvesting of the antibiotic in order to enrich the inoculum. Employing media as described above, fresh medium can be inoculated with the germinated culture previously prepared (5% weight/volume) and incubated at about 22 to about 37° C. for about 2 to about 4 days. Usually, a temperature of 28° C. over a period of 3 days is found most satisfactory.

In order to produce the Megalomicin Complex, a 5 percent (weight/volume) inoculum taken from the inoculum stage may be added to aqueous fermentation medium which, prior to autoclaving, has been adjusted to about pH 7.1 to 7.8. Air, usually about 3 to about 6

liters per minute, is introduced into the fermentation which, generally, is conducted at a temperature between about 22 to 40° C. and at a pH of from 7.0 to 8.5, preferably at a temperature of from 28 to 37°C. and at a pH of from 7.2 to 8.2 over a period of about 2 to about 6 days with continuous agitation. Particularly with reference to Medium III, described below, it has been found that maximum yields of Megalomicin Complex are obtained following fermentation for 66 hours at 31° C. with introduction of air at a rate of 4.5 liters per minute and with impeller agitation at 250 rotations per minute (rpm). Using 14-liters-fermenters containing 10 liters of medium, yields ranging from 300 to 800 µg/ml have been obtained under these conditions.

Media useful in the fermentation stage include, for example, the following: *Medium III*: sucrose, 30.0 g; dextrose, 2.5 g; trypticase, 17.0 g; NaNO₃, 3.0 g; K₂HPO₄, 3.5 g; MgSO₄·7H₂O, 0.5 g; KCl, 0.5 g; FeSO₄, 0.01 g; NaCl, 5.0 g; tap water, 1000 ml; *Medium IV*: Bacto-Yeast Extract (Difco), 5.0 g; dextrose, 10.0 g; starch, 20.0 g; casein hydrolysate, 5.0 g; CaCO₃, 4.0 g; tap water, 1000 ml; *Medium V*: sucrose, 20.0 g; peptone, 5.0 g; cotton seed protein hydrolysate, 5.0 g; CaCO₃, 4.0 g; tap water, 1000 ml; *Medium VI*: sucrose, 30.8 g; peptone, 8.0 g; NaNO₃, 3.0 g; K₂HPO₄, 1.0 g; MgSO₄·7H₂O, 0.5 g; FeSO₄, 0.01 g; tap water, 1000 ml; *Medium VII*: trypticase, 17 g; NaCl, 5.0 g; K₂HPO₄, 2.5 g; dextrose, 2.5 g; Czapek-Dox broth, 35.0 g; tap water, o.s. 1000 ml.

For recovery of Megalomicin Complex from the fermentation broth, one may proceed as follows: The whole broth is adjusted to about pH 9.5 with base such as, for example, sodium hydroxide. The whole broth then is extracted with about 2 volumes for each volume of broth of a suitable water-immiscible organic solvent, such as ethyl acetate, methylene dichloride, butyl acetate, chloroform and butanol. The solvent phase is next concentrated under vacuum to a small volume, approximately 100-fold concentration, and is purified by column chromatography employing, for example, LH 20 Sephadex, an alkylated cross-linked dextran, (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden; "Sephadex" is a Registered Trade Mark) suspended in about 95 percent aqueous ethanol. The column is eluted with the aqueous ethanol. Column fractions are combined according to their anti-bacterial activities as evidenced by agar disc testing against *Staphylococcus aureus* (ATCC 6538P), concentrated to dryness, dissolved in a small amount of ethyl ether or acetone and added to an excess volume of petroleum ether (b.p. about 30 to about 60° C.). The mixture is then transferred to a dry-ice/acetone bath (approximately -50° C) and allowed to stand for about 20 minutes

after which it is allowed to return to room temperature. The mother liquor is separated from any oily residue by decanting and is concentrated to dryness. Material produced in this manner is usually found to have potency of about 232 µg/mg according to the bioassay procedure described below.

The assay is a cylinder cup assay using *Sarcina Lutea* (ATCC 9341) as the test organism. The conditions of the assay are similar to the cylinder plate assay described for erythromycin (Grove and Randall, 1955, Med. Encyclopedia, Inc., N.Y.). A µg of Megalomicin Complex activity is the amount of material which produces a zonal response of 20.8 ± 0.5 mm under the conditions of this assay.

Alternatively, Megalomicin Complex may be prepared by utilizing the following fermentation and isolation techniques:

Employing Medium I for the preparation of an inoculum, fermentation flasks containing this medium are inoculated with a suitable culture (approximately 5% weight/volume) of *Micromonospora megalomicea*. The flasks are placed on a rotary shaker running at about 280 strokes per minute and are incubated at 28° C. for 72 hours. At the end of this time, fermentors, each charged with fermentation medium (Medium VII for example), adjusted to pH 7.15 to 7.25, are inoculated with approximately 5% (volume/volume) of the 72 hour inoculum culture. The fermentation medium is agitated at a rate of about 500 rpm at a temperature of 31° C. Air flow is induced through the medium at a rate of about 0.5 liters of air/liter of broth/minute. The agitation rate may be increased to 600 rpm after 24 hours and to 700 rpm after 48 hours. Fermentation is terminated at the end of about 69 hours. The broth from all fermentors is pooled for extraction and recovery of Megalomicin Complex as described below.

The pooled whole fermentation broth is adjusted to about pH 9.25 with aqueous sodium hydroxide (50%) and is extracted with about 2 volumes of ethyl acetate and the extracts are concentrated to about one to two percent of the original volume. The ethyl acetate concentrate is then extracted at least twice with half volumes of hydrochloric acid (about 0.14N). Alternatively, the acid extraction can be carried out with other mineral acids at about the same normality. The combined acid extracts are made slightly alkaline with 5% aqueous sodium hydroxide (to about pH 8.5 to 9.0) and are extracted at least twice with about equal volumes of ethyl acetate. The combined ethyl acetate extracts are concentrated to dryness under reduced pressure to obtain the Megalomicin Complex. The complex at this stage will show a potency of 450 to 550 µg/mg in the assay described above.

(2) *Characterization of Megalomycin Complex*
Megalomycin Complex was compared by paper chromatography with a variety of antibiotics, including several macrolides. In a series of solvent systems, as shown in Table 3

below, Megalomycin complex moves as a single spot and is differentiated from all other antibiotics except the macrolides which were tested (i.e. magnamycin, oleandomycin, spiramycin and erythromycin).

TABLE 3

COMPARATIVE R_f VALUES OF MEGALOMICIN COMPLEX

R_f 's of Antibiotics

Paper Chromatographic Systems	Megalomycin Complex	Magnamycin	Oleandomycin	Erythromycin	Spiramycin	Penicillin	Kanamycin	Chloramphenicol	Chlortetracycline	Gentamicin	Streptomycin	Tetracycline
80% Methanol plus 3% Sodium Chloride (weight/volume) 1:1 descending *	0.90	0.90	0.90	0.90	0.90	0.63	0.30	0.84	0.74	0.59	0.57	0.72
Propanol:Pyridine:Acetic Acid:Water (6:4:1:3) ascending (volume/volume)	0.93	0.93	0.93	0.93	0.93	0.91	0.18	0.91	0.75	0.26	0.40	0.70
Butanol:Acetic Acid:Water (4:1:5) ascending (volume/volume)	0.78	0.78	0.78	0.78	0.78	0.85	0.00	0.92	0.46	0.00	0.00	0.38
80% Phenol ascending (volume/volume)	0.86	0.86	0.86	0.86	0.86	0.81	0.17	0.93	0.87	0.50	0.06	0.83

*) Paper buffered with 0.95 molar $\text{Na}_2\text{SO}_4 + 0.05$ molar NaHSO_4 .

- Differentiation of Megalomicin Complex from these important macrolide antibiotics was made by thin layer chromatography on silica gel GF plates (Analtech Inc., Wilmington, Delaware). Zones were detected by the following two methods:
- Plates are sprayed with a mixture of concentrated sulfuric acid-methanol (1:1 volume/volume) and developed by heating at 105° C. for several minutes. The zones of organic material appear as colored areas against a white background.
 - Plates are allowed to remain in contact with an agar layer seeded with *S. aureus*. A sheet of Whatman No. 1 paper is placed between the seeded agar layer and the plate to insure that the silica gel does not adhere to the agar. The paper and silica gel are removed after 15 minutes. The agar plate is incubated at 37° C. overnight and the zones of inhibition evaluated.
- Results of these experiments are shown in Table 4 below.

