

1225047

PATENT SPECIFICATION

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(54) ANTIBIOTIC QUINTOMYCIN AND PROCESS FOR ITS PREPARATION

(71) We, KOWA COMPANY LIMITED, a Japanese Body Corporate, of 6-29, 3-chome, Nishiki, Naka-ku, Nagoya, Japan, 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 an antibiotic substance, "Quintomycin", which belongs to the aminocyclitol group and which is produced by a novel soil ray fungus, *Streptomyces lividus* or a quintomycin-producing mutant thereof, and to a process for obtaining it.

The above new fungus was deposited under the name of "No. 2230-N₁ strain" at Fermentation Laboratory of Technical Institute, 2, 5, 4-chome, Inage-Higashi, Chiba-shi, Japan, with the deposit number of "No. 50 FLTT". The same fungus was later deposited under the name of *Streptomyces lividus* at American Type Culture Collection, with the deposit number of ATCC 21178.

The antibiotic substance was first termed "No. 2230 substance" and later called "Quintomycin". It includes a novel antibiotic substance Quintomycin-A expressed by the molecular formula $C_{29}H_{55}N_5O_{19}$, the pentahydrochloride of which has a specific rotation $[\alpha]_D^{22}$ of $+59^\circ$; a novel antibiotic substance Quintomycin-B expressed by the molecular formula $C_{29}H_{55}N_5O_{18}$, the pentahydrochloride of which has a specific rotation of $[\alpha]_D^{22}$ of $+50^\circ$; and a novel antibiotic substance Quintomycin-D expressed by the molecular formula

$C_{29}H_{45}N_5O_{18}$, the pentahydrochloride of which has a specific rotation of $[\alpha]_D^{22}$ of $+36^\circ$, and also Quintomycin-C which is regarded as a synonym for a known antibiotic substance "paromomycin" produced by *Streptomyces rimosus forma paromomycinus*.

The principal ingredients of the antibiotic substance Quintomycin of the invention are Quintomycin-B and Quintomycin-A. By virtue of its content of these, especially Quintomycin-B, the Quintomycin of the invention exhibits a high biological activity against *Pseudomonas aeruginosa*, and is exceedingly low in toxicity to warm-blooded animals. Known antibiotic substances having a high biological activity against the above bacterium are not satisfactory in respect of toxicity.

The *Streptomyces lividus* of the invention has the following microbiological properties.

[I] Morphology:—

Streptomyces lividus n.s.p. first exhibits the growth of a light gray on synthetic media, which gradually becomes dark blue to black. The production of a soluble pigment is none, or in a rare substance, a soluble pigment of light rose or raspberry is formed. Aerial hyphae are short and irregularly branched in a single form. The formed sporophores are generally straight but, in a rare instance, of flexuous loops (whorls or spirals are not formed). Three to ten spores occur at the end of a sporophore. The structure of the spore surface as observed by an electron-microscope is oval or ellipsoidal ($0.2 \times 0.3 - 0.5 \mu$).

[II] Cultural characteristics on various media:—

(Synthetic media)

Glycerol Czapek's agar

G: moderate, light gray to dark blue or black

A: moderate, velvety, light gray to dark blue or black

S: none, rarely light rose or raspberry

Glucose-asparagine agar

G: moderate, cream to dark blue or charcoal gray

A: moderate, velvety, light gray to dark blue

S: none

Calcium malate agar

G: moderate, white to shadow blue

A: very scant, white or light blue

S: none

(Organic media)

Nutrient agar

G: good, wrinkled moist yellowish brown or black

A: scant, light gray

S: none

Starch agar

G: good, light gray to dark blue or black

A: velvety, white or light gray

S: none

Potato glucose agar

G: moderate, dark blue or dark gray, slightly penetrating into medium

A: scant, light gray

S: none, rarely light rose

Peptone glucose agar

G: good, dark blue or dark gray

A: scant, white to gray

S: brick red

Tyrosine agar

G: moderate, pale brown to dark gray

A: slightly white

S: none or pale brown

[II] Cultural characteristics on various media:—

(Organic media)

Gelatin stab

G: good, white or light gray

A: none

S: none

Litmus milk

C: moderate, cream on surface

A: none

S: no change

[Note]:

G: Growth; A: Aerial mycelium; S: Soluble pigment.

