

PATENT SPECIFICATION

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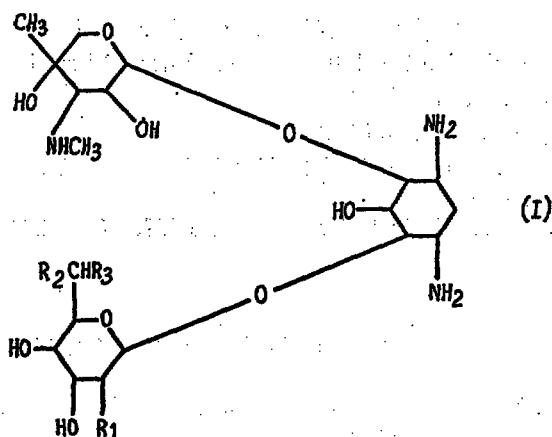
(54) GENTAMICIN ANTIBIOTICS

(71) We, SOHERICO 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 new amino glycoside antibiotics designated gentamicin B, gentamicin B₁ and gentamicin X, to their pharmaceutically acceptable derivatives and to processes for their preparation. This invention also relates to pharmaceutical compositions containing at least one of the said antibiotics, to methods for the preparation of such compositions and methods of use of these antibiotics and of a further amino glycoside antibiotic designated gentamicin A.

United Kingdom Patent Specification No. 1,021,401 describes and claims a process for the production of the antibiotic identified as gentamycin and for the antibiotics identified as BA—3 (Fractions A and B), which comprises incubating a micro-organism of the species *Micromonospora purpurea*, or the species *Micromonospora echinospora*, or a gentamycin- and/or BA—3-producing mutant or variant thereof, until at least one compound having substantial antibiotic activity is produced and isolating at least one of the compounds thus produced. Gentamycin, now known as gentamicin, was described in U.K. Specification No. 1,021,401 as the principal antibiotic component produced in the fermentation and is referred to herein as the C-component or gentamicin C. In the said specification reference is made to co-produced antibiotics BA—3 (Fraction A) and BA—3 (Fraction B) which were separated from gentamicin C and which in admixture with each other exhibited a low order of antibacterial activity. By the utilization of a particular chromatographic system described later herein, it has now been found that the substances described in U.K. Patent Specification No. 1,021,401 as co-produced antibiotics BA—3 (Fraction A) and BA—3 (Fraction B) are not single entities but together they represent a mixture of a least four components. An improved process for the resolution of this mixture of the co-produced antibiotics into its components (hereinafter called gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X), has led to the discovery that each member of this group possesses surprising and unexpected useful characteristics.

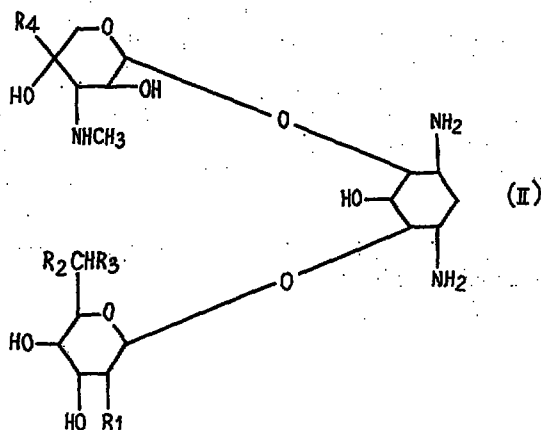
Thus in accordance with one aspect of the present invention there are provided the amino glycoside antibiotics designated gentamicin B, gentamicin B₁ and gentamicin X and having a gross molecular formula



in which, in the case of gentamicin B, R_1 is hydroxy, R_2 is hydrogen and R_3 is amino; in the case of gentamicin B_1 , R_1 is hydroxy, R_2 is methyl and R_3 is amino; and in the case of gentamicin X, R_1 is amino, R_2 is hydrogen and R_3 is hydroxy, the antibiotics each being in substantially pure form.

Gentamicin A has been previously isolated in pure form, investigated chemically and biologically and found to have rather weak antibiotic activity. Now a rather surprising useful characteristic of gentamicin A has been discovered namely, its utility as an anthelmintic and antiprotozoal agent. Gentamicin B, gentamicin B_1 and gentamicin X which heretofore have never been isolated, have been discovered to possess certain valuable properties as antibiotics, anthelmintic and antiprotozoal agents. In addition gentamicin B and B_1 have significant antibacterial properties. Therefore it is advantageous to use gentamicin B and gentamicin B_1 for treating bacterial infections over a protracted period of time. In the treatment of helminthiasis, which is aggravated by a bacterial infection or a protozoal infestation, the antibiotics gentamicin A, gentamicin B, gentamicin B_1 and gentamicin X can be of immeasurable value.

The antibiotics gentamicin B, gentamicin B_1 , gentamicin X and gentamicin A are structurally similar to each other as shown in formula (II) which depicts gross molecular arrangement without regard to spatial considerations.



Gentamicin A	$R_1 = \text{NH}_2$	$R_2 = \text{H}$	$R_3 = \text{OH}$	$R_4 = \text{H}$
Gentamicin B	$R_1 = \text{OH}$	$R_2 = \text{H}$	$R_3 = \text{NH}_2$	$R_4 = \text{CH}_3$
Gentamicin B_1	$R_1 = \text{OH}$	$R_2 = \text{CH}_3$	$R_3 = \text{NH}_2$	$R_4 = \text{CH}_3$
Gentamicin X	$R_1 = \text{NH}_2$	$R_2 = \text{H}$	$R_3 = \text{OH}$	$R_4 = \text{CH}_3$

The compounds of this invention readily form hydrates and solvates which are extremely difficult to break. Consequently, the physical constants for gentamicin A, gentamicin B and gentamicin B₁ are based upon relatively stable hydrates or solvates. Further, although gentamicin B and gentamicin B₁ form crystalline ethanol solvates they do not give reproducible melting points but rather tend to soften and eventually become viscous liquids over quite a wide temperature range. Gentamicin X has not yet been obtained in crystalline form; it is isolated as a white amorphous solid which likewise has no discrete melting point.

The antibiotics described herein have physical properties substantially as set forth

in Table I below:

(* All oxygen values are by difference)

TABLE I

Physical Properties

Compound	Elemental		Analyses		Sulfate Salt	
	Calculated*	Found	[α] _D ²⁵		Analyses	
Gentamicin A Calculated for C ₁₉ H ₃₈ N ₄ O ₁₀ ·1.5H ₂ O	C = 43.63 H = 7.93 N = 11.31 O = 37.13	C = 43.60 H = 7.86 N = 11.56 O = 37.16	+ 136° (C = 1.0, H ₂ O)		+ 96° (C = 0.3, H ₂ O)	
Gentamicin B Calculated for C ₁₉ H ₃₈ N ₄ O ₁₀ ·C ₂ H ₅ OH	C = 47.70 H = 8.41 N = 10.60 O = 33.29	C = 47.78 H = 8.38 N = 10.59 O = 33.25	+ 155° (C = 0.3, H ₂ O)		+ 116° (C = 0.3, H ₂ O)	
Gentamicin B ₁ Calculated for C ₂₀ H ₄₀ N ₄ O ₁₀ ·C ₂ H ₅ OH	C = 47.22 H = 8.34 N = 10.41 O = 34.03	C = 48.68 H = 8.56 N = 10.32 O = 32.44	+ 161° (C = 0.3, H ₂ O)		+ 117° (C = 0.3, H ₂ O)	
Gentamicin X C ₁₉ H ₃₈ N ₄ O ₁₀		C = 43.54 H = 7.22 N = 9.90 O = 39.34	+ 154° (C = 0.3, H ₂ O)		+ 121° (C = 0.3, H ₂ O)	

Gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X have characteristic infrared absorption spectra in mineral oil (Nujol) as shown by Figure 1 (gentamicin A), Figure 3 (gentamicin B), Figure 5 (gentamicin B₁) and Figure 7 (gentamicin X) of the accompanying drawings. These spectra are of the respective antibiotics as sulfate salts.