TABLE 4
COMPARATIVE THIN LAYER CHROMATOGRAPHY OF MEGALOMICIN COMPLEX

System	Antibiotic	R _f and Color by H ₂ SO ₄ Spray	R _f of Inhibition Zone
Chloroform:Methanol: 17% Ammonia 2:1:1 (volume/volume)	Megalomicin Complex oleandomycin erythromycin magnamycin	blue black 0.98 green 0.98 yellow tan 0.98 blue purple 0.98	0.98 0.98 0.98 0.98
Butanol:Acetic Acid: Water 3:1:1 (volume/volume)	Megalomicin Complex erythromycin spiramycin oleandomycin magnamycin	red purple 0.13 green brown 0.26 purple; gray 0.06;0.30 green 0.19 purple 0.40;0.47 red purple	0.13 0.26 0.06 0.19 0.40

- Further, by comparison of the physical, chemical and biological properties of Megalomicin Complex, and of its separated fractions, as hereinafter described with properties of known macrolides as reported in the literature, Megalomicin Complex and its components are distinguishable from all known macrolide antibiotics.
- Megalomicin Complex gives a positive color reaction in the Ninhydrin, Starch—KI and Biuret tests and a negative color reaction in the Stannous Chloride, Molisch and Sakaguchi tests. The complex is essentially transparent in the ultra-violet region. The activity of Antibiotic W847 Complex is not significantly altered when a solution thereof is subjected to a temperature of 100° C. for 30 minutes throughout the pH range of 2 to 10 (stability is tested by dissolving the antibiotic in ethanol, diluting with buffer, and disc testing against *S. aureus* and *Escherichia coli*). The complex shows no loss in activity after treatment with trypsin, chymotrypsin, pepsin, α -amylase or penicillinase in buffered solutions (optional pH for each enzyme) at 37° C. for up to 24 hours exposure. Megalomicin Complex is soluble in

most polar organic solvents such as ethyl acetate, methanol, ethanol and acetone, methylene dichloride, butyl acetate, chloroform and butanol.

- (3) *Resolution of Megalomicin Complex*
- Bioautography of thin layer silica gel plates on agar seeded with *Sarcina lutea* following chromatography in a solvent system consisting of chloroform:methanol (60:40 volume/volume) indicates that Megalomicin Complex consists of at least four antibiotic components which will be identified hereafter as Megalomicins A, B, C₁ and C₂. These antibiotics, in the solvent system described above, show R_f values of 0.19 (Megalomicin A), 0.38 (Megalomicin B), 0.52 (Megalomicin C₁) and 0.65 (Megalomicin C₂) respectively.
- Separation of the constituent antibiotics may be achieved by dissolving Megalomicin complex in acetone mixed with silicic acid, evaporating the acetone and adding the dry mixture to the top of a silicic acid column. The column is eluted with chloroform:methanol (60:40 volume/volume) and fractions are collected. The column is monitored by disc test-

ing each fraction against *S. aureus* and *E. coli*. The active fractions are chromatographed on silica gel thin layer plates run for about 1.5 hours in the same solvent system used for the column. The patterns of the antibiotic fractions are detected by the two methods previously described. Fractions are combined according to their chromatographic patterns and are concentrated.

By this method Megalomicin C₂ as a hydrochloride salt is separated and isolated as a white powder by dissolving the pooled active fractions in methanol and precipitating with about 10 volumes of ethyl ether. Megalomicin C₂ hydrochloride at this stage exhibits a potency of about 1100 µg/mg.

During chromatography Megalomicin-C₂ base is converted to a hydrochloride salt by reaction with small quantities of hydrogen chloride present in the solvent. The hydrochloride, as would be expected, does not exhibit appreciable solubility in ethyl ether and is, therefore, precipitated by the addition thereof.

The free base form of Megalomicin-C₂ may be obtained by adding dilute alkali such as 0.1N NaOH to an aqueous solution of the hydrochloride to about pH 8.5—9.5 and separating the free base by filtration from the aqueous suspension.

Later fractions collected from the column described above when pooled and concentrated to dryness yield a white powder, Megalomicin-C₁ fraction. At this stage, the Megalomicin-C₁ powder obtained displays a potency of about 880 µg/mg in the previously described cylinder cup assay.

Using the bioautograph techniques previously described and chloroform:methanol (60:40 volume/volume) as the solvent system, Megalomicin-C₁ isolate has broad spectrum antibacterial activity *in vitro* against the following gram-positive and gram-negative organisms:

Bacillus subtilis ATCC 6633
Sarcina lutea ATCC 9341
Staphylococcus aureus ATCC 6538P
Pseudomonas aeruginosa ATCC 8689

The remaining active fractions from the silicic acid column may be combined and further resolution achieved by Florisil (magnesium silicate) column chromatography as follows: The column is prepared by activating the Florisil ("Florisil" is a Registered Trade Mark) at 100° C. for 16 hours; fixing in hexane and pouring the resulting slurry into a glass column. The column is charged with residual Megalomicin Complex (after removal of Megalomicins C₁ and C₂) dissolved in methylene chloride and is developed progressively with one hold-up volume (i.e. that volume of solvent which is held by the absorb-

ent) of hexane, one hold-up volume of ethyl ether, one hold-up volume of ethyl acetate and then with increasing amounts of acetone in ethyl acetate. Separation is monitored by disc testing the fractions against *S. aureus* and *E. coli* followed by thin layer chromatography as previously described.

Employing this technique, separation of Megalomicin A and Megalomicin B can be accomplished. Both are isolated as off-white powders by concentration of the pooled chromatographic fractions to dryness. Megalomicins A and B assayed at 145 µg/mg and 71 µg/mg respectively, in the *S. lutea* assay.

Alternatively, a mixture of Megalomicins C₁ and C₂ may be isolated from the ethyl acetate concentrate prepared as described herein above, as follows: The residue is dissolved in acetone and poured into cold water with vigorous stirring. The suspension is permitted to warm to room temperature and filtered. The precipitate comprises a mixture of Megalomicins C₁ and C₂ and is hereafter referred to as Megalomicin-C complex and, in this instance, is in the form of the free base. An acid addition salt of the complex may conveniently be prepared by the titration of a methanol solution of the base with the appropriate acid and by precipitating the salt by the addition of ethyl ether. When the foregoing procedure is utilized to obtain the Megalomicin-C Complex, Megalomicins-A and -B may be extracted from the aqueous filtrate, and the extract subsequently concentrated to a residue and chromatographed on magnesium silicate as described above.

(4) Chemical Characterization of Megalomicins A, B, C₁ and C₂.

The antibiotics of this invention are nitrogen bases; consequently, each fraction is capable of forming acid addition salts in a manner similar to that described for Megalomicin-C Complex. Typical of such salts are those formed with hydrochloric, sulfuric, phosphoric, stearic, propionic, tartaric and maleic acids. As would be anticipated, such salts also display antibiotic activity similar to that of the free antibiotic, usually differing in potency and solubility characteristics.

The antibiotics of this invention are also hydroxylated compounds; consequently, they are capable of being esterified, preferably with a hydrocarbon carboxylic acid. Exemplary of the same are such esters as the acetates, propionates, cyclopropyl-carboxylates, succinates and benzoates. Such esters generally exhibit antibiotic activity similar to that of the free antibiotic usually differing in potency and solubility characteristics.

In Table 5 below is set forth the chemical and physical properties of Megalomicins A, B, C₁ and C₂.

TABLE 5

CHEMICAL AND PHYSICAL PROPERTIES OF CONSTITUENT ANTIBIOTICS OF MEGALOMICIN COMPLEX					
	Megalomicin A	Megalomicin B	Megalomicin C ₁	Megalomicin C ₂	
Optical Rotation [α] _D ²⁵ (1% in ethanol)	−90°	−92°*	−104°	−102°	
Melting point	255—259°C. (dec.)	125—135°C. (dec.)*	243—246°C. (dec.)	146—150°C. (dec.)	
pK _a	9.1	8.8	8.8	8.6	
Neutralization Equivalent	442	490	488	492	
Elemental Analysis:					
Carbon	Found 60.23 Calculated 60.25	Found 58.90 Calculated 60.11	Found 60.23 Calculated 59.98	Found 58.93 Calculated 60.35	
Hydrogen	9.28	8.94	8.93	8.73	8.89
Nitrogen	3.30	2.88	2.94	2.88	2.87
Oxygen (by difference)	27.19	29.18	27.90	29.46	27.89
Empirical Formula	C ₄₄ H ₈₀ N ₂ O ₁₅	C ₄₈ H ₈₂ N ₂ O ₁₆	C ₄₈ H ₈₄ N ₂ O ₁₇	C ₄₉ H ₈₆ N ₂ O ₁₇	
Molecular Weight	877.10	919.14	961.17	975.20	

*) Monohydrate

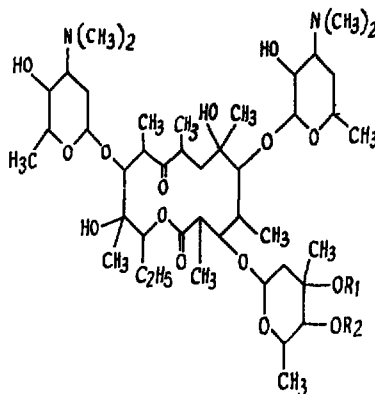
The infrared spectra of Megalomicins A, B, C₁ and C₂ are shown in the drawings as Figures 1, 2, 3 and 4 respectively. The wave lengths (λ) are indicated in μ, the frequencies (ν) in cm^{−1}; T stands for transmittance, A for absorbance. — The spectra of the respective antibiotic fractions were run in mineral oil (Nujol, "Nujol" is a Registered Trade Mark) and the more significant absorption peaks are set forth in Table 6 with the following designations: VS=very strong, S=strong, M=moderate to strong, W=weak, brd=broad, shp=sharp, shd=shoulder and sb=side band. Peaks which are accountable to the Nujol medium rather than the respective Megalomicin are designated "N" (for Nujol).