[III] Biochemical properties:—

Reaction	
Chromogenic formation	negative
Tyrosinase	negative
Nitrate reduction	positive
Cellulose decomposition	negative
Milk coagulation	negative
Milk peptonization	positive
Hydrolysis of starch	positive (strong)
Gelatin liquefaction	positive (considerable)

[IV] The utilization of carbon sources:—

Carbon source	Utilization	Carbon source	Utilization
D-Xylose	—	Sorbitol	++
L-Arabinose	—	D-Mannitol	++
D-Glucose	++	Dextrin	+
D-Galactose	++	Starch	+
D-Fructose	+	Rhamnose	—
D-Lactose	—	Inulin	—
D-Maltose	++	Dulcitol	—
Sucrose	—	Glycerol	++
D-Raffinose	+	Sodium citrate	+
Trehalose	++	Sodium acetate	+
L-Inositol	++	Calcium malate	+

- Reference to the classification given in Bergey's Manual of Determinative Bacteriology, 7th Ed. indicates that the new fungus of the present invention resembles *S. gedanensis* (*S. ged.*) in the following respects: Psychrophilic to mesophilic growth; no soluble pigment produced in organic media; proteolytic action strong; growth on synthetic media dark to black to almost bluish black; and a serial mycelium white to gray. *S. ged.* in a potato medium, however, shows a cream-coloured to brownish growth, and produces no aerial mycelium and soluble pigment, while *S. lividus* (*S. liv.*) exhibits a dark gray or grayish black growth with a scant aerial mycelium and does not usually produce a soluble pigment although exhibiting a light rose color in a rare instance. On a starch medium, *S. ged.* exhibits a yellow to cream coloured growth, and *S. liv.* exhibits a light gray to dark blue or black growth. *S. ged.* proves to be negative in milk peptonization and nitrate reduction, while *S. liv.* proves to be positive in both.
- As strains which exhibit a psychrophilic to mesophilic growth and produce no soluble pigment in organic media, there are *S. griseolus* (*S. gris.*) which exhibits a mouse-gray growth on synthetic media, produces a white to gray aerial mycelium, and forms straight sporo-

phores, and *S. fasciculus* which forms broom shaped sporophores. Comparison of these two strains with the *S. liv.* of the invention has revealed the following. In a nutrient agar, *S. gris.* exhibits a brownish growth with a smooth surface, produces a deep dull gray aerium mycelium, and produces no soluble pigment, while *S. liv.* exhibits a wrinkled moist yellowish brown growth and produces no soluble pigment with hardly any aerial mycelium. In a potato medium, *S. gris.* exhibits a cream-coloured to black growth, forms a aerial mycelium of white greenish tinge and produces a brown to black soluble pigment, while *S. liv.* exhibits a dark grey or grayish black growth, forms a scant aerial mycelium, and produces no soluble pigment although exhibiting a light rose colour in a rare instance. In gelatin, *S. gris.* gives rise to a yellowish flaky pellicle and sediment, and forms a white aerial mycelium and faint brown medium, while *S. liv.* exhibits a white or light gray growth, but produces no aerial mycelium and soluble pigment. In milk, *S. gris.* exhibits an abundant pink pellicle growth and slow coagulating, while *S. liv.* grows in cream on surface and does not coagulate.

S. fasciculus (*S. fasc.*) exhibits a good growth lichenoid and forms an aerial mycelium

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which is colourless, covered with dark gray, powdery or velvety, while *S. liv.* exhibits a light gray to dark blue or black growth, and forms a white or white gray aerial mycelium which is short and not so abundant. In a milk medium, *S. fasc.* proves to be positive both in coagulation and peptonization and exhibits a good growth on cellulose, while *S. liv.* proves to be positive only in peptonization in milk medium, and does not grow on a cellulose medium.

With reference to S. A. Waksman, "The Actinomycetes", Vol. 2, comparison of characteristic properties of various series of Streptomyces will indicate that there is nothing which *S. liv.* belongs to. *S. intermedius*, *S. parvullus*, *S. craterifer* (*S. crat.*) and *S. cellulosa* belonging to the series of Cinerus, however, might be regarded as being similar to *S. liv.* in that the spiral formation is + or -, melin is - and an aerial mycelium is white to gray.