These antibiotics also have characteristic nuclear magnetic resonance spectra (NMR) as shown by Figure 2 (gentamicin A), Figure 4 (gentamicin B), Figure 6 (gentamicin B₁), and Figure 8 (gentamicin X) of the accompanying drawings. All of the NMR spectra are of the antibiotic free bases; however, the samples of gentamicin A and gentamicin X were hydrated and those of gentamicin B and gentamicin B₁ are of the ethanol solvate. These spectra were obtained by the use of a Varian A-60-A spectrometer (Varian Associates, Palo Alto, California — "VARIAN" is a Registered Trade Mark) on about 0.4 ml. of a solution (conc. about 20 mg/ml) of the antibiotic in deuterium oxide (D₂O). The spectra are recorded in parts per million (PPM) from 3-(trimethylsilyl)-propanesulfonic acid sodium salt, the internal standard.

In Table II below are set forth the more characteristic peaks from Figures 1, 3, 5 and 7, respectively.

TABLE II

Significant Infrared Absorption Bands

Gentamicin A Sulfate

2.9 — 3.8 μ (S, V brd)	6.85 μ (Nujol)
3.35 μ (Nujol)	7.25 μ (Nujol)
3.55 μ (Nujol)	8.50 — 10.75 μ (V, S, V brd)
4.83 μ (W, brd)	12.95 μ (V, W, brd)
6.17 μ (M)	13.88 μ (W, brd)
6.53 μ (M)	

Notations: M = medium; S = strong; V = very; W = weak; brd = broad

Gentamicin B Sulfate

2.95 — 3.25 μ (M—S, brd)	7.26 μ (Nujol)
3.40 μ (Nujol)	7.75 μ (W)
3.51 μ (Nujol)	8.75 — 9.75 μ (S, V brd)
4.85 μ (W, brd)	10.28 μ (W—M)
6.16 μ (M)	12.95 μ (VW, brd)
6.55 μ (M)	13.90 μ (W, brd)
6.83 μ (Nujol)	

Gentamicin B₁ Sulfate

2.95 — 3.25 μ (M—S, brd)	7.25 μ (Nujol)
3.38 μ (Nujol)	7.75 μ (W)
3.50 μ (Nujol)	8.75 — 9.75 μ (S, V brd)
4.85 μ (W, brd)	10.27 μ (W—M)
6.16 μ (M)	10.75 μ (W)
6.55 μ (M)	13.05 μ (V—W, brd)
6.83 μ (Nujol)	13.90 μ (W, brd)

Gentamicin X Sulfate

3.0 — 3.3 μ (S, brd)	7.25 μ (Nujol)
3.37 — 3.51 μ (Nujol)	7.75 μ (W—M)
3.65 — 4.33 μ (N, brd)	8.7 — 10.0 μ (V—S, brd)
4.82 μ (W, brd)	10.30 μ (Brd, shd)
5.94 μ (W, shd)	11.50 μ (W, brd)
6.17 μ (M)	13.03 μ (V—W, brd)
6.54 μ (M)	13.90 μ (Nujol)
6.83 μ (Nujol)	15.08 μ (V—W)

Notations: M = medium; S = strong; shd. = shoulder; V = very; W = weak; brd. = broad.

Gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X have amine or substituted amine groups which are capable of forming acid addition salts with organic and inorganic acids. If such a salt is derived from a polybasic acid it may contain free acid groups and/or acid groups neutralised by other bases, such as for example the alkali metal bases. The term non-toxic salt used in this specification is defined herein as salts that do not give rise to detrimental side effects when administered at the usual dosage level. Generally, the inorganic acid salts may be formed by adding an amount of dilute (1.0N) acid to an aqueous solution of antibiotic base to adjust the pH of the solution to about 4.5 and lyophilizing the resulting solution. Thus, by the foregoing procedure acid addition salts are formed with hydrochloric acid, sulfuric acid and phosphoric acid including the alkali metal salts of the dibasic and tribasic acids. In general, the salts of inorganic acids are water soluble but are substantially insoluble in organic solvents. However, such salts (e.g. hydrochlorides) may have very limited solubility in polar organic solvents such as for example lower alcohols. Salts of organic acids such as, for example, hydrocarbon carboxylic acids including dibasic acids, such as for example succinic, tartaric, maleic, fumaric and glutaric acid are usually prepared by reacting an aqueous solution of the antibiotic with a stoichiometric quantity of acid and either lyophilizing the resulting solution or precipitating the product with a miscible organic non-solvent for the salt, such as for example acetone, dioxane, tetrahydrofuran or methanol. Also solvates of acid addition salts of the antibiotics can be prepared.

In accordance with a further aspect of the present invention, there is provided a process for producing the antibiotics identified as gentamicin B, gentamicin B₁ and gentamicin X, in free form or in the form of antibiotically active, pharmaceuticals.

acceptable derivatives, which comprises subjecting a mixture comprising at least one of said antibiotics or derivatives to chromatography on a column of solid adsorbent using a liquid solvent as mobile phase, recovering a fraction of said solvent which contains one of the said antibiotics or derivative, isolating the antibiotic or derivative from said fraction, and, if desired, transforming the isolated antibiotic into an antibiotically active, pharmaceutically acceptable derivative or the isolated derivative into the free form of the antibiotic or into another antibiotically active, pharmaceutically acceptable derivative. Preferably silica gel is used as an adsorbent.

The said mixture may comprise, for example, gentamicin B and/or gentamicin B₁ and/or gentamicin X in admixture with at least one further gentamicin antibiotic such as for example gentamicin A and/or gentamicin C.

Thus, for example, a mixture comprising gentamicin B, B₁, X, A and C may for example be obtained by the fermentation of *Micromonospora purpurea* or *Micromonospora echinospora* as described in U.K. Patent Specification No. 1,021,401. The so obtained mixture is preferably subjected to a column chromatographic separation process utilizing the lower layer of a solvent system consisting of methanol, chloroform, and ammonium hydroxide as the eluting solvent. Prior to chromatography, equal volumes of the solvents are mixed, allowed to settle and the layers separated. Gentamicin C, also known as Garamycin (Trademark of Schering Corporation, Bloomfield, New Jersey) (known to be composed of three components, C₁, C_{1a} and C₂) is first eluted and is followed in turn by gentamicin B₁, gentamicin X, gentamicin B and lastly, gentamicin A.

It will be appreciated that the above-described separation enables antibiotically active compositions comprising antibiotics gentamicin B, gentamicin B₁ and gentamicin X to be produced in which at least one of antibiotics gentamicin B and gentamicin B₁ is substantially absent from the composition, or alternatively in which the composition comprises only one of said antibiotics gentamicin B, gentamicin B₁ and gentamicin X, and other substances coproduced therewith in the fermentation are substantially absent. Such compositions, together with corresponding compositions in which the antibiotics are in the form of antibiotically active pharmaceutically acceptable derivatives form further aspects of the invention.

A process for separating individual gentamicins from a mixture containing gentamicin antibiotics will now be described in more detail.

At the end of the fermentation, the antibiotics are extracted from the fermentation broth by adsorbing the total antibiotic complex from the fermentation broth onto a cation exchange resin, preferably Amberlite IRC-50.