Megalomicins A, B, C₁ and C₂ have characteristic nuclear magnetic resonance spectra (NMR) which are shown in the drawings as Figures 5, 6, 7, and 8 respectively. The NMR spectra were observed on a Varian A-60—A spectrometer on a solution (ca. 0.4 ml, concentration ca. 20 mg/ml) of the sample of each fraction in deuterated chloroform. The spectra are recorded in parts per million (PPM) from tetramethylsilane, the internal standard.

TABLE 6
INFRARED SPECTRA OF CONSTITUENT ANTIBIOTICS OF MEGALOMICIN COMPLEX

Megalomicin A		Megalomicin B		Megalomicin C ₁		Megalomicin C ₂	
Wave-length- (μ)	Peak Strength	Wave-Length (μ)	Peak Strength	Wave-Length (μ)	Peak Strength	Wave-Length (μ)	Peak Strength
2.82-2.90	M-S, brd	2.87	M	2.82	s b	2.83	W-M
3.35-3.50	N	2.97	shd	2.87	W	3.33-3.48	N
5.77	S	3.35-3.50	N	3.35-3.50	N	5.70	S
5.82	(instrument artifact)	5.72	S	5.72	S	5.80	(instrument artifact)
5.88	M-S	5.83	S	5.87	W-M	5.88	shd
6.82	N	6.82	N	6.82	N	6.80	N
7.24	N	7.25	N	7.25	N	7.23	N
7.36	sb	8.05	S	7.45	M	8.00	S
7.82	M-S, shp	8.45	S, brd	7.57	M	8.53	S, brd
8.45	VS, brd	8.59	S	7.82	M	8.93	S
8.92	VS	8.94	S	8.03	S	9.08	shd
9.02	VS	9.30	S	8.13	VS	9.27	M-S
9.12	VS	9.62	S	8.60	S	9.65	S
9.32	M-S	9.91	M-S	8.94	M-S	10.00	M-S, brd
9.53	VS	10.02	M-S	9.05	S	10.38	M
9.63	VS	10.29	M	9.56	S	10.97	M
9.98	VS	10.84	W-M	9.70	S	11.15	M
10.27	S	11.08	W-M	10.13	M-S	11.52	W
11.02	M-S	11.20	W-M	10.48	M	11.92	W-M
11.17	M-S			11.02	W-M		
				11.18	W-M		

On the basis of the foregoing chemical and physical properties, the constituent antibiotics of megalomicin complex are believed to have gross molecular structures as follows:



- 5 wherein in megalomicin A, R₁=R₂=hydrogen;
 in megalomicin B, R₁=hydrogen, R₂=acetyl;
 in megalomicin C₁, R₁=R₂=acetyl; and
 in megalomicin C₂, R₁=acetyl, R₂=propionyl.

10 The stabilities of Megalomicins A, B, C₁
 and C₂ are as follows: At pH 2.0 to 6.0 and
 a temperature of 100° C., Megalomicins A and
 B show a significant loss of activity in 30
 minutes; they are stable, however, when sub-
 jected to the same conditions at pH 7.0 to
 10.0. Megalomicin C₁ in contrast is stable at
 15 pH 2.0 to 8.0 for 30 minutes at 100° C.,
 but is unstable under the same conditions at
 pH 10.0. Megalomicin C₂ is unstable under
 both acid (pH 2.0) and alkaline (pH 10.0)
 20 conditions at 100° C., but is stable at pH
 4.0 to 8.0 for 30 minutes at 100° C.

(5) *Biological Properties of Megalomicin
 Complex, Megalomicins A, B, C₁, C₂ and
 Megalomicin-C Complex.*

A. *In Vitro Tests*

25 Megalomicin Complex, Megalomicins A,
 B, C₁ and C₂ and Megalomicin-C Complex
 all possess a broad spectrum anti-bacterial
 activity *in vitro* against a variety of gram-
 positive and gram-negative organisms. Either

as a complex or as individual fractions, there- 30
 fore, they may be used to clean and sterilize
 laboratory glassware, to clean and sterilize
 surgical instruments and the like. Also, these
 antibiotics may be used in combination with
 soaps and detergents to clean and sensitize 35
 areas used for food preparation such as kit-
 chens, dining halls and the like.

The *in vitro* antibacterial spectrum of Mega-
 lomycin Complex, Megalomicins A, B, C₁ and
 C₂ and Megalomicin-C Complex against 40
 typical gram-positive and gram-negative
 organisms is shown in Table 7 below.
 The susceptibility of the test organisms
 to the antibiotics was determined by
 a tube dilution assay in yeast-beef broth, 45
 adjusted to pH 8.0 with sodium hydroxide.
 The strains of microorganisms tested are iden-
 tified by collection numbers; here and in other
 Tables which follow, those numbers preceded
 by the letters "DA" refer to the collection of 50
 Schering Corporation, Bloomfield (N.J.),
 U.S.A.

TABLE 7

ANTIBACTERIAL SPECTRUM OF MEGALOMICIN COMPLEX, MEGALOMICINS A, B, C₁, C₂ AND MEGALOMICIN C-COMPLEX

Microorganism	Minimal Inhibitory Concentration (μg/ml)						
	Complex (Base)	Complex (HCl salt)	A	B	C ₁	C ₂	C-Complex
<i>Bacillus megatherium</i> DA 7064	0.3	0.3	0.6	1.2	0.3	0.6	0.3
<i>Bacillus subtilis</i> ATCC 6633	0.03	0.3	0.005	0.05	0.03	0.005	0.005
<i>Diplococcus pneumoniae</i> DA 700	—	—	1.2	1.2	—	0.6	—
<i>Diplococcus pneumoniae</i> ATCC 10015	—	—	0.5	0.5	—	0.05	—
<i>Diplococcus pneumoniae</i> DA 150	6.0	6.0	>2.7	>2.7	12.0	>2.7	>2.7
<i>Enterococcus</i> sp. DA 800	0.3	0.75	0.6	0.6	0.3	0.08	0.3
<i>Enterococcus</i> sp. DA 801	0.3	0.75	0.6	0.6	0.3	0.6	0.3
<i>Enterococcus</i> sp. DA 802	0.3	>1.0	0.2	0.2	0.3	0.03	0.3
<i>Sarcina lutea</i> ATCC 9341	0.0075	0.0075	0.005	0.005	0.0075	0.0005	0.00075
<i>Staphylococcus aureus</i> ATCC 12715	0.3	0.75	0.08	0.2	0.3	0.005	0.03
<i>Staphylococcus aureus</i> ATCC 6538P	0.3	0.03	0.6	0.2	0.03	0.005	0.03
<i>Staphylococcus aureus</i> ATCC 11631*	0.3	0.075	0.6	0.6	0.3	0.6	0.3
<i>Staphylococcus aureus</i> (Gray)	0.3	0.3	0.6	0.6	0.3	0.6	0.5
<i>Staphylococcus aureus</i> DA 2001	0.03	0.3	0.6	0.2	0.03	0.03	0.03
<i>Staphylococcus aureus</i> DA 2003	0.03	0.03	0.6	0.6	0.075	0.6	0.3
<i>Staphylococcus aureus</i> DA 2010	0.3	0.3	0.6	0.6	0.3	0.2	0.25
<i>Staphylococcus aureus</i> DA 2014	0.3	0.3	0.6	0.6	0.03	0.6	0.03
<i>Staphylococcus aureus</i> DA 2018	0.3	0.3	0.6	0.6	0.075	0.1	0.1
<i>Staphylococcus aureus</i> DA 2032	0.075	0.3	0.6	0.6	0.075	0.08	0.08
<i>Staphylococcus aureus</i> DA 2033**	>16	>16	>2.7	>2.7	>32.0	>2.7	>2.7
<i>Streptococcus faecalis</i> DA 20	0.3	0.3	0.03	0.1	0.3	0.005	0.075
<i>Streptococcus pyogenes</i> DA 21	0.75	>1.0	5.0	5.0	0.3	0.6	0.3
<i>Streptococcus pyogenes</i> DA 11	0.3	—	0.6	0.2	0.3	0.03	0.3
<i>Streptococcus pyogenes</i> DA 12	—	—	0.2	0.2	—	0.03	—
<i>Mycobacterium smegmatis</i> ATCC 10143	0.75	0.3	5.0	5.0	0.3	0.05	0.3
<i>Escherichia coli</i> ATCC 10536	6.0	>16	6.0	12.0	12.0	12.0	12.0
<i>Klebsiella pneumoniae</i> ATCC 10031	12.0	>16	6.0	24.0	12.0	24.0	12.0
<i>Proteus vulgaris</i> DA 121	6.0	>16	12.0	24.0	24.0	24.0	24.0
<i>Pseudomonas aeruginosa</i> ATCC 8689	6.0	12.0	6.0	12.0	6.0	6.0	6.0
<i>Salmonella schottmuelleri</i> DA 10	6.0	12.0	6.0	12.0	12.0	12.0	12.0