In a nutrient agar, *S. crat.* exhibits a colourless growth, and in a starch agar, it exhibits a spreading, thin and colourless growth and forms no aerial mycelium. In a potato medium, *S. crat.* exhibits a cream-coloured growth and forms a white to mouse-gray aerial mycelium. On the other hand, *S. liv.* exhibits a good growth of wrinkled moist yellowish brown in a nutrient agar; exhibits a good growth of light gray to dark blue or black, and forms a velvety, white or light gray aerial mycelium in a starch agar; and exhibits a dark gray growth in a potato medium.

S. cellulosa grows well on cellulose and rapidly coagulates milk, and exhibits a yellow or lemon-yellow growth in a synthetic media. These are the points that differentiate it from *S. liv.*

S. parvullus differs from *S. liv.* in that it forms sporophores twisting into long closed spirals, exhibits a yellow growth in synthetic media, produces a yellow soluble pigment in gelatin, produces a brown soluble pigment and an abundant gray aerial mycelium in milk,

and exhibits a very slow peptonization.

S. liv. of the invention also differs from *S. intermedius* in that the latter exhibits a good growth of olive-green in cellulose, exhibits a folded growth of brown to greenish-brown in a potato medium and produces an olive-green soluble pigment, and exhibits slow liquefaction in gelatin and produces a greenish-brown soluble pigment.

Except *S. griseolus*, *S. cellulosa* and *S. parvullus*, the above-mentioned known strain do not produce an antibiotic substance.

Reference will now be made to fungi of the genus Streptomyces which produce antibiotic substances belonging to the aminocyclitol group. *S. fradiae*, according to Waksman, exhibits a thin, smooth, colourless, occasionally orange-yellow substrate growth on synthetic media, and forms an aerial mycelium which is light pink, seashell-pink or salmon-coloured. These will differentiate it from the *S. liv.* of the invention. Waksman's classification also indicates that *S. albogriseolus* exhibits a pink to reddish purple growth on a potato medium and the sporophores produce spirals. Consequently, it differs from the *S. liv.*, *S. rimosus* f. *paromomycinus*, *S. chrestomycetices*, *S. kanamycetius* and *S. pulveraceus* all exhibit a yellow or cream-coloured to brown growth, and do not exhibit dark gray to dark blue or black growth as in *S. liv.*

Consequently, the Quintomycin-producing strain of the present invention has been identified as a novel strain belonging to the genus Streptomyces, and has been named *S. lividus*.

For the sake of reference, the details of microbiological properties of strains belonging to genus Streptomyces which will produce antibiotic substances belonging to the aminocyclitol group will be shown below under paragraphs (I) to (VI). The microbiological properties of strains belonging to other genera which will produce antibiotic substances belonging to the aminocyclitol group are also given under paragraphs (VII) and (VIII).

[I]

Strain	<i>Streptomyces rimosus forma paromomycinus</i>
Antagonistic properties	Paromomycin
Morphology	Sporophores form dense clusters of spirals, and aerial mycelium is short and straight but rarely spiral
Glycerol-asparagine agar	G: cream-colored, becoming brownish to orange-brown with age A: white S: none
Calcium malate agar	G: yellowish brown A: white S: none
Nutrient agar	G: light cream-colored to yellowish-brown A: none S: none
Starch agar	G: cream-colored, with deeper brown center A: absent or white S: none
Potato agar	G: lichenoid, cream-colored to reddish-brown A: white to gray to dark brown S: yellowish-brown
Gelatin	G: cream-colored to brownish A: white S: none
Milk	G: heavy surface pellicle, cream-colored to yellowish A: grayish-white
Nitrate reduction	positive
Cellulose decomposition	negative
Milk coagulation	negative
Milk peptonization	positive
Hydrolysis of starch	positive (limited)
Gelatin liquefaction	positive (slowly)

(18)

[III]

Strain	<i>Streptomyces kanamyceticus</i>
Antagonistic properties	Kanamycin
Morphology	The aerial mycerium develops from the submerged mycelium on a few media and its branching is not profuse and it bears the sporophores at the end. Spirals and whorls are not observed in general.
Glycerol Czapek's agar	G: colorless, later changed to lemon yellow A: white to yellow and occasionally bore greenish or faint pinkish tinge S: occasionally faint brown
Glucose-asparagine agar	G: colorless to yellow with faint pinkish white A: scant, white, faint pinkish white, greenish yellow or yellow S: occasionally faint brown
Calcium malate agar	G: yellow A: white-yellow
Nutrient agar	G: cream-colored A: absent or white S: none
Potato agar	G: wrinkled, faint yellowish brown to yellow A: scant, white S: none
Nitrate reduction	positive
Milk coagulation	doubtful
Milk peptonization	doubtful
Hydrolysis of starch	positive
Gelatin liquefaction	positive