The resin is advantageously utilized in the ammonium cycle and the crude antibiotic mixture eluted with dilute ammonium hydroxide. The eluate is decolorized by passage through a column containing decolorizing carbon, or, preferably, by passage through an anion exchange resin column, especially, a resin such as an Amberlite IRA. For decolorizing the IRC-50 resin eluate IRA-401S resin in the hydroxyl cycle is preferred. After decolorization, the eluate is concentrated *in vacuo* to a solution containing from about 30% to about 60% solids and held for further processing. At this juncture, the solution contains all of the antibiotics produced in the fermentation and in substantially the ratio produced therein. The gentamicin "C" components constitute from about 60% to about 80% of the mixture and the co-produced antibiotics constitute the remainder. Thus, in order to obtain the co-produced antibiotics it is advantageous to at least partially remove the gentamicin C. There are a substantial number of resins that are suitable for this purpose. Exemplary of such resins are anion exchange resins such as for example Amberlite IRA-400, Amberlite IRA-401, Dowex 1-X2, and Dowex 2-X4 (Amberlite being a Registered trademark of Rohm and Haas Company, Philadelphia, Pennsylvania and Dowex being a Registered trademark of Dow Chemical Company, Midland, Michigan.) In general, porous strongly basic quaternary amine type resins are of utility. The Applicants prefer to utilize Dowex 1-X2 in the hydroxyl cycle.

The antibiotic mixture is absorbed onto the resin and then gentamicin C components are selectively desorbed therefrom by elution with deionized water having from about 50,000 to about 150,000 ohm metres specific resistance. The column may advantageously be connected in series and through a conductivity bridge to an automatic fraction collector so that a sharp rise in conductance of the effluent will actuate the fraction collector. Similarly, a drop in conductance (rise in resistance) followed by a sharp rise in conductance will start the collection of a new fraction. The care with which this column is run, to a substantial degree, controls the quantity of gentamicin "C", which remains in the mixture of co-produced antibiotics and to some

degree affects the efficiency of the subsequent column which is used to separate the co-produced antibiotics one from the other.

The remaining co-produced antibiotics are eluted with de-ionised water from the Dowex 1—X2 column subsequent to the gentamicin C components and the aqueous eluate concentrated *in vacuo* to a suitable volume and lyophilized. At this stage, it is advantageous to dissolve the antibiotic mixture in hot methanol and cool while exposing the solution to air. Gentamicin A adsorbs carbon dioxide from the air forming a sparingly soluble carbonate salt which crystallizes from the methanol solution and is removed by filtration and dried *in vacuo*. The filtrate is concentrated to a residue and dissolved in about 3 volumes of the lower phase of a solvent mixture consisting of methanol: chloroform: ammonium hydroxide in the volume ratio of 1:1:1.

The foregoing solution is placed atop a suitably sized column of preferably silica gel and the column eluted using the lower phase of the above-described solvent mixture and a controlled flow rate. Gentamicin B and gentamicin B₁ may be obtained in crystalline form by dissolving the residues from the appropriate column fractions (See Example 1, Step D) in ethanol and concentrating *in vacuo* to a residue, redissolving the residue in ethanol and re-evaporating the solution until a crystalline suspension is obtained. This procedure yields substantially pure gentamicin B and gentamicin B₁, as ethanol solvates having about 1 mole of ethanol per mole of antibiotic. Gentamicin X is usually obtained as an amorphous solid.

Alternatively, the total antibiotic mixture from the IRA—401S column which includes the gentamicin C components may be subjected to silica gel chromatography. However, the separation effected by this technique is not as sharp as that obtained when most of the gentamicin "C" and the gentamicin A are removed prior to silica gel chromatography.

Example 1.

A. Isolation of gentamicin C and the co-produced antibiotics from the fermentation

Adjust the pH of a fermentation broth obtained as described in U.K. Patent Specification No. 1,021,401 to pH 2.0 with 6N sulfuric acid. Stir the mixture for about 30 minutes and filter through a suitably sized filter which has been precoated with about a 2.5 cm layer of filter aid. Re-adjust the pH of the filtrate to about 7 with 6N ammonium hydroxide and add oxalic acid to lower the pH to about 4. This quantity of oxalic acid is usually sufficient to precipitate the calcium ions in the fermentation broth as the insoluble calcium oxalate. Adjust the pH to about 7 with 6N ammonium hydroxide and stir the mixture for from about 1 to about 4 hours at about 20°—35°C. Refilter the mixture using a suitably sized pre-coated filter and collect the filtrate. Adsorb the antibiotic mixture in the filtrate on IRC—50 (ammonium cycle) using about 10 grams of resin per liter of the original broth volume. Wash the resin color free with deionized water and elute the antibiotics with 2N ammonium hydroxide. Concentrate the eluate *in vacuo* to a solution containing about 50% solids.

B. Separation of gentamicin C components from gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X

Charge 1.3 m³ of Dowex 1—X2 resin (hydroxyl cycle) into a clean chromatographic column which is 91 cm in diameter, at least 244 cm high and is connected through a conductivity bridge to a fraction collector. Pump deionized water through the resin bed until the effluent has a resistance of at least 65,000 ohms. Charge an aqueous solution of about 120 liters containing about 60 kilograms of solids (from Step A) to the column and adsorb the antibiotic mixture by permitting the antibiotic solution to pass through the column at the rate of about 2 liters per minute.

Develop the column with deionized water at the rate of about 2 liters per minute and collect fractions in accordance with the conductance of the eluate. Typically, about seven fractions are collected which vary from about 14 to about 750 liters in volume. The first five fractions comprising about 500 liters contain the bulk of the gentamicin C components. The remaining two fractions comprising about 1500 to 1600 liters contain the bulk of gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X. The latter two fractions are combined, concentrated *in vacuo* to a small volume and either lyophilized or spray dried.

C. Isolation of gentamicin A

Dissolve 19.8 kilograms of fermentation product from which most of the gentamicin C has been removed (by Step B) in 45 liters of hot methanol. Clarify the solution by filtration. Cool the solution at room temperature with exposure to air and seed with gentamicin A. Gentamicin A crystallizes as its colorless carbonate salt which is

filtered, washed sparingly with methanol and dried at 80°C in vacuo to obtain 1119 grams of gentamicin A. The mother liquor and washes are combined, concentrated to a residue *in vacuo* and chromatographed as described in step D.

D. Chromatographic separation of gentamicin B, gentamicin B₁ and gentamicin X

Two 152 cm×10 cm chromatographic columns connected in series are filled with silica gel (e.g. Baker Silica Gel for Chromatography) which has previously been conditioned by contact with the lower phase of a 1:1:1 mixture of chloroform, methanol and concentrated ammonium hydroxide. Prepare a solution of 750 grams of the residue from step C in a minimum volume (about 2 liters) of the lower layer of the above-described solvent mixture. Place the antibiotic solution atop the first column and elute the column at the rate of about 3 to about 4.5 liters per hour, collecting fractions of approximately two (2) liters. Monitor the effluent by thin layer chromatography on silica gel plates using the same solvent system that is being used on the column. Combine the fractions containing like antibiotics and evaporate to a residue. When the amount of gentamicin B eluted tapers off, the column is eluted with a mixture comprising equal volumes of methanol and concentrated ammonium hydroxide to strip the column. The fractions containing gentamicin B and gentamicin B₁ are separately dissolved in ethanol and concentrated to obtain, on repeating these steps, crystalline suspensions of the ethanol solvates of gentamicin B and gentamicin B₁. When the foregoing chromatographic separation is effected, the products are obtained in the amounts and order substantially as shown below:

Gentamicin C	}	198.6 gms	
Gentamicin B ₁			
Gentamicin B ₁		42.0 gms	$[\alpha]_D^{26^\circ} = 157^\circ$
Gentamicin B ₁	}	67.8 gms	
Gentamicin X			
Gentamicin X		12.3 gms	$[\alpha]_D^{26^\circ} = 153.8^\circ$
Gentamicin X	}	74.9 gms	
Gentamicin B			
Gentamicin B		85.8 gms	$[\alpha]_D^{26^\circ} = 132^\circ$
Gentamicin B	}	165.0 gms	
Gentamicin A			

The fractions containing mixtures of antibiotics may be rechromatographed to afford more of the individual fractions.