*) a penicillin resistant strain

**) an erythromycin resistant strain

- 5 The antibacterial activity of Megalomicin Complex is compared with that of erythromycin, penicillin G, and methicillin in Table 8 below (susceptibility being determined by the tube dilution assay technique described above). The tests have been made utilizing the following media: Megalomicin Complex and erythromycin in yeast-beef broth at pH 8.0; penicillin G and methicillin in yeast-beef broth at pH 6.8. The results show that Megalomicin Complex compares favourably in activity to the other antibiotics tested against gram-positive bacteria. In addition, Megalomicin Complex is active against *Mycobacterium smegmatis* at a lower concentration than erythromycin, while penicillin G and methicillin are inactive up to 10 µg/ml.

TABLE 8

COMPARISON OF THE ANTIBACTERIAL SPECTRUM OF MEGALOMICIN COMPLEX WITH ERYTHROMYCIN, PENICILLIN G AND METHICILLIN

	Megalomicin Complex	Erythromycin	Penicillin G	Methicillin
<i>Bacillus cereus</i>	0.2	0.05	0.12	4.8
<i>Bacillus subtilis</i>	0.005	0.005	0.01	0.03
<i>Diplococcus pneumoniae</i>	0.2	0.05	0.12	1.8
<i>Sarcina lutea</i>	0.0005	0.005	0.06	0.3
<i>Staphylococcus aureus</i> (3 different Strains)	0.005—0.2	0.005—0.05	0.003—0.06	0.12—0.6
<i>Staphylococcus aureus</i> (7 different Strains)*	0.03—0.6	0.005—0.5	1.2—9.4	1.2—7.5
<i>Staphylococcus aureus</i> (1 Strain)**	2.7	50.0	—	—
<i>Streptococcus faecalis</i>	0.005	0.0005	0.75	25.0
<i>Streptococcus pyogenes</i> (4 different Strains)	0.03—0.2	0.05	0.01—0.02	0.6
<i>Mycobacterium smegmatis</i>	0.5	5.0	10	10

Megalomicin Complex and erythromycin in yeast-beef broth at pH 8.0; penicillin G and methicillin in yeast-beef broth at pH 6.8.

* Clinical isolates including penicillin resistant organisms.

** Clinical isolates resistant to erythromycin.

B. *In Vivo* Tests

- 20 As was noted above, Megalomicin Complex, Megalomicin-C Complex, and Megalomicins A, B, C₁ and C₂ exhibit a broad range of anti-microbial activity against gram-positive and gram-negative microorganisms. Included in the gram-positive group are pathogenic microorganisms including species of the genus *Streptococcus*, *Staphylococcus* and *Diplococcus* which are known to cause many disease manifestations. Various species of *Staphylococcus* and *Streptococcus* are the organisms responsible for causing bovine mastitis. These species are readily controlled and treated by means of the antibiotics disclosed herein after a relatively brief regimen of administration.
- Megalomicin Complex, Megalomicins A, B, C₁, C₂ and Megalomicin-C Complex are also active against gram-negative organisms including species of the genus *Escherichia*, *Salmonella*, *Proteus* and *Pseudomonas*. These organisms are responsible for many serious disease syndromes including urinary tract infections and diarrheas. Such syndromes are quite common in domestic animals such as cattle, horses, sheep, swine, dogs and cats and may be effectively controlled and treated by means of the antibiotics described herein.
- Megalomicin Complex suspended in peanut oil gives an LD₅₀ (lethal dose for 50 per cent of the population tested) of 270 mg/kg in mice by the intraperitoneal route. On sub-

cutaneous and oral administration, the LD₅₀ is greater than 5000 mg/kg. The PD₅₀ (protective dose for 50 per cent of the population tested) is 150 mg/kg by the subcutaneous route (peanut oil suspension) against a lethal infection of *S. aureus* in mice. Against *Streptococcus pyogenes* by the same route, the PD₅₀ in mice is 100 mg/kg.

5

In addition to the foregoing, Megalomycin Complex administered at 17.5 mg/kg to dogs is detectable in the blood serum for 7 days.

10

This is surprising in view of the fact that when a comparable dose of erythromycin is given it does not persist throughout a 24 hour interval.

15

Acute toxicity data for the constituent antibiotics of Megalomycin Complex and the complexes are tabulated in Table 10 below.

Tables 11, 12 and 13 are set forth to illustrate the animal protection exhibited by the antibiotics of the instant invention.

20

TABLE 10

TOXICITY OF MEGALOMICIN CONSTITUENT ANTIBIOTICS AND COMPLEXES IN MICE (LD₅₀ IN MG/KG)

Route	Megalomicin Complex Base in peanut oil	Megalomicin Complex HCl in H ₂ O	Megalomicin A Base*	Megalomicin B Base in peanut oil	Megalomicin C Complex
Oral	>1160	>2500	7500	—	>500
Subcutaneous	>1160	2500	7000	>500	>500
Intraperitoneal	75	300	350	—	400
Route	Megalomicin—C ₁ Base*	Megalomicin—C ₂ HCl in H ₂ O			
Oral	>1000	>500			
Subcutaneous	>1000	>500			
Intraperitoneal	500	>500			

* In 0.5% aqueous carboxymethyl-cellulose.

TABLE 11

ACTIVITY OF MEGALOMICIN COMPLEX AGAINST LETHAL INTRAPERITONEAL INFECTIONS
IN MICE (PD₅₀ IN MG/KG)

	Megalomicin Complex in peanut oil	Megalomicin Complex HCl in H ₂ O	Megalomicin C ₂ HCl in H ₂ O
Subcutaneous Treatment			
Staphylococcus aureus Gray	35	4.3	5
Staphylococcus aureus DA 2030	—	4.3	—
Staphylococcus aureus DA 2000	—	3.5	—
Staphylococcus aureus Smith	—	2.3	—
Streptococcus pyogenes C DA 21	23	4.6	45
Streptococcus pyogenes DA 32	—	1.5*	—
Streptococcus pyogenes DA 31	—	1.5*	—
Streptococcus pyogenes DA 12	—	1.5*	—
Streptococcus pyogenes DA 9	—	1.5*	—
Diplococcus pneumoniae DA 150	35	23.2	150
Diplococcus pneumoniae DA 150 (2 day treatment)	23/day	11.6/day	30/day
Diplococcus pneumoniae DA 151	—	11.6	—
Diplococcus pneumoniae ATCC 10015	—	1.5*	—
Oral Treatment			
Staphylococcus aureus Gray	>116	>116	50
Streptococcus pyogenes DA 21	—	>116	—
Diplococcus pneumoniae DA 150	—	116	—

TABLE 12

ACTIVITY OF MEGALOMICIN CONSTITUENT ANTIBIOTICS AGAINST LETHAL INTRAPERITONEAL INFECTIONS IN MICE (PD₅₀ IN MG/KG)

	Treatment Route	Megalomicin A Base*	Megalomicin C ₁ Base*
Streptococcus pyogenes C 203	Oral	300	207
Streptococcus pyogenes C 203	S.C. ¹	220	118
Streptococcus pyogenes C	Oral	300	300
Streptococcus pyogenes C	S.C.	134	180
Streptococcus pyogenes No. 22	Oral	180	>250
Streptococcus pyogenes No. 22	S.C.	180	200
Streptococcus pyogenes No. 9	Oral	250	>250
Streptococcus pyogenes No. 9	S.C.	167	250
Staphylococcus aureus Smith	Oral	250	180
Staphylococcus aureus Smith	S.C.	75	117
Staphylococcus aureus Gray	Oral	300	>250
Staphylococcus aureus Gray	S.C.	20	62
Staphylococcus aureus W	Oral	117	150
Staphylococcus aureus W	S.C.	20	25
Staphylococcus aureus No. 41	Oral	150	170
Staphylococcus aureus No. 41	S.C.	20	25
Diplococcus pneumoniae eye	Oral	300	300
Diplococcus pneumoniae eye	S.C.	90	167
Diplococcus pneumoniae No. 2	Oral	250	200
Diplococcus pneumoniae No. 2	S.C.	70	170
Enterococcus No. 802	Oral	300	>250
Enterococcus No. 802	S.C.	160	180
Enterococcus No. 804	Oral	250	250
Enterococcus No. 804	S.C.	180	250
Escherichia coli	Oral	200	250
Escherichia coli	S.C.	100	250
Klebsiella pneumoniae	Oral	250	>250
Klebsiella pneumoniae	S.C.	150	>250
Pseudomonas aeruginosa	Oral	>250	>250
Pseudomonas aeruginosa	S.C.	161	>250

* in 0.5% aqueous carboxymethylcellulose (¹) S.C. = subcutaneous.