[III]

Strain	<i>Streptomyces fradiae</i>
Antagonistic properties	Neomycin
Morphology	Sporophores branched monopodially, straight or flexible, but no true spirals. On certain media, spirals are formed
Glucose-asparagine agar	G: restricted, glossy, buff-colored, lichenoid margin A: late, seashell-pink
Malate glycerol agar	G: orange A: seashell-pink
Nutrient agar	G: restricted, yellowish, becoming orange-yellow to buff A: none S: none
Starch media	G: spreading, colorless A: seashell-pink
Potato agar	G: restricted, orange-colored A: white to rose or pink S: absent or faint brown
Gelatin	G: dense, cream-colored to brownish A: white S: none
Litmus milk	G: cream-colored ring S: becoming alkaline
Nitrate reduction	negative
Cellulose decomposition	negative
Milk coagulation	positive
Milk peptonization	positive (rapid)
Hydrolysis of starch	positive

[IV]

Strain	<i>Streptomyces albogriseolus</i>
Antagonistic properties	Neomycin or Chalcomycin
Morphology	Sporophores monopodially branched, producing short, compact spirals, averaging 4 to 6 turns. Spores spherical or oval, covered with numerous long, fine hairs
Nutrient agar	A: white to ash-gray
Starch agar	A: white to dark gray
Milk	G: orange-colored ring
Nitrate reduction	positive
Milk peptonization	positive
Hydrolysis of starch	positive
Gelatin liquefaction	positive

[V]

Strain	<i>Streptomyces pulveraceus</i>
Antagonistic properties	Zygomycin (identical with Paromomycin)
Morphology	Aerial mycelium generally develops well. The sporophore forms spiral and the spore is spherical-ellipsoidal. The spiral adhering state.
Glycerol Czapek's agar	G: colorless, later faint brown A: powdery, light drab to light grayish olive S: none
Glucose-asparagine agar	G: orange to xanthin orange A: powdery, light grayish olive S: none or becoming ochraceous-buff
Calcium malate agar	G: yellow, penetrating into the medium A: none or scant, smoke gray S: none
Nutrient agar	G: colorless, folded A: none S: none
Starch agar	G: colorless to yellow ocher, penetrating deep into the medium A: scant, light grayish olive S: none
Tyrosine agar	G: colorless to faint brown A: light grayish olive S: none
Milk	G: colorless, surface growth S: brown
Nitrate reduction	positive (strong)
Cellulose decomposition	negative
Milk coagulation	negative
Milk peptonization	positive
Hydrolysis of starch	positive
Gelatin liquefaction	positive

[VI]

Strain	<i>Streptomyces chrestomyceticus</i>
Antagonistic properties	Aminosidin (identical with Paromomycin)
Morphology	Aerial mycelium are generally strength, but observed rarely hooks or spirals
Glucose-asparagine agar	G: yellow A: absent
Potato glucose agar	G: colorless A: scant white
Peptone glucose agar	G: good, white or cream-colored A: abundant white
Tyrosinase	positive
Milk coagulation	positive
Milk peptonization	negative (neutral)
Hydrolysis of starch	positive
Gelatin liquefaction	positive

[VII]

Strain	<i>Micromonospora purpurea</i>
Antagonistic properties	Gentamicin
Morphology	No aerial mycerium, colony raised, convolute abundant growth, waxy, no diffusible pigment. Surface:terra cotta. Reverse: russet. Mycelium long, branched, regular, nonseptate, 0.5 μ in diameter. Sporophores single, spores borne terminally, spores spherical to ellipsoidal, 1.0 μ in diameter
Glucose-asparagine agar	G: fair, brite peach
Peptone glucose agar	G: good, burgundy
Nitrate reduction	positive
Gelatin liquefaction	positive (weak)

Strain

Micromonospora echinospora

Antagonistic properties

Gentamicin

Morphology

No aerial mycelium, colony raised, crenate-convolute good growth, waxy, slight amber diffusible pigment. Surface: deep red-brown. Reverse: russet. Mycelium long, branched, regular nonseptate. No spores

Glucose-asparagine agar

G: poor.