Example 2.

Preparation of gentamicin A sulfate

Dissolve 5.0 grams of gentamicin A in 25 ml. of water and adjust the pH of the solution to 4.5 with 1N sulfuric acid. Treat the solution with about 50 milligrams of decolorizing carbon for about 15 minutes and filter. Pour the filtrate into about 300 milliliters of methanol with vigorous agitation, continue the agitation for about 10—20 minutes and filter. Wash the precipitate with methanol and dry at about 60°C *in vacuo* to obtain the title product of this Example.

In like manner, the sulfate salt of gentamicin B, gentamicin B₁, and gentamicin X may be prepared. In order to prepare sulfate salts of a mixture of gentamicin A, B, B₁ and X, the lyophilized or spray dried product of Example 1, step B, is treated as described in this example.

Example 3.

Preparation of gentamicin B₁ hydrochloride

Dissolve 5.0 grams of gentamicin B₁ in 1000 milliliters of water. Prepare a column containing 500 grams of weakly basic ion exchange resin in the chloride cycle, preferably, Amberlite IR-45 (Trademark, product of Rohm and Haas, Philadelphia, Pennsylvania). Pass the antibiotic solution through the column followed by an additional 500 milliliters of water. Concentrate the effluent to about 100 milliliters *in vacuo* and lyophilize to obtain the title product of this example.

In like manner, the hydrochloride salt of gentamicin A, gentamicin B, and gentamicin X may be also prepared.

Gentamicin A and gentamicin X, and to a lesser extent, gentamicin B and gentamicin B₁, have been found to be effective against protozoa such as, for example, *Entamoeba histolytica*, *Histomonas meleagridis*, *Trichomonas foetus* and *Trichomonas vaginalis*. *Histomonas meleagridis* is the protozoal parasite which causes enterohepatitis, the so-called "blackhead disease" in turkeys. This disease causes serious economic problems in the turkey raising industry. *Trichomonas vaginalis* is the pathogen most frequently associated with cases of human vaginal infections and is usually a most difficult pathogen to eradicate.

Gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X are also effective as anthelmintic agents against pinworms such as for example *Syphacia obveata*.

Thus in accordance with a further aspect of the present invention, there are provided processes for combatting protozoal infections in a non-human animal having such infection and processes for combatting helminthiasis in a non-human animal afflicted with helminthiasis, which comprise administering to said animals a therapeutically effective quantity of at least one of antibiotics gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X, and their antibiotically active pharmaceutically acceptable derivatives.

That these substances, gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X, possess valuable antiprotozoal properties is surprising. It has been reported that gentamicin C, which is structurally related to the aforementioned antibiotics, exhibits only slight activity against *T. vaginalis* at somewhat high dose ranges. It has been further reported by Albach et al in the American Journal of Tropical Medicine and Hygiene, 15, 885 (1965) that gentamicin C is relatively ineffective against *E. histolytica*. All of these gentamicin antibiotics are members of a chemical class of compounds sometimes referred to as amino-glycosides. This class includes such known antibiotics as neomycin, kanamycin, streptomycin and paromomycin. Only paromomycin is known to exhibit antiprotozoal activity to any substantial degree. Furthermore, with the exception of paromomycin, gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X are the only amino-glycosides now known to exhibit anthelmintic activity in reasonably useful dosage ranges.

In view of these properties gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X may be used *in vitro* in wash solutions for sanitary purposes, such as in the cleaning of laboratory equipment contaminated with protozoa of the type named above. This is especially true of laboratory table tops and animal cages and pens from which it is desired to eradicate susceptible parasitic protozoal and bacterial species. By virtue of their *in vivo* activity, the compounds of this invention may be used in admixture with drinking water or animal feeds to treat laboratory or domestic animals having intestinal bacterial infections, intestinal protozoal infections, and helminthic infestations, or combination of the foregoing wherein the infecting organisms are susceptible to the compounds of this invention. The compounds are especially useful for intestinal disorders in view of the fact that they are not appreciably absorbed from the gastro-intestinal tract. Thus, substantial concentrations of therapeutic agent may be ingested without causing toxic manifestations to the host, and at the same time be present in quantities sufficient to destroy, or at least to substantially inhibit, the infecting organism. For combatting generalized (systemic) bacterial infections administration of antibiotic is effected by other than oral means, preferably by injection.

ANTIBIOTIC ACTIVITY — IN VITRO

In Table III below is set forth the *in vitro* minimal inhibitory concentration (MIC) of gentamicin B, gentamicin B₁ and gentamicin X in comparison with the known antibiotic kanamycin, all in the free base form against a variety of gram positive and gram negative microorganisms. The experiments were carried out in a yeast beef broth medium at pH 7.4.

TABLE III

MIC (mcg/ml)

Organism	Gentamicin B	Gentamicin B ₁	Gentamicin X	Kanamycin
<i>Aerobacter aerogenes</i>	0.08	0.08	—	3
<i>Bacillus subtilis</i>	0.8	3.0	0.03	0.3
<i>Escherichia coli</i>	0.03—0.3	0.08—0.3	0.3	5
<i>Klebsiella pneumoniae</i>	3.0	3.0	0.75	1
<i>Pseudomonas aeruginosa</i>	0.03—0.08	0.08	0.3—0.75	62.5
<i>Sarcina lutea</i>	0.3	0.3	—	4
<i>Staphylococcus aureus</i>	0.3—3.0	0.3—3.0	0.08—0.75	0.25
<i>Streptococcus pyogenes</i>	3.0	3.0	0.3—0.75	50
<i>Shigella</i> sp M324	5.0	5.0	—	
<i>Salmonella</i> sp. 1	0.8	0.3	0.75	
<i>Salmonella</i> sp. 2	0.8	0.3	—	
<i>Salmonella</i> sp. B	0.8	0.3	—	

ANTIBIOTIC ACTIVITY — IN VIVO

The protective activity of gentamicin B, gentamicin B₁ and gentamicin X is determined by administering the respective compound subcutaneously one-hour after administration of an infecting dose of pathogen and by counting the survivors 48 hours after infection. Infected non-treated controls die in about 18 to 24 hours. Generally 5—7 dose levels are administered to mice weighing about 20 grams in groups of 7—10. The PD₅₀ values are set forth in Table IV below:

TABLE IV

Protective Activity of Gentamicin B, B₁ and X in Mice

Infecting Microorganism	PD ₅₀ (mg/kg)		
	Gentamicin B	Gentamicin B ₁	Gentamicin X
Staphylococcus aureus Gray	20.0	30.0	4.0
Staphylococcus aureus W	50.0	50.0	—
Staphylococcus aureus Smith	5.0	2.5	—
Streptococcus pyogenes C	15.0	18.0	—
Salmonella paratyphi B	5.0	4.0	—
Pseudomonas aeruginosa SC	2.0	2.0	7.0
Escherichia coli SC	—	—	1.5

ACUTE TOXICITY

The acute toxicity of gentamicin B, gentamicin B₁ and gentamicin X is determined in male CF—1 (Carworth Farms) mice weighing about 20 grams each. The results of this test are set forth in Table V below:

TABLE V

Acute Toxicity of Gentamicin B, Gentamicin B₁ and Gentamicin X

Route	LD ₅₀ (mg/kg)		
	Gentamicin B	Gentamicin B ₁	Gentamicin X
I.V.	228	230	225
I.P.	1750	1600	>1000
S.C.	>1500	>1500	>1500
Oral	>250	>250	>250

IN VITRO ACTIVITY AGAINST TRICHOMONAS VAGINALIS

In Table VI below is set forth the in vitro activity of the compounds of this invention against *T. vaginalis*. The test procedure used was substantially as described by R. J. Schnitzer on pages 289—331 in *Experimental Chemotherapy*, Volume 1, Academic Press, New York (1963).