TABLE 13

ACTIVITY OF MEGALOMICIN CONSTITUENT ANTIBIOTICS AND COMBINATIONS THEREOF
AGAINST LETHAL INTRAPERITONEAL INFECTIONS IN MICE (PD₅₀ IN MG/KG)

	Megalomicin-B Base in peanut oil	Megalomicin-C ₁ Base*	Megalomicin-C ₂ HCl in H ₂ O	Megalomicin-C Complex Base*	Megalomicin-C Complex HCl in H ₂ O
Subcutaneous Administration of Antibiotics					
Organism					
<i>Staphylococcus aureus</i> Gray	150	200	45	50	50
<i>Streptococcus pyogenes</i> C	—	150	10	50	50
<i>Staphylococcus aureus</i> Smith	—	—	—	86	—
<i>Streptococcus pyogenes</i> No. 5	—	—	—	39	—
Oral Administration of Antibiotics					
Organism					
<i>Staphylococcus aureus</i> Gray	—	>250	100	390	>250
<i>Streptococcus pyogenes</i> C	—	>250	100	310	>250
<i>Staphylococcus aureus</i> Smith	—	—	—	310	—
<i>Streptococcus pyogenes</i> No. 5	—	—	—	390	—

* Ultrasonicated suspension in aqueous 0.5% carboxymethyl-cellulose.

- 5 Megalomicin A suspended in 0.5% aqueous carboxymethylcellulose and dispersed by ultrasonication is active by subcutaneous administration in mice against *S. aureus* Gray with a PD₅₀ (protective dose for 50 per cent of the population tested) of 20 mg/kg and against *P. aeruginosa* with a PD₅₀ of 161 mg/kg. The LD₅₀ in mice by the subcutaneous route is 7000 mg/kg. 25
- 10 Megalomicin B suspended in peanut oil is active by subcutaneous administration in mice against *S. aureus* with a PD₅₀ of 150 mg/kg and against *P. aeruginosa*, 350 mg/kg delays death by 24 hours. The LD₅₀ in mice by the subcutaneous route is greater than 500 mg/kg. 30
- 15 Megalomicin C₁ suspended in 0.5% aqueous carboxymethyl cellulose and dispersed by ultrasonication is active in mice against *S. aureus*. When administered subcutaneously against *S. aureus* Gray it has a PD₅₀ of 62 mg/kg. By the same route it has a PD₅₀ of 180 mg/kg against *S. pyogenes* C. The antibiotic is somewhat less active orally having a PD₅₀ against *S. aureus* Gray and *S. pyogenes* C. of greater than 250 mg/kg. The LD₅₀ in mice by the subcutaneous route is greater than 1000 mg/kg. Megalomicin-C₁ also exhibits anti-malarial activity against *Plasmodium berghei* and when administered to rodents (100 mg/kg/day) 24 hours post-infection causes a significant suppression in the blood parasite count. 35
- 20 Megalomicin C₂ as a suspension in aqueous carboxymethyl-cellulose is active by subcutaneous administration. It is, however, more advantageously used as an aqueous solution of its hydrochloride salt and when administered subcutaneously to mice has a PD₅₀ of 45 mg/kg against *S. aureus* Gray and a PD₅₀ of 10 mg/kg against *S. pyogenes* C. By the oral route it has a PD₅₀ of 100 mg/kg against both organisms. The LD₅₀ in mice in intraperitoneal administration is greater than 500 mg/kg. 40

EXAMPLES

The following examples will more fully illustrate preferred techniques for the preparation of the antibiotics described above.

5 (1) *Fermentation of the Microorganism*EXAMPLE 1
*Shake Flask Fermentation*A. *Germination Stage*

10 Add a 0.5 ml lyophilized culture of *Micro-
monospora megalomicea* (NRRL 3274 or
3275) to a 300 ml Erlenmeyer flask containing
100 ml of the following medium which has
been adjusted to pH 7.5 with dilute sodium
hydroxide prior to sterilization:

15	Bacto-Beef Extract (Difco)	3.0 g
	Bacto-Tryptose (Difco)	5.0 g
	Dextrose	1.0 g
	Starch (potato)	24.0 g
	Bacto-Yeast Extract (Difco)	5.0 g
20	Calcium Carbonate	2.0 g
	Tap Water	1000.0 ml

Incubate the flask and its contents for 3
days at 37° C. on a rotary shaker (280 r.p.m.;
5 cm stroke).

25 B. *Inoculum Stage*

30 Aseptically transfer a 25 ml inoculum from
the germination stage to a 2 liter Erlenmeyer
flask containing 500ml of the sterile medium
used above for germination. Incubate the flask
and its contents for 3 days at 28° C. on a
rotary shaker (280 r.p.m.; 5 cm stroke).

C. *Fermentation Stage*

35 Aseptically transfer 25 ml aliquots from
the inoculum stage to 2 liter Erlenmeyer flasks
containing 500 ml of the following sterile
medium which has been adjusted to pH 7.8
with dilute aqueous sodium hydroxide prior
to sterilization.

	Sucrose	30.0 g
40	Sodium Nitrate	3.0 g
	Dipotassium Phosphate	1.0 g
	Magnesium Sulfate	0.5 g
	Ferrous Sulfate	0.01 g
	Peptone	8.0 g
45	Tap Water	1000.0 ml

50 Incubate the flasks and their contents for 4
days at 28° C. on a rotary shaker (280 r.p.m.;
5 cm stroke). Pool the contents of the flasks
and adjust an aliquot of the whole broth to pH
9.5 and extract with ethyl acetate (approx-
imately 2 volumes per volume of broth). Sep-
arate the solvent phase and concentrate 100-
fold under vacuum. Test the concentrate for
activity by the agar disc diffusion technique
55 using *S. aureus* (ATCC 6538P) as the test

organism. The concentrate will give a zone of
inhibition of approximately 20—35 mm.

EXAMPLE 2
*Tank Fermentation*A. *Germination Stage*

60

Carry out the germination stage and the
inoculum stage in the manner described in
Example 1.

B. *Fermentation Stage*

65

Aseptically transfer 500 ml of inoculum
to a 14 liter fermentor containing 10 liters of
sterile medium as described in Example 1 to
which has been added 6 ml of anti-foam, Dow
Corning B (Dow Corning Corporation, Mid-
land, Michigan). Allow fermentation to pro-
ceed for 66 hours under the following con-
ditions:

Temperature	31° C.
Sterile Air Input	4 liters/minute
Agitation	250 r.p.m.
Anti-Foam (Dow Corning B)	add as needed

75

At the end of this period, the potency of
the produced antibiotic reaches a peak which
remains substantially constant. Throughout
fermentation, the pH of the fermentation mix-
ture remains substantially in the range of 7.2
to 8.2. The packed cell volume reaches a
constant value of 3.5—4.5 ml. The activity of
the produced antibiotic in the whole broth
gives a zone diameter of 15—25 mm when
disc tested against *S. aureus* or *P. aeruginosa*.

80

85

EXAMPLE 3
*Tank Fermentation*A. *Preparation of Inoculum*

90

To four 2 liter flasks each containing 500
ml of sterile broth as described in Example
1A, add 0.5 ml of a lyophilized culture of
Micromonospora megalimicea (NRRL 3274 or
3275). Place the flasks on a rotary shaker
running at 280 strokes per minute (5 cm
stroke) and incubate at 28° C. for 72 hours.