Peptone glucose agar

G: good, burgundy

Nitrate reduction

variable

Gelatin liquefaction

positive

The utilization of carbon sources of the above-mentioned fungi excepting [IV] are shown below together with the similar data for the novel *S. lividus* of the invention.

Micromonospora

Micromonocera

Stratification

Streptomyces

	<i>Streptomyces lividus</i>	<i>Streptomyces kanamyceticus</i>	<i>Streptomyces fradiae</i>	<i>Streptomyces pulveraceus</i>	<i>Streptomyces rimosus</i> f. <i>paromomycinus</i>	<i>Streptomyces chrestomyceticus</i>	<i>Micromonospora purpurea</i>	<i>Micromonospora echinospora</i>
L-Arabinose	-	+	+	±	±		+	+
Rhamnose	-	-	-	+	-		+	+
D-Xylose	-	-	+	+	-		+	+
D-Glucose	++		+		+	+	+	+
D-Galactose	++	+	±	+	+	+	±	±
D-Fructose	+	+	-	+	+	+		
D-Lactose	-	-	-	+	+	+	±	±
D-Maltose	++	+	+	+	+		+	+
Sucrose	-	+	-		-	+	+	-
Trehalose	++				+			
D-Raffinose	+	+	-	+	+		-	
Dextrin	+	+		+	+	+		
Inulin	-	-	-	-	-	+		
Starch	+	+			+		+	+
Dulcitol	-		+	-	-		-	-
Glycerol	++	+			+	+	-	-
L-Inositol	++	-		-	+		-	-
D-Mannitol	++	+	-	-	+	+	-	-
Sorbitol	++	±	-	+	+	+	-	-

In another aspect the invention consists in a process for the preparation of a novel and useful antibiotic substance (Quintomycin), which comprises culturing *Streptomyces lividus* ATCC 21178 or a quintomycin-producing mutant thereof under aerobic conditions in a medium containing an assimilable carbon source and an assimilable nitrogen source, and recovering Quintomycin from its culture broth. The said culture medium may contain an inorganic substance (mineral) besides the carbon source and nitrogen source.

Various substances known in the culturing art can be used as the assimilable carbon and assimilable nitrogen sources. Examples of carbon sources are starch, glucose, glycerol, maltose, inositol, fructose, dextrin, sucrose and galactose. As the nitrogen source, we can mention a yeast extract, polypeptone, dry yeast, meat extract, cornsteep liquor, soybean meal, inorganic nitrates and ammonium salts.

Examples of the mineral substances are sodium chloride, dipotassium hydrogen phosphate, magnesium sulphate, iron sulphate, calcium chloride, calcium carbonate, calcium hydroxide, iron chloride, zinc sulphate and cobalt chloride.

The culturing can be carried out under aerobic conditions either in a solid culture medium or a liquid medium. It is preferable to effect the culturing in a liquid medium under aerobic conditions, if desired using an antifoaming agent such as liquid paraffin, a fatty oil or a silicone oil.

The culturing temperature is about 20 to 39°C., preferably about 25 to 37°C. It is advisable that the culturing should be carried out while the pH of the culture medium is adjusted to about 6.6—7.5, preferably 7.0 $\pm$ 0.3. The period of culturing is usually at least 2 days. For instance, a period of 2 days to 2 weeks, or 2 days to 1 week, will suffice. Culturing for a longer period is possible, but not necessary. When the production of Quintomycin in the culture broth is sufficient, preferably when it reaches almost a maximum, the culturing is stopped, and Quintomycin in the broth is recovered. The recovery can be effected either by a procedure in which the quintomycin is adsorbed by an adsorbent and then eluted by a suitable eluting liquid, or by a solvent extraction procedure, the former being preferable. When the adsorbing and eluting procedure is employed, a suitable adsorbent is added to the culture broth to adsorb the quintomycin sufficiently under stirring, for instance, and after the removal of waste broth with mycelium, the adsorbent may be subjected to the eluting operation. It is also possible to add an adsorbent to the culture broth from which mycelium has been removed by a suitable means such as filtration and centrifugal separation, and then to follow the same procedure. Or such mycelium-free broth can be passed through a column packed with a suit-

able adsorbent, and then eluted.