TABLE VI

Compound	Minimal	(mcg/ml)	Minimal	(mcg/ml)
	99% Suppression Level 24 hours		Cidal Level 24 hours	
Gentamicin A	10	5—10	25	10
Gentamicin B	25	25	>250	25
Gentamicin B ₁	25	10—25	>250	25
Gentamicin X	—	2.5	—	10
Garamycin (R)	250	100	>250	100

STS — Simplified Trypticase Serum Medium, Baltimore Biological Laboratories, Baltimore, Maryland.

Against *Entamoeba histolytica*, gentamicin X has a minimal inhibitory concentration of 4 mcg/ml (48 hours) and a minimal cidal concentration of 7 mcg/ml (48 hours).

ANTHELMINTIC ACTIVITY IN VIVO

Gentamicin B and gentamicin X exhibit anthelmintic activity against *Syphacia obvelata* when tested in groups of seven male Millerton mice by substantially the procedures described by O.D. Standen on pages 701—892 in *Experimental Chemotherapy*, Volume 1.

TABLE VII

Compound	mg/kg/day	% in Diet	% Infected	Mean Worm Burden
Gentamicin B	259	0.25	0	0
Gentamicin B	100	0.0625	0	0
Gentamicin B	39	0.02	0	0
Gentamicin B	8	0.008	67	29.5
Gentamicin X	310	0.25	0	0
Gentamicin X	62	0.05	83	27
Controls	0	0	100	53
Controls	0	0	100	22
Controls	0	0	75	6.8

The data set forth in Table VII shows that gentamicin B has anthelmintic activity against *Syphacia obvelata* at a minimum dosage of 39 mg/kg/day when administered to male Millerton mice. Gentamicin X is totally effective at 310 mg/kg/day and partially effective at 62 mg/kg/day under substantially the same test conditions.

DIMINISHED INCIDENCE OF ATAXIA

Gentamicin B₁ and to a lesser extent, gentamicin B₁, exhibit a property that is quite unusual in amino-glycoside antibiotics. When subjected to the test procedures described by Weinstein, Wagman and Taber in *Antimicrobial Agents and Chemotherapy*, 1965, pages 227—231, each of these compounds required substantially longer periods of administration at high doses to cause impairment of the "righting reflex" and the appearance of ataxia. There appears to be a casual relationship between high doses of amino-glycoside type antibiotics given over an extended period of time and vestibular damage wherein one of the early manifestations is ataxia. The test results, in Table VIII, show that gentamicin B and gentamicin B₁ as compared with gentamicin C (Garamycin) may be used at dosages that are substantially above the normal therapeutic dose. Moreover, they may be used at such dosages for a time interval substantially beyond that normally required to eliminate infection without causing ataxia; and with minimal, if any, toxic manifestations.

TABLE VIII

Chronic Toxicity in Cats After Daily Subcutaneous Dosing

Antibiotic	Dose (mg/kg/day)	No. of Cats	Average No. Days to become ataxic
Gentamicin B ₁	240	1	21.0
	60	4	6 9.0
	40	4	108.0
Garamycin (B)	60	8	13.5
	40	11	17.5
	20	5	31.4
Gentamicin B	240	1	21.0
	60	4	81.0
	40	4	≥312*

* Study terminated 2 of the 4 cats had not become ataxic at the end of 385 days; number given is the truncated median.

PROPHYLACTIC ACTIVITY OF GENTAMICIN A IN TURKEYS AGAINST HISTOMONAS MELEAGRIDIS

Gentamicin A was administered to groups of turkeys at dosage levels of 5, 10, and 15 mg. per turkey per day in aqueous solution. Two days after the initiation of the drug, the turkeys were inoculated orally with 500 *Heterakis gallinae* ova (the intermediate host carrier of *Histomonas*). A single group of turkeys received 0.035% of gentamicin A in their feed for six days. Untreated infected controls were also carried in each experiment. The test is continued for twelve days and the birds are autopsied on the thirteenth day. Infection is usually manifested principally in two areas, the cecum and the liver which are the areas examined during the autopsy.

According to the results obtained in the foregoing test, gentamicin A exhibits prophylactic activity in turkeys against *Histomonas meleagridis* at a minimum dosage level of 10 mg. per bird; whereas the untreated controls became infected.

Gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X are suitable for oral, topical or parenteral administration. When administered orally they may be compounded in the form of tablets, capsules or elixirs or may even be admixed with animal feed. It is in these dosage forms that the compounds of this invention are most effective as anthelmintic and antiprotozoal agents. This is especially true wherein they are being used to treat intestinal amoebiasis or non-specific diarrheas. The compounds of this invention may be incorporated into a dosage unit as the sole active ingredient or they may be compounded into dosage units in admixture with other active ingredients, such as for example anti-anxiety agents, it being known that infections and infestations often cause anxiety reactions in animal species. When the animal species is being treated orally, the compounds of this invention are administered at from about 10 to about 100 mg. preferably 20 to 70 mg per kilogram of body weight per day, preferably divided into from about 2 to about 4 doses.

For topical administration, the compounds of this invention may be compounded into creams, ointments or suppositories and the like. Such dosage forms should contain from about 1 to about 10% of antibiotic and should be administered to the site of the infection from about 2 to about 4 times a day.

When treating bacterial infections the antibiotics gentamicin B, gentamicin B₁ and gentamicin X can be administered in quantity of about 2 mg to about 20 mg, preferably 5 to 15 mg per kilogram of body-weight.

As shown hereinabove, (see Table VIII) gentamicin B and gentamicin B₁, by virtue of their low ataxic potential, may preferably be used to treat deep-seated bacterial infections especially those which have proved to be refractory to other antibacterial agents. It is preferred that gentamicin B and/or gentamicin B₁ be administered parenterally in dosages ranging between 2 and about 10 mg. per kilogram of body weight per day in divided doses.

Whenever the administration of the antibiotics gentamicin A, gentamicin B, gentamicin B₁ and X is described, the administration of their pharmaceutically acceptable acid addition salts and solvates as well as hydrates of all of these compounds is included.

As mentioned before the antibiotics can be applied in the form of compositions and in accordance with a further aspect of the present invention there are provided pharmaceutical compositions comprising at least one of the amino glycoside antibiotics gentamicin B, gentamicin B₁ and gentamicin X having the gross molecular formula (I) defined herein in admixture with a suitable pharmaceutical carrier, at least one of gentamicin B and gentamicin B₁ being substantially absent from the composition, and pharmaceutical compositions comprising an antibiotically active pharmaceutically acceptable derivative of at least one of the amino glycoside antibiotics gentamicin B, gentamicin B₁ and gentamicin X having the gross molecular formula (I) defined herein in admixture with a suitable pharmaceutical carrier, antibiotically active pharmaceutically acceptable derivatives of at least one of gentamicin B and gentamicin B₁ being substantially absent from the composition.