95

B. *Fermentation Stage.*

To each of four fermentors, add 10 liters
of the following sterile production medium
adjusted to pH 7.15 to 7.25 prior to steriliza-
tion:

100

Trypticase	170.0 g
Sodium Chloride	50.0 g
Dipotassium Phosphate	25.0 g
Dextrose	25.0 g
Czapek-Dox Broth	350.0 g
G.E.—60 Antifoam	as needed
(General Electric Company)	
(Schenectady, New York)	
Tap Water q.s.	10.0 liters

105

110

- Inoculate each fermentor with 500 ml of the 72 hour seed culture prepared above. Bring the temperature of the fermentation medium to 31° C. and agitate at 500 r.p.m. while introducing air flow through the medium at the rate of 0.5 liters of air/liter of broth/minute. Increase the agitation rate to 600 r.p.m. after 24 hours and to 700 r.p.m. after 48 hours. Terminate the fermentation at the end of 69 hours. Pool the broths from the four fermentors for extraction (about 37 liters).

(2) *Extraction of Megalomicin Complex*

EXAMPLE 4

15 *Extraction of Megalomicin Complex from Laboratory Fermentation (Chromatographic Technique)*

- Adjust 60 liters of whole broth prepared according to the procedure described in Example 2 to pH 9.5 with dilute aqueous sodium hydroxide. Extract with 2 volumes of ethyl acetate for each volume of broth. Separate the solvent phase and concentrate under vacuum to obtain an oily residue (approximately 30.0 g). The biological activity (1/20 dilution) of this oily residue will give a zone inhibition diameter of about 20—30 mm against *S. aureus* and about 15—25 mm against *P. aeruginosa*. Purify the oily residue by column chromatography according to the following technique:

- 30 Prepare a column using 1000 g of LH20 Sephadex (Pharmacia Fine Chemical, Inc.) suspended in 95 per cent aqueous ethanol. Transfer the oily residue to the column and elute with 95 per cent ethanol at a flow rate of 200 ml/hour. Collect 50 ml fractions. Combine fractions according to their antibacterial activity (determined by agar disc testing against *S. aureus* (ATCC 6538P). Concentrate fractions having peak activity to dryness. Dissolve the solid residue in a small amount of acetone and pour into an excess volume of petroleum ether (b.p. 30—60° C.). Transfer the mixture to a dry-ice/acetone bath (approximately -50° C.) and allow to stand 20 minutes. Allow the mixture to return to room temperature and separate the mother liquor from the oily residue by decanting. Concentrate the mother liquor to dryness to obtain purified Megalomicin Complex.

- 50 Megalomicin Complex so produced will have a potency of about 225 to 275 µg/mg when assayed according to the cylinder cup technique described above.

EXAMPLE 5

55 *Extraction of Megalomicin Complex From Tank Fermentation (Solvent Extraction Technique)*

- 60 Adjust the pooled whole broth (about 37 liters) obtained as described in Example 3 to pH 9.5 with 50% aqueous sodium hydroxide. Extract with two volumes of ethyl acetate and

evaporate the extract to a volume of 2150 ml. Isolate the Megalomicin Complex by one of the following techniques:

A. *Hydrochloric Acid Extraction*

65 Extract a 500 ml aliquot of the ethyl acetate concentrate twice with 250 ml portions of 0.5 per cent (0.14N) hydrochloric acid. Make the combined acid extracts slightly alkaline by the addition of 5% aqueous sodium hydroxide (about pH 8.5 to 9.0) and extract twice with 250 ml portions of ethyl acetate. Combine the ethyl acetate extracts and concentrate under reduced pressure to dryness. The complex so produced will have a potency of about 75 525 µg/mg when assayed by the previously described cylinder cup technique.

B. *Sulfuric Acid Extraction*

Utilize the extraction described in Example 5A substituting 0.1 N sulfuric acid for the hydrochloric acid. The complex so produced will have a potency of about 550 µg/mg in the cylinder cup assay.

(3) *Resolution of Megalomicin Complex and Preparation of Derivatives*

EXAMPLE 6

Isolation of Megalomicin C₂

Prepare a column (105 cm long, 7.6 cm diameter) using 2000 g of silicic acid (Analytical Reagent, Mallinckrodt) by packing small segments into a compact bed. Dissolve 13.0 g of Megalomicin Complex as prepared in Example 4 in acetone mixed with silicic acid and evaporate the acetone under vacuum. Add the dry mixture to the top of the column. Elute the column with a mixture of chloroform (60 parts by volume) and methanol (40 parts by volume) at a flow rate of 100 ml/hour. Collect 200 ml fractions. Monitor the column by disc testing each fraction against *S. aureus* and *E. coli*. Chromatograph the active fractions on silicic acid thin layer plates run for 1.5 hours in the same solvent system used for the column. Combine fractions according to their chromatographic patterns as determined by the two methods previously described (see Characterization of Megalomicin Complex). Concentrate the fractions under vacuum. By combining fractions as indicated in Table 14 below, Megalomicin C₂ Hydrochloride is isolated as a white powder by dissolving the combined residues in one volume of methanol and precipitating with 10 volumes of ethyl ether. This material at this stage has a potency of about 1000 µg/mg in the cylinder cup assay previously described.

Isolation of Megalomicin C₁ Material

By combining later fractions as indicated in Table 14 below, an Megalomicin Fraction C₁ isolate is obtained as a white powder by

concentrating the selected column fractions to dryness. At this stage, this material exhibits a potency of about 900 $\mu\text{g}/\text{mg}$ in the cylinder cup assay previously described. The remaining active material is eluted from the column, pooled and concentrated to a residue.

EXAMPLE 7

Isolation of Megalomicins A and B

10 Activate 500 g of Florisil at 100 ° C. for 16 hours; fix in hexane and slurry into a glass column (105 cm long; 3.8 cm/diameter; hold-

up volume 700 ml). Charge the column with 2.8 g of the residue from Example 6, dissolved in 10 ml of methylene chloride. Elute the column progressively with 700 ml of hexane, 700 ml of ether, 700 ml of ethyl acetate and increasing amounts of acetone in ethyl acetate (flow rate 200 ml/hour). Collect 100 ml fractions. The column is monitored as previously described by testing fractions against *S. aureus* and *E. coli* followed by thin layer chromatography. Combine the fractions as indicated in Table 14 below.

TABLE 14

RESULTS OF COLUMN SEPARATIONS

Antibiotic	Column Cut	Weight	Thin Layer Pattern R_f Chloroform-Methanol (60/40)	Potency ($\mu\text{g}/\text{mg}$)
Silicic Acid Column				
Megalomicin $\text{C}_2 \cdot \text{HCl}$	28—48	1.2 g	0.65	900—100
Megalomicin C_1	56—58	625 mg	0.52	850—100
Florisil Column				
Megalomicin B	164—227 (30% acetone in ethyl acetate)	540 mg	0.38	125—175
Megalomicin A	370—397 (80% acetone in ethyl acetate)	283 mg	0.19	50—100

25

EXAMPLE 8

Megalomicin Complex Hydrochloride Salt

Suspend about 140 mg of Megalomicin Complex, as isolated in Example 5 above, in about 50 ml of water. Adjust the pH of the suspension to about 6.5 to 7.0 by the addition of dilute (n/l) hydrochloric acid and stir vigorously. Remove the insolubles, if any remain, by filtration and lyophilize the filtrate to obtain a Megalomicin hydrochloride weighing approximately 90 mg and assaying about 550 $\mu\text{g}/\text{mg}$.

By the utilization of substantially the pro-

cedure of this example on either the Megalomicin Complex or the various fractions thereof and by substituting equivalent quantities of other acids such as, for example, sulfuric or phosphoric acid, the respective salts may also be prepared.

Table 15 below sets forth the activity of the hydrochloride, sulfate and phosphate salts made from Megalomicin Complex with a potency of 232 $\mu\text{g}/\text{mg}$ of Megalomicin Complex against a variety of gram-positive and gram-negative organisms, in a yeast-beef broth medium at pH 8.0.