Any solid adsorbent which adsorbs the quintomycin and from which it can be eluted by a suitable eluting liquid may be used, but the use of cation exchangers is preferable. Especially preferable is the use of weakly acidic cation exchange resins. Specific examples of the cation exchangers are Amberlite IRC-50, Amberlite IRC-84, Amberlite CG-50 (tradenames) and carboxymethyl cellulose. Besides these, other solid adsorbents such as cellulose powder activated carbon, alumina gel, silica gel and alumina/silica gel are usable.

As the eluting liquid, there is preferably used an aqueous solution of an inorganic or organic acid or of alkaline-reacting substance such as an alkali metal hydroxide, ammonia, or an ammonium salt.

Suitable acids include mineral acids such as hydrochloric acid, sulphuric acid and phosphoric acid and lower aliphatic acids such as acetic acid and formic acid. It is advisable that these acids should be used in the form of aqueous solutions having a concentration of about 0.1—1.0 N. Examples of suitable alkaline-reacting substances are sodium and potassium hydroxides, ammonia and ammonium formate. It is advisable that these alkaline-reacting substances should be used in concentrations of about 0.1—3.0 N. The use of mineral acids or aqueous ammonia is specially recommended.

The pH of the eluate is, if desired, adjusted to a value in the vicinity of neutrality, and by removing the liquid content at the lowest possible temperature, the Quintomycin can be obtained as a solid powder. The removal of the liquid content can be effected by any of the various known means, such means as lyophilising and spray drying being preferable. If the operation is performed at the lowest possible temperature, heating under reduced pressure can also be used. Or a concentration-vacuum drying means may be employed under the conditions such as to form a stable salt like a hydrochloride and sodium salt. Furthermore, Quintomycin can be obtained as a solid powder by extracting the eluate with a suitable extracting solvent, and removing the solvent at the lowest possible temperature.

When activated carbon is used as a solid adsorbent, an aqueous solution of an acid, or acetone or a lower aliphatic alcohol such as methanol and ethanol acidified to a pH of less than 5, preferably less than 4, is preferably used as the eluting liquid.

When the Quintomycin is recovered from the culture broth by solvent extraction, higher fatty acids such as lauric acid and stearic acid can be used as assistants. As the solvent, water-immiscible aliphatic alcohols are usable, preferably n- or iso-butanol or amyl alcohol. An alcohol layer is separated by decantation or other known means of separation into two layers, and the alcohol is evaporated at the

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lowest possible temperature, preferably by spray drying or heating under reduced pressure.

The novel antibiotic Quintomycin so obtained is a white to white-gray solid substance and can be used as an antibiotic in a wide range of fields in the crude form or if desired, after having been further refined by repeated application of the above-mentioned recovery means. If desired, the obtained Quintomycin can be separated into the four antibiotic substances already mentioned, namely Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D, Quintomycin-B being the chief constituent.

Methods for separating the Quintomycin into these four substances will now be described.

When the Quintomycin separated from the culture broth is again dissolved into water, or is eluted with an aqueous solution of an acid or an alkaline-reacting substance as mentioned above, it can be separated by chromatography into Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D while the pH of the eluting liquid is maintained at 6.2—12, with or without adjustment.

Both cation exchangers and anion exchangers are usable. For instance, with the use as a column of a cation exchanger, preferably a weakly acidic cation exchanger, such as Amberlite IRC-50, Amberlite IRC-84, Amberlite CG-50 (tradenames), carboxymethyl cellulose and CM-Sephadex (trade name), an aqueous solution of the Quintomycin adjusted to a pH of 6.2—12 is caused to be adsorbed by the column, followed by developing. Subsequently, with the use of aqueous ammonia or an aqueous solution of ammonium formate as an eluting liquid, the Quintomycin is separated by a gradient or stepwise method.

According to the gradient method, under the operating conditions given in Example 4 below, Quintomycin-A is eluted in sections 45—50; Quintomycin-B in sections 53—58; Quintomycin-C in sections 88—90; and thereafter, Quintomycin-D is eluted. According to the stepwise method, Quintomycin-A is first eluted by 0.1 N aqueous ammonia, and then Quintomycin-B by aqueous ammonia water of the same normality. Quintomycin-C is eluted by 0.15 N aqueous ammonia, and then Quintomycin-D, by 0.3 N aqueous ammonia.