These compositions can be prepared by mixing the active ingredient with a suitable pharmaceutical carrier such as for example gelatin, lactose, talc, stearic acid, acacia, gums or a cellulosic material, polyalkylene glycols, petroleum jelly, disintegrating agents such as maize or starch, tableting lubricant or mould release agents such as magnesium stearate or calcium carbonate, aromatic water, benzylalcohols, syrup, pharmaceutical mucilage, an elixir, colouring agents, tinctures, spirits, oily media, sterile water or isotonic saline solution, preservatives such as methylparaben, propylparaben, benzoic acid, and nontoxic antimicrobial agents.

The examples below set forth some of the various dosage forms into which the compounds of this invention may be compounded.

Formulation I

Parenteral Solution	mg/ml	ml/10 ml.
Gentamicin B (as the sulfate)	40 to 200 mg*	400 — 2000
Methylparaben	1.8 mg.	18 mg.
Propylparaben	0.2 mg.	2 mg.
Water for Injection q.s.	1.0 ml.	10.0 ml.

* based on theoretical 100% purity.

Place 70% of the water for injection into a suitable mixing vessel and heat to 70°C. Add the methylparaben and propylparaben and mix until dissolved. Cool the above solution to 25°C—30°C. Pass a stream of nitrogen gas through the solution. Add the gentamicin B (as the sulfate) and mix until dissolved. Bring the solution to final volume. Pass the solution through a suitable sterilizing filter, employing appropriate aseptic techniques. Fill the solution into suitable sterile containers employing appropriate aseptic filling techniques.

Formulation II.

Parenteral Solution	mg/ml	mg/10 ml.
Gentamicin A (as the Sulfate)	40 to 200 mg*	400 — 2000
Methylparaben	1.80	18.00
Propylparaben	0.20	2.00
Sodium Bisulfite	3.20	32.0
Disodium Edetate, Dihydrate	0.10	1.00
Glacial Acetic Acid	1.25	12.50
Sodium Acetate	2.50	25.00
Propylene Glycol	100 to 400	1000 — 4000
Water for Injection q.s.	1.0 ml	10.00 ml

* Based on theoretical 100% purity.

Place 70% of the water for injection into a suitable mixing vessel and heat to 70°C. Add the methylparaben and propylparaben and mix until dissolved. Cool the above solution to 25°C—30°C. Pass a stream of nitrogen gas through the solution. Add the disodium edetate, dihydrate, sodium acetate and glacial acetic acid; mix thoroughly. Stop the nitrogen sparging, add the sodium bisulfite and mix until dissolved. Add the propylene glycol and mix thoroughly. Add the gentamicin A (as the sulfate) and mix until dissolved. Bring the solution to final volume. Pass the solution through a suitable sterilizing filter, employing appropriate aseptic techniques. Fill the solution into suitable sterile containers employing appropriate aseptic techniques.

Formulation III.

Parenteral Solution	mg/ml	mg/100 ml
Gentamicin B ₁ (as the Sulfate)	40 to 200 mg*	4000 — 20,000
Methylparaben	1.80	180
Propylparaben	0.20	20
Sodium Bisulfite	3.20	320
Disodium Edetate, Dihydrate	0.10	10
Glacial Acetic Acid	1.25	125
Sodium Acetate	2.50	250
Water for Injection q.s.	1.00 ml	100 ml

* Based on theoretical 100% purity.

Place 70% of the water for injection into a suitable mixing vessel and heat to 70°C. Add the methylparaben and propylparaben and mix until dissolved. Cool the above solution to 25°—30°C. Pass a stream of nitrogen gas through the solution. Add the disodium edetate, dihydrate, sodium acetate and acetic acid; mix thoroughly. Stop the nitrogen gas, add the sodium bisulfite and mix until dissolved. Add the gentamicin B₁ (as the sulfate) and mix until dissolved. Bring the solution to final volume. Pass the solution through a suitable sterilizing filter, employing appropriate aseptic techniques. Fill the solution into suitable sterile containers employing appropriate aseptic filling techniques.

Formulation IV.

Oral Syrup	Per Liter
Gentamicin A (as the sulfate)	100 g.*
Standard Granulated Sugar	550
Sorbitol Solution	200
Water, to make	1.0 liter

* Based on theoretical 100% purity.

Add the gentamicin A (as the sulfate), granulated sugar and sorbitol solution, to approximately 350 mls of water contained in a suitable mixing vessel. Mix until a solution is obtained. Add sufficient water to make 1 liter. Pass the solution through a suitable clarifying filter.

Formulation V.

Oral Syrup	Per Liter
Gentamicin B (as the sulfate)	100 g.*
Granulated Sugar	100
Glucose Syrup	44
Sorbitol Solution	400
Glycerin	50
Alcohol	50
Water, to make	1.0 liter

* Based on theoretical 100% purity.

Add the gentamicin B (as the sulfate), granulated sugar, glucose syrup, sorbitol solution, glycerin, alcohol to approximately 500 ml. of water contained in a suitable mixing vessel. Mix until a solution is obtained. Add sufficient water to make 1 liter. Pass the solution through a suitable clarifying filter.

Formulation VI.

Topical Cream	Per Kg.	
Gentamicin A (as the sulfate)	10 g.—100 g.*	
Stearic Acid	60 g.	
Propylene Glycol Monostearate	100	5
Isopropyl Myristate	80	
Polyoxyethylene (20) Sorbitan Monopalmitate	60	
Sorbitan Solution	20	
Water to make	1.0 kg.	
* Based on theoretical 100% purity.		

Add the stearic acid, propylene glycol monostearate, isopropyl myristate and polyoxyethylene (20) sorbitan monopalmitate to a suitable mixing vessel. Heat to 80°C to melt. Mix.

Formulation VII.

Topical Cream	Per Kg.	
Gentamicin B (as the sulfate)	10 g.—100 g.*	
Ethoxylated Cetyl/Stearyl Alcohol	20 g.	
Cetyl Alcohol	35	
Stearyl Alcohol	35	
Petrolatum	200	20
Mineral Oil	50	
Water to make	1.0 kg.	
* Based on theoretical 100% purity.		

Add the cetyl alcohol, stearyl alcohol, ethoxylated cetyl/stearyl alcohol, petrolatum and mineral oil to a suitable mixing vessel. Heat to 80°C to melt. Mix. Add the gentamicin B (as the sulfate) in approximately 95% of the water heated to 80°C in a suitable mixing vessel. Mix. Add the melted wax phase to the aqueous phase and mix while cooling to approximately 40°C. Add sufficient water to make 1 kg. Mix until cool.

Formulation VIII.

Topical Ointment	Per Kg.	
Gentamicin B ₁ (as the sulfate)	10 g.—100 g.*	
White Petrolatum, to make	1.0 g.	
* Based on theoretical 100% purity.		

Melt and heat the petrolatum to 50°C in a suitable mixing vessel. Remove a portion of the melted petrolatum and make a slurry of the gentamicin B₁ (as the sulfate). Pass the slurry through a suitable colloid mill and mill until a uniform dispersion is obtained. Add the milled slurry to the remainder of the melted petrolatum and mix until cool.

Formulation IX.

Parenteral Solution	mg/ml	
Gentamicin X (as the sulfate)	40 to 200 mg.*	
Methylparaben	1.8 mg.	
Propylparaben	0.2 mg.	
Water for injection q.s.	1.0 ml.	
* Based on theoretical 100% purity.		