TABLE 15

ANTIBACTERIAL SPECTRUM OF WATER-SOLUBLE ACID ADDITION SALTS OF MEGALOMICIN COMPLEX

Microorganism	Minimum Inhibitory Concentration ($\mu\text{g/ml}$)		
	Hydrochloride Salt	Sulfate Salt	Phosphate Salt
<i>Bacillus subtilis</i> ATCC 6633	0.01	0.01	0.01
<i>Staphylococcus aureus</i> ATCC 6538P	0.1	0.01	0.1
<i>Staphylococcus aureus</i> ATCC 11163	0.1	0.1	0.1
<i>Sarcina lutea</i> ATCC 9341	0.001	0.001	0.001
<i>Streptococcus faecalis</i> ATCC 10541	0.1	0.1	0.1
<i>Escherichia coli</i> ATCC 10536	2.8	2.8	2.8
<i>Klebsiella Pneumoniae</i> ATCC 10031	1.4	1.4	2.8
<i>Proteus vulgaris</i> DA 121	5.6	11.1	11.1
<i>Pseudomonas aeruginosa</i> ATCC 8689	1.4	1.4	1.4
<i>Salmonella schottmuelleri</i> DA 10	1.4	2.8	2.8

EXAMPLE 9

Isolation of Megalomicin-C Complex

5 Dissolve 542 g of Megalomicin Complex
(See Example 5) in 5.4 liters of acetone and
treat the resulting solution with 81 g of de-
colorizing carbon for about 30 minutes at
about room temperature. Remove the decolor-
izing carbon by filtration and concentrate the
10 filtrate *in vacuo* to approximately 2.0 liters.
Prepare a slurry of about 80 liters of ice and
water and with vigorous stirring add the acet-
one solution. Allow the temperature of the
resulting suspension to rise to about 25° C.
15 with stirring and collect the product by filtra-
tion. Wash the solids with a small quantity of
water and dry at about 50° C. *in vacuo* to
yield about 212 g of Megalomicin-C complex
assaying about 1075 $\mu\text{g/mg}$.

20 Additional product (about 85 grams) may
be obtained by concentration of the mother
liquor, however, this product may be con-
taminated with Megalomicins A and B.

25 Megalomicin-C Complex as prepared above
may advantageously be used in the prepara-
tion of acid addition salts as shown below.

EXAMPLE 10

*Preparation of a Tartrate Salt of an Megalo-
micin Complex*

To a solid composition comprising 2.0 g of 30
a complex of Megalomicin-C₁ and Megalo-
micin-C₂, obtained in Example 9 above, add 30
ml of diethylether with stirring. Add to the
resulting solution 0.5 g of tartaric acid dis- 35
solved in 2.5 ml of ethanol. Stir the mixture
for about 15 minutes, then filter the precipitat-
ed salt. Wash the precipitate with diethyl-
ether and dry the product to obtain about 2.0
g of tartrate salt whose bioassay is about 575 40
 $\mu\text{g/mg}$ and whose rotation is -57° measured
at a 1% solution in ethanol.

EXAMPLE 11

Megalomicin-C Complex Propionate Ester

Dissolve 1.0 g of Megalomicin-C Complex 45
in 5.0 ml of dry acetone and add 0.35 ml
of propionic anhydride. Stir the mixture at
room temperature for 4 hours, then slowly and
with stirring add 8 ml of 1.2% aqueous am-
monia. Filter the resulting precipitate and 50
wash with 40% aqueous acetone, then with

water to obtain the desired product. The product obtained in this manner has the following constants:

5 m.p. = 207—210° C.; $[\alpha]_D^{25} = -63^\circ$ (C = 1% in ethanol).

EXAMPLE 12

Megalomicin-C Complex Diacetate Ester

10 Dissolve 11.0 g of Megalomicin-C Complex in 55 ml of pyridine and add 5.5 ml of acetic anhydride. Stir the reaction mixture at room temperature for about 18 hours, then pour it into 2.0 liters of ice water containing 50 ml of 14% aqueous ammonia with vigorous stirring. Filter the resulting precipitate, wash with 15 water and dry to yield the desired product melting 237—241° C. and having a rotation of about -86° (C = 1% in ethanol).

Alternatively, the precipitated product may be extracted with ethyl acetate, the extract 20 washed with water and evaporated *in vacuo* to a residue. The residue is subsequently dissolved in acetone, treated with decolorizing carbon and precipitated by the addition of an equal volume of water to yield the title product.

25 In view of the foregoing examples, it becomes obvious to those skilled in the art that the antibiotics of this invention will form other esters.

(4) *Pharmaceutical Dosage Forms*

30 The following Examples are directed to some of the pharmaceutical dosage forms wherein the antibiotics may be used. The formulations, excluding the topicals, are designed to permit the administration of from about 5 to 50 mg of antibiotic per kilogram of body weight per day. It is, of course, contemplated that the medicaments be given in divided doses from 2 to 8 times in a 24 hour period.

40 The topical formulations are generally applied to the infected area from 2 to 4 times daily.

45 In the utilization of the formulations set forth below the dosage of antibiotic given and the frequency of administration depends in large part on the stage and severity of the infection, the type of infection and the individual characteristics of the animal species being treated.

EXAMPLE 13

Capsule:

Megalomicin Complex	250.00 mg
Lactose	248.75 mg
Magnesium Stearate	1.25 mg
55	500.00 mg

Procedure:

1. Blend the Megalomicin Complex and the lactose.
2. Add the magnesium stearate and mix.
3. Fill capsules.

60

EXAMPLE 14

Oral Suspension:

Megalomicin A	25.0 g	
Magnesium aluminium silicate	9.5 g	
Sodium Carboxymethylcellulose, USP	2.5 g	65
Sodium Citrate, USP	25.0 g	
Flavor	q.s.	
Color	q.s.	
Methylparaben, USP	0.9 g	
Propylparaben, USP	0.2 g	70
Polysorbate 80, USP	1.0 g	
Sorbitol Solution, USP	500.0 g	
Water	q.s.	1000.0 ml

Procedure:

1. Heat 200 ml of water to boiling and dissolve in it one half of the parabens. Cool to about 70° C., then mix in the Polysorbate 80. Sprinkle in the silicate, stirring until a uniform smooth suspension results, which is premixture 1.

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2. Heat an additional 200 ml of water to boiling, and dissolve in it the remainder of the parabens. Disperse the carboxymethylcellulose in this until a smooth gel results. Mix in the Sorbitol Solution. Then dissolve the sodium citrate, to obtain premixture 2.

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3. Add premixture 2 to premixture 1 slowly, with constant stirring. Cool the resulting mixture to 25° C. Add the Megalomicin A, flavor and color, mixing thoroughly. Add sufficient quantity of water to make the total volume 1000 ml.

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EXAMPLE 15

Topical Cream:

Megalomicin B	10 g	95
Stearic Acid	200 g	
Sorbitan monostearate	104 g	
Sorbitan monooleate	20 g	
Polyoxyethylene sorbitan monolaurate	56 g	
Water	q.s.	1000 g

100

Procedure:

1. Heat the stearic acid, sorbitan monostearate, sorbitan monooleate, and polyoxyethylene sorbitan monolaurate to 65° C., so as to obtain a premixture 1.
2. Heat about 90% of the required water to 70° C., then add it to premixture 1 and mix to form the cream base.
3. Slurry the Megalomicin B with about 10% of the water, pass through a colloid mill, then add the milled slurry to the molten cream base and mix. Allow to cool.

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EXAMPLE 16

Topical Ointment:

Megalomicin C ₁	10 g
Petrolatum	990 g
	1000 g

Procedure:

1. Melt the petrolatum.
2. Slurry the Megalomicin C₁ with about 10% of the petrolatum and pass through a colloid mill.
3. Mix the milled slurry with the remainder of the molten petrolatum. Allow to cool.

EXAMPLE 17

Parenteral Solution:

Megalomicin A Tartrate	250 mg/vial
Citric Acid	} serving as 14 mg/vial
Disodium Phosphate	
Sterile distilled water	36 mg/vial
	5.0 mg/vial

Procedure:

1. Mix sterile Megalomicin A Tartrate with sterile citrate/phosphate buffer aseptically.
2. Fill into sterile vials.
3. Add water and shake well immediately before using.

EXAMPLE 18

Parenteral Suspension:

Megalomicin C ₂	250 mg/vial
Sodium Carboxymethylcellulose	3 mg/vial
Polysorbate 80, USP	1 mg/vial
Methylparaben	3.6 mg/vial
Propylparaben	0.4 mg/vial
Water	q.s. 2.0 ml/vial

Procedure:

1. Prepare a solution of the above required sodium carboxymethylcellulose, Polysorbate 80, methylparaben, and propylparaben. Autoclave the solution.
2. Aseptically, slurry sterile Megalomicin-C₂ with a portion of the above vehicle and pass through a colloid mill.
3. Mix the milled slurry with the remainder of the vehicle.
4. Fill into sterile vials.

With reference to Examples 13—18 above, it is to be noted that the antibiotic components thereof may be replaced by any other antibiotic complex, antibiotic or mixture of antibiotics according to the invention, in the free base or in the form or esters or salts, except that parenteral solutions such as represented by Example 17 desirably contain the antibiotic complex, antibiotic or mixture of antibiotics in the form of an acid addition salt.