It is also possible to separate the Quintomycin by adsorbing it from aqueous solution on a column of an anion exchanger, preferably a strongly basic anion exchanger, such as Dowex 1 × 2 OH type (tradename) or Amberlite 400 (tradename), and then eluting it with water, whereby Quintomycin-D, Quintomycin-C, Quintomycin-B and Quintomycin-A are sequentially eluted.

Each of the separated components can be further refined by chromatography with the use of activated carbon, alumina or cellulose

as a column, and acetone or a lower alcohol acidified with a mineral acid such as hydrochloric acid, sulphuric acid and nitric acid or a lower aliphatic acid such as acetic acid as an eluting liquid. It can also be purified by converting it into its picrate or Reinecke's salt, and changing it into a proper inorganic salt by a salt exchange means.

The novel antibiotic substance of the invention can be characterised as follows:

(1) *Characteristics:*

Quintomycin-A, Quintomycin-B and Quintomycin-D are basic substances. The free base and the hydrochlorides and sulphates are white amorphous powders.

(2) *Solubility:*

Quintomycin-A, Quintomycin-B and Quintomycin-D, in their free-base, hydrochloride and sulphate form, are soluble in water, aqueous solutions of sodium or potassium hydroxide, lower aliphatic acids and mineral acids. They are soluble in methyl alcohol in their free base form, but are very difficultly soluble for insoluble in C₄ or higher aliphatic alcohols, ketones and ethers.

(3) *Colour Reaction:*

Quintomycin-A, Quintomycin-B and Quintomycin-D prove to be positive in Molisch reaction, Elson-Morgan's reaction, Abderhalden's reaction (ninhydrin reaction) and Bile's reaction; exhibit a deep red purple colour in Tollens reaction (phloroglucine hydrochloride); and prove to be negative in Fehling reaction, Benedict reaction, Biuret reaction, Ehrlich reaction and Sakaguchi reaction.

Quintomycin-A and Quintomycin-B exhibit a purple colour in Skatol reaction, but Quintomycin-D does not.

(4) *Stability:*

Quintomycin-A, Quintomycin-B and Quintomycin-D in 1 N aqueous hydrochloric acid solution are unstable when heated to 100°C. for 30 minutes, but are stable at room temperature. They are stable in 1 N aqueous sodium hydroxide solution both when heated to 100°C. for 30 minutes and at room temperature.

(5) *R_f value:*

The R_f value determined by alumina thin-layer chromatography (R_f values in the claims are as determined by this method) with the use of an upper layer of mixed solvent consisting of chloroform/methanol/17% aqueous ammonia (in a volume ratio of 2:1:1) as a developing liquid is 0.23 for Quintomycin-A, 0.50 for Quintomycin-B and 0.75 for Quintomycin-D. Some known antibiotic substances have the following R_f values as determined by the above procedure. Kanamycin (product of *Streptomyces kanamyceticus*), 0.75; Paromomycin (product of *Streptomyces rimosus* f. *paromomycinus*), 0.70; Neomycin (product of *Streptomyces fradiae* or *Streptomyces albobrunneus*), 0.74; and Gentamicin (product of *Micromonospora echinospora*), 0.81.

The R_f value determined by paper chromatography with the use of a solvent consisting of methanol and 3% NaCl aqueous solution (in a volume ratio of 2:1) as a developing liquid and Toyo Filter Paper No. 51A (product of Toyo Filter Paper Co., Ltd., Japan) is 0.25 for Quintomycin-A, and 0.27 for Quintomycin-B. The R_f values determined by this procedure of some known antibiotic substances are as follows: Kanamycin, 0.36; Paromomycin, 0.31; Neomycin, 0.20; and Gentamicin, 0.53.

Other properties of the antibiotic substances of the invention are shown in the following Table together with those of other known antibiotic substances.