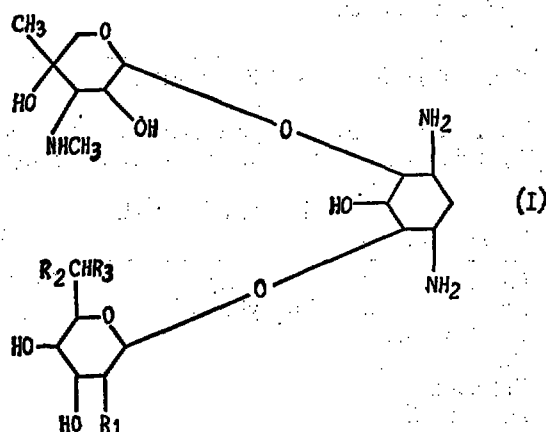
Place 70% of the water for injection into a suitable mixing vessel and heat to 70°C. Add the methylparaben and propylparaben and mix until dissolved. Cool the above solution to 25°—30°C. Pass a stream of nitrogen gas over the solution. Add the gentamicin X (as the sulfate) and mix until dissolved. Bring the solution to final volume. Pass the solution through a suitable sterilizing filter, employing appropriate aseptic techniques. Fill the solution into suitable sterile containers employing appropriate aseptic filling techniques.

It will be noted that gentamicin A, B, B₁ and X contain a garosamine ring and attention is directed to claim 1 of our U.K. Patent No. 1,314,058 which claims an oxa-

zolidine derivative of a gentamicin antibiotic containing a garosamine ring wherein the oxazolidine ring is fused to said garosamine ring.

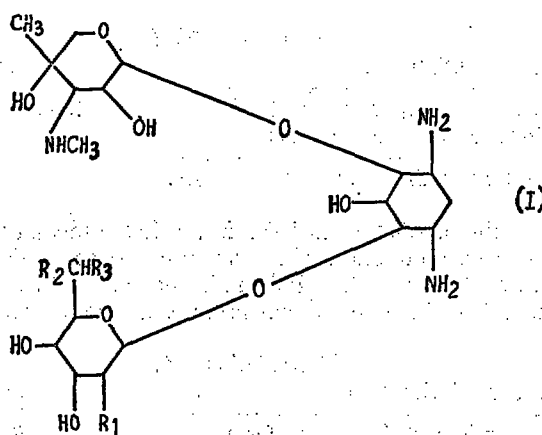
WHAT WE CLAIM IS:—

1. An amino glycoside antibiotic designated gentamicin B, gentamicin B₁ or gentamicin X and having a gross molecular formula



in which, in the case of gentamicin B, R₁ is hydroxy, R₂ is hydrogen and R₃ is amino; in the case of gentamicin B₁, R₁ is hydroxy, R₂ is methyl and R₃ is amino; and in the case of gentamicin X, R₁ is amino, R₂ is hydrogen and R₃ is hydroxy, the antibiotic being in substantially pure form.

2. An amino glycoside antibiotic designated gentamicin B, gentamicin B₁ or gentamicin X and having a gross molecular formula



in which, in the case of gentamicin B, R₁ is hydroxy, R₂ is hydrogen and R₃ is amino; in the case of gentamicin B₁, R₁ is hydroxy, R₂ is methyl and R₃ is amino; and in the case of gentamicin X, R₁ is amino, R₂ is hydrogen and R₃ is hydroxy, the antibiotic being substantially free from substances co-produced therewith.

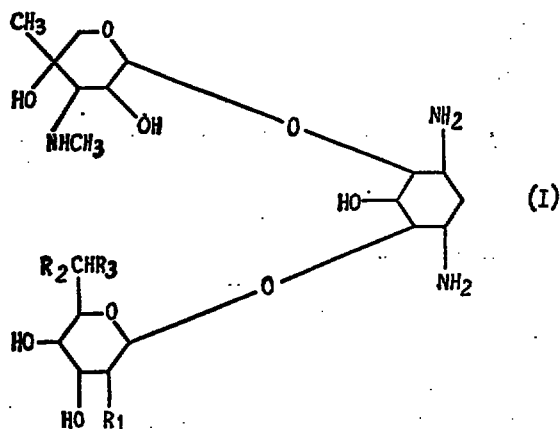
3. The amino glycoside antibiotic designated gentamicin B, being in a state of purity such that (i) its ethanol solvate has a nuclear magnetic resonance spectrum in deuterium oxide substantially as shown in Figure 4 of the drawings and (ii) its sulphate has an infra-red absorption spectrum in mineral oil, substantially as shown in Figure 3 of the drawings.

4. The amino glycoside antibiotic designated gentamicin B₁ being in a state of purity such that (i) its ethanol solvate has a nuclear magnetic resonance spectrum in deuterium oxide substantially as shown in Figure 6 of the drawings and (ii) its sulphate has an infra-red absorption spectrum in mineral oil, substantially as shown in Figure 5 of the drawings.

zolidine derivative of a gentamicin antibiotic containing a garosamine ring wherein the oxazolidine ring is fused to said garosamine ring.

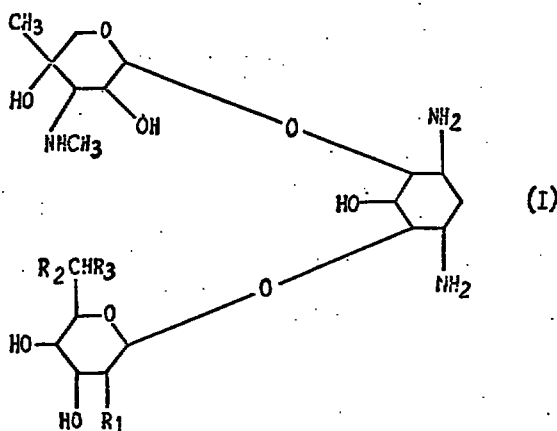
WHAT WE CLAIM IS:—

1. An amino glycoside antibiotic designated gentamicin B, gentamicin B₁ or gentamicin X and having a gross molecular formula



in which, in the case of gentamicin B, R₁ is hydroxy, R₂ is hydrogen and R₃ is amino; in the case of gentamicin B₁, R₁ is hydroxy, R₂ is methyl and R₃ is amino; and in the case of gentamicin X, R₁ is amino, R₂ is hydrogen and R₃ is hydroxy, the antibiotic being in substantially pure form.

2. An amino glycoside antibiotic designated gentamicin B, gentamicin B₁ or gentamicin X and having a gross molecular formula



in which, in the case of gentamicin B, R₁ is hydroxy, R₂ is hydrogen and R₃ is amino; in the case of gentamicin B₁, R₁ is hydroxy, R₂ is methyl and R₃ is amino; and in the case of gentamicin X, R₁ is amino, R₂ is hydrogen and R₃ is hydroxy, the antibiotic being substantially free from substances co-produced therewith.

3. The amino glycoside antibiotic designated gentamicin B, being in a state of purity such that (i) its ethanol solvate has a nuclear magnetic resonance spectrum in deuterium oxide substantially as shown in Figure 4 of the drawings and (ii) its sulphate has an infra-red absorption spectrum in mineral oil, substantially as shown in Figure 3 of the drawings.

4. The amino glycoside antibiotic designated gentamicin B₁ being in a state of purity such that (i) its ethanol solvate has a nuclear magnetic resonance spectrum in deuterium oxide substantially as shown in Figure 6 of the drawings and (ii) its sulphate has an infra-red absorption spectrum in mineral oil, substantially as shown in Figure 5 of the drawings.