WHAT WE CLAIM IS:—

1. Process for the production of an antibiotic complex identified as megalomicin com-

plex or a constituent antibiotic thereof, which comprises incubating a megalomicin complex-producing microorganism (or strain) of the species *Micromonospora megalomicea* in an aqueous nutrient medium, under aerobic conditions.

2. Process according to claim 1 wherein the microorganism utilised is *Micromonospora megalomicea* var. *megalomicea*.

3. Process according to claim 2 wherein the microorganism utilised is *Micromonospora megalomicea* var. *megalomicea* NRRL 3274 or a megalomicin producing mutant or descendant thereof.

4. Process according to claim 1 wherein the microorganism utilised is *Micromonospora megalomicea* var. *nigra*.

5. Process according to claim 4 wherein the microorganism utilised is *Micromonospora megalomicea* var. *nigra* NRRL 3275 or a megalomicin producing mutant or descendant thereof.

6. Process according to any preceding claim wherein the incubation is performed under submerged aerobic conditions.

7. Process according to any preceding claim wherein the microorganism is incubated at a temperature of from about 22 to 40° C. and at a pH of from about 7.0 to 8.5.

8. Process according to claim 7 wherein the temperature is from 28 to 37° C. and the pH is from 7.2 to 8.2.

9. Process according to any preceding claim which includes recovering at least one antibiologically active compound or substance.

10. Process according to claim 9, wherein the antibiologically active compound or substance is recovered in free base form.

11. Process according to claim 9, wherein the antibiologically compound or substance is recovered in the form of an ester.

12. Process according to claim 9, wherein the antibiologically active compound or substance is recovered in the form of a salt.

13. Process according to claim 12, wherein the salt is an acid addition salt.

14. Process according to any of claims 9 to 13, including the steps of making the broth alkaline, extracting it with a water-immiscible solvent and isolating the antibiologically active compound or substance from the extract.

15. Process according to any of claims 9 to 14 including the steps of resolving the recovered antibiologically active substance into antibiologically active compounds and isolating at least one such compound.

16. Process according to claim 15, wherein the compound isolated is megalomicin A.

17. Process according to claim 16, wherein the compound isolated is megalomicin B.

18. Process according to claim 15, wherein the compound isolated is megalomicin C₁.

19. Process according to claim 15, wherein the compound isolated is megalomicin C₂.

20. Process according to any of claims 15 to

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EXAMPLE 16

Topical Ointment:

Megalomicin C ₁	10 g
Petrolatum	990 g
	1000 g

Procedure:

1. Melt the petrolatum.
2. Slurry the Megalomicin C₁ with about 10% of the petrolatum and pass through a colloid mill.
3. Mix the milled slurry with the remainder of the molten petrolatum. Allow to cool.

EXAMPLE 17

Parenteral Solution:

Megalomicin A Tartrate	250 mg/vial
Citric Acid	} serving as 14 mg/vial
Disodium Phosphate	
Sterile distilled water	5.0 mg/vial

Procedure:

1. Mix sterile Megalomicin A Tartrate with sterile citrate/phosphate buffer aseptically.
2. Fill into sterile vials.
3. Add water and shake well immediately before using.

EXAMPLE 18

Parenteral Suspension:

Megalomicin C ₂	250 mg/vial
Sodium Carboxymethylcellulose	3 mg/vial
Polysorbate 80, USP	1 mg/vial
Methylparaben	3.6 mg/vial
Propylparaben	0.4 mg/vial
Water	q.s. 2.0 ml/vial

Procedure:

1. Prepare a solution of the above required sodium carboxymethylcellulose, Polysorbate 80, methylparaben, and propylparaben. Autoclave the solution.
2. Aseptically, slurry sterile Megalomicin-C₂ with a portion of the above vehicle and pass through a colloid mill.
3. Mix the milled slurry with the remainder of the vehicle.
4. Fill into sterile vials.

With reference to Examples 13—18 above, it is to be noted that the antibiotic components thereof may be replaced by any other antibiotic complex, antibiotic or mixture of antibiotics according to the invention, in the free base or in the form or esters or salts, except that parenteral solutions such as represented by Example 17 desirably contain the antibiotic complex, antibiotic or mixture of antibiotics in the form of an acid addition salt.

WHAT WE CLAIM IS:—

1. Process for the production of an antibiotic complex identified as megalomicin com-

plex or a constituent antibiotic thereof, which comprises incubating a megalomicin complex-producing microorganism (or strain) of the species *Micromonospora megalomicea* in an aqueous nutrient medium, under aerobic conditions.

2. Process according to claim 1 wherein the microorganism utilised is *Micromonospora megalomicea* var. *megalomicea*.

3. Process according to claim 2 wherein the microorganism utilised is *Micromonospora megalomicea* var. *megalomicea* NRRL 3274 or a megalomicin producing mutant or descendant thereof.

4. Process according to claim 1 wherein the microorganism utilised is *Micromonospora megalomicea* var. *nigra*.

5. Process according to claim 4 wherein the microorganism utilised is *Micromonospora megalomicea* var. *nigra* NRRL 3275 or a megalomicin producing mutant or descendant thereof.

6. Process according to any preceding claim wherein the incubation is performed under submerged aerobic conditions.

7. Process according to any preceding claim wherein the microorganism is incubated at a temperature of from about 22 to 40° C. and at a pH of from about 7.0 to 8.5.

8. Process according to claim 7 wherein the temperature is from 28 to 37° C. and the pH is from 7.2 to 8.2.

9. Process according to any preceding claim which includes recovering at least one antibiotically active compound or substance.

10. Process according to claim 9, wherein the antibiotically active compound or substance is recovered in free base form.

11. Process according to claim 9, wherein the antibiotically compound or substance is recovered in the form of an ester.

12. Process according to claim 9, wherein the antibiotically active compound or substance is recovered in the form of a salt.

13. Process according to claim 12, wherein the salt is an acid addition salt.

14. Process according to any of claims 9 to 13, including the steps of making the broth alkaline, extracting it with a water-immiscible solvent and isolating the antibiotically active compound or substance from the extract.

15. Process according to any of claims 9 to 14 including the steps of resolving the recovered antibiotically active substance into antibiotically active compounds and isolating at least one such compound.

16. Process according to claim 15, wherein the compound isolated is megalomicin A.

17. Process according to claim 16, wherein the compound isolated is megalomicin B.

18. Process according to claim 15, wherein the compound isolated is megalomicin C₁.

19. Process according to claim 15, wherein the compound isolated is megalomicin C₂.

20. Process according to any of claims 15 to

- 19, wherein the resolution is achieved by techniques including chromatographic separation.
21. Process according to any of claims 15 to 20, wherein the resolution is achieved by techniques including solvent extraction.
22. A process for the preparation of anti-biotically active compounds or substances belonging to the antibiotic complex identified as megalomicin complex substantially as here-inbefore described with particular reference to Examples 1 to 12.
23. An antibiotically active compound, substance or complex whenever obtained by a process according to any of claims 1 to 22.
24. The antibiotic complex identified as megalomicin complex.
25. Megalomicin A.
26. Megalomicin B.
27. Megalomicin C₁.
28. Megalomicin C₂.
29. A mixture of megalomicin C₁ and C₂, identified as megalomicin C complex.
30. Salts of the antibiotically active products of claims 25 to 28, and mixtures thereof.
31. Esters of the antibiotically active products of claims 25 to 28, and mixtures thereof.
32. The hydrochlorides of the products of claims 25 to 28.
33. The tartrates of the products of claims 25 and 28.
34. Megalomicin C₂ Hydrochloride.
35. Megalomicin A Hydrochloride.
36. Megalomicin A Tartrate.
37. Method for the preparation of an anti-biotically active composition, characterized in that at least one antibiotically active compound or substance belonging to the antibiotic complex identified as megalomicin complex, in free base form or in the form or an ester or salt, is brought into a form suitable for therapeutical administration.
38. Method according to claim 37, wherein the said antibiotically active compound or substance is mixed with a suitable pharmaceutical carrier.
39. Antibiotically active composition, containing at least one antibiotically active compound or substance belonging to the antibiotic complex identified as megalomicin complex in free base form or in the form of an ester or salt and a suitable pharmaceutical carrier.
40. Composition according to claim 39, substantially as hereinbefore described with particular reference to Examples 13 to 18.
41. A dosage unit such as a tablet or capsule, characterized by a content of at least one antibiotically active compound or substance belonging to the antibiotic complex identified as megalomicin complex, in free base form or in the form or an ester or salt.
42. Composition according to any of claims 39 to 41 wherein the said compound is megalomicin A.
43. Composition according to any of claims 39 to 41 wherein the said compound is megalomicin B.
44. Composition according to any of claims 39 to 41 wherein the said compound is megalomicin C₁.
45. Composition according to any of claims 39 to 41 wherein the said compound is megalomicin C₂.

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