TABLE (1)

Name of Antibiotic substance	Quintomycin-A	Quintomycin-B	Quintomycin-C	Quintomycin-D	Kanamycin-A	Kanamycin-B	Kanamycin-C
Produced by	S.lividus	S.lividus	S.lividus	S.lividus	S.kanamyceticus	S.kanamyceticus	S.kanamyceticus
Melting point (M.P.) °C (dec.)	(Free) 197—203 (-HCl) 190—200	(Free) 197—203 (-HCl) 185—195	(-HCl) 198—208	(Free) 178—184 (-HCl) 198—208	(Free) 263—268	(Free) 170—190	(Free) ca. 270
[α] _D + °	HCl)[α] _D ²² =59 (c 1, H ₂ O)	(-HCl)[α] _D ²² =50 (c 1, H ₂ O)	(same as the paromomycin)	(-HCl)[α] _D ²² =36 (c 0.5, H ₂ O)	(Free)[α] _D ²⁴ =146 (c 1, 0.1 N H ₂ SO ₄)	(Free)[α] _D ²⁴ =126 (c 0.7, H ₂ O)	(Free)[α] _D ²⁰ =126 (c 1, H ₂ O)
Molecular formula	C ₂₉ H ₅₃ N ₅ O ₁₉	C ₂₉ H ₅₅ N ₅ O ₁₈	C ₂₃ H ₄₅ N ₅ O ₁₄	C ₂₃ H ₄₅ N ₅ O ₁₃	C ₁₈ H ₃₆ N ₄ O ₁₁	C ₁₈ H ₃₇ N ₅ O ₁₀	C ₁₈ H ₃₆ N ₄ O ₁₁
Molecular Weight (M.W.)	777.77	761.77	615.63	599.63	484.51	482.51	484.51
Analysis (%)	c. f. C:44.77 C:45.16 H: 7.07 H: 7.05 N: 9.01 N: 8.73	c. f. C:45.72 C:46.23 H: 7.22 H: 7.70 N: 9.19 N: 9.14	(same as the paromomycin)	c. f. C:46.07 C:46.40 H: 7.56 H: 7.59 N:11.68 N:11.33	c. f. C:44.62 H: 7.49 N:11.56	c. C:44.80 H: 7.51 N:14.52	c. C:44.62 H: 7.49 N:11.56
(Components of the substance)			(same as the paromomycin)				
Mannose	+	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+
Deoxystreptamine	+	+	+	+	+	+	+
Monoamino sugar	+	+	+	+	+	+	+
Diamino sugar	+	+	+	+	+	+	+

TABLE (2)

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TABLE (2)

Names of Antibiotic substance	Quintomycin-A	Quintomycin-B	Quintomycin-C	Quintomycin-D	Kanamycin-A	Kanamycin-B	Kanamycin-C
(Biol. activity, M.I.C. mcg/ml.) Staph. aureus 209p	1.56	0.8	(same as the paromomycin)	0.4	0.8		
S. citreus	3.12	0.8		1.56	3.12		
S. albus	0.8	0.4		0.1	0.8		
Strept. faecalis	100	100		50	12.5		
Sarcina lutea	100	>100		1.56	3.12		
Bacillus subtilis	0.8	0.8		0.8	0.2		
B. cereus	1.56	3.12		0.8	1.56		
B. anthracis	1.56	1.56		0.4			
Escherichia coli	6.25	6.25		3.12	1.56		
Kreb. pneumoniae	6.25	3.12		6.25	3.12		
Prot. vulgaris	12.5	12.5		12.5	12.5		
Salmonella typhi	6.25	1.56		1.56	1.56		
Shigella flexneri	6.25	12.5		12.5	6.25		
Pseudo. aeruginosa	100	3.12		3.12	25		
Mycobacterium 607	5.12	1.56		1.56	1.56		
M. phlei	3.12	1.56		1.56	1.56		
Mycobacterium tuber		3.12		3.12	3.12		

TABLE (3)

Names of Antibiotic substance	Quintomycin-A	Quintomycin-B	Quintomycin-C	Quintomycin-D	Kanamycin-A	Kanamycin-B	Kanamycin-C
(Toxicity in mice, LD ₅₀ mg/kg)							
Intravenous	198	193		190	374.2		
Intraperitoneal							
Subcutaneous	1878	1210			2282		
Synonym			Paromomycin				

TABLE (4)

Name of Antibiotic substance	Neomycin-A	Neomycin-B	Neomycin-C	Paromomycin-I	Paromomycin-II	Gentamicin complex
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TABLE (4)

Name of Antibiotic substance	Neomycin-A	Neomycin-B	Neomycin-C	Paromomycin-I	Paromomycin-II	Genamycin complex