5. The amino glycoside antibiotic designated gentamicin X being in a state of purity such that (i) its hydrate has a nuclear magnetic resonance spectrum in deuterium oxide substantially as shown in Figure 8 of the drawings and (ii) its sulphate has an infra-red absorption spectrum in mineral oil, substantially as shown in Figure 7 of the drawings. 5
6. An antibiotically active pharmaceutically acceptable derivative of an amino glycoside antibiotic as claimed in any preceding claim.
7. A derivative according to claim 6, said derivative being a solvate.
8. A derivative according to claim 6, said derivative being a hydrate.
10. 9. A derivative according to claim 6, said derivative being an ethanol solvate. 10
10. A derivative according to claim 6, said derivative being a non-toxic acid addition salt, which when derived from a polybasic acid may contain free acid groups and/or acid groups neutralized by other bases.
11. A salt according to claim 10 containing acid moieties of at least one of the following acids: hydrochloric, sulfuric, phosphoric, succinic, tartaric, maleic, fumaric and glutaric acid. 15
12. An acid addition salt according to claim 10 or claim 11 which contains acid groups neutralized by alkali metal bases.
13. An antibiotically active composition comprising at least one of antibiotics gentamicin B, gentamicin B₁ and gentamicin X having a gross molecular formula (I) as defined in claim 1, at least one of antibiotics gentamicin B and gentamicin B₁ being substantially absent from the composition. 20
14. An antibiotically active composition comprising an antibiotically active pharmaceutically acceptable derivative of at least one of antibiotics gentamicin B, gentamicin B₁ and gentamicin X having a gross molecular formula (I) as defined in claim 1, antibiotically active pharmaceutically acceptable derivatives of at least one of antibiotics gentamicin B and gentamicin B₁ being substantially absent from the composition. 25
15. A process for producing an amino glycoside antibiotic as claimed in any one of claims 1 to 5 or a derivative as claimed in claim 6, which comprises subjecting a mixture comprising at least one of said antibiotics or derivatives to chromatography on a column of solid adsorbent using a liquid solvent as mobile phase, recovering a fraction of said solvent which contains one of said antibiotics or derivatives, isolating the antibiotic or derivative from said fraction, and, if desired, transforming the isolated antibiotic into an antibiotically active pharmaceutically acceptable derivative or the isolated derivative into the free form of the antibiotic or into another antibiotically active pharmaceutically acceptable derivative. 30
16. Process according to claim 15, characterised in that silica gel is used as adsorbent. 35
17. Process according to claim 15 or claim 16, wherein the mixture comprises gentamicin B and/or gentamicin B₁ and/or gentamicin X in admixture with at least one further gentamicin antibiotic. 40
18. Process according to claim 17, wherein the further antibiotic is gentamicin A and/or gentamicin C.
19. Process according to any one of claims 15 to 18, wherein the mixture comprises gentamicin A, gentamicin B, gentamicin B₁, gentamicin X and gentamicin C. 45
20. Process according to claims 15 or claim 16 wherein the mixture comprises gentamicin B, gentamicin B₁ and gentamicin X.
21. Process according to any one of claims 15 to 20 characterised in that the solvent for the chromatographic separation is the lower phase of a solvent system consisting of methanol: chloroform: ammonium hydroxide in the volume ratio of 1:1:1. 50
22. Process according to any one of claims 15 to 21 characterised in that the compounds gentamicin B and/or gentamicin B₁ and/or gentamicin X are isolated in the form of their acid addition salts, or of the solvates or hydrates of the free antibiotics or the acid addition salts of said solvates or hydrates.
23. An amino glycoside antibiotic as claimed in any one of claims 1 to 5 or a derivative thereof as claimed in claim 6, whenever produced by a process according to any one of claims 15 to 22. 55
24. A pharmaceutical composition comprising at least one of the amino glycoside antibiotics gentamicin B, gentamicin B₁ and gentamicin X having the gross molecular formula (I) defined in claim 1 in admixture with a suitable pharmaceutical carrier, at least one of gentamicin B and gentamicin B₁ being substantially absent from the composition. 60
25. A pharmaceutical composition comprising an antibiotically active pharmaceutically acceptable derivative of at least one of the amino glycoside antibiotics gentamicin



B, gentamicin B₁ and gentamicin X having the gross molecular formula (I) defined in claim 1 in admixture with a suitable pharmaceutical carrier, pharmaceutically acceptable derivatives of at least one of gentamicin B and gentamicin B₁ being substantially absent from the composition.

5 26. A composition according to claim 24 or claim 25 in the form of a dosage unit.

27. A composition according to claim 24 or claim 25 in the form of a shaped dosage unit.

28. A composition according to claim 27 in the form of a tablet, capsule or suppository.

10 29. Process for the production of a pharmaceutical composition as claimed in claim 24 or 25, which comprises admixing at least one of the amino glycoside antibiotics gentamicin B, gentamicin B₁ and gentamicin X having the gross molecular formula (I) defined in claim 1, in its free form or in the form of an antibiologically active pharmaceutically acceptable derivative, with a suitable pharmaceutical carrier.

15 30. Process for the production of a pharmaceutical composition, which comprises admixing an amino glycoside antibiotic as claimed in any one of claims 1 to 5 and 23, a pharmaceutically acceptable derivative thereof as claimed in claims 6 to 12 and 23, or an antibiologically active composition as claimed in claim 13 or claim 14, with a suitable pharmaceutical carrier.

20 31. A method of combatting protozoal infections which comprises administering to a non-human animal having such an infection a therapeutically effective quantity of at least one of the antibiotics gentamicin A, gentamicin B, gentamicin B₁ and gentamicin X and their antibiologically active pharmaceutically acceptable derivatives.

25 32. A method of combatting helminthiasis in a non-human animal afflicted with helminthiasis which comprises administering to the animal a therapeutically effective quantity of at least one of the antibiotics gentamicin A, gentamicin B, gentamicin B₁, gentamicin X and their antibiologically active pharmaceutically acceptable derivatives.

33. A method as claimed in claims 31 or 32 in which the therapeutically effective quantity administered is from 10 mg. to 100 mg. per kilogram of bodyweight per day.

30 34. A method of combatting bacterial infections which comprises administering to a non-human animal having such an infection a therapeutically effective quantity of at least one of the antibiotics gentamicin B, gentamicin B₁ and gentamicin X and their antibiologically active pharmaceutically acceptable derivatives.

35 35. A method as claimed in claim 34 in which the therapeutically effective quantity administered is from 2 mg. to 20 mg. per kilogram of body weight per day.

36. A method as claimed in claim 35 in which the therapeutically effective quantity of gentamicin B and gentamicin B₁, when parenterally administered, is from 2 mg. to 10 mg. per kilogram of body weight per day.

40 37. A method as claimed in any one of claims 31 to 36 characterised in that the pharmaceutically acceptable derivatives used therein are acid addition salts or solvates of the free antibiotics or of their acid addition salts.

38. A method according to claim 37 in which the solvate is a hydrate.

45 39. An amino glycoside antibiotic designated gentamicin B, gentamicin B₁ or gentamicin X, according to claim 1 and substantially as hereinbefore described.

40. An antibiologically active pharmaceutically acceptable derivative of an amino glycoside antibiotic designated gentamicin B, gentamicin B₁ or gentamicin X, according to claim 1 and substantially as hereinbefore described.

41. An antibiologically active composition according to claim 13 and substantially as hereinbefore described.

50 42. A process for producing an amino glycoside antibiotic according to claim 15 and substantially as hereinbefore described.

43. A pharmaceutical composition according to claim 24 and substantially as hereinbefore described.

55 44. A process for producing a pharmaceutical composition, according to claim 29 and substantially as hereinbefore described.

45. A method of combatting protozoal infections according to claim 31 and substantially as hereinbefore described.

46. A method of combatting helminthiasis according to claim 32 and substantially as hereinbefore described.

60 47. A method of combatting bacterial infections according to claim 34 and substantially as hereinbefore described.



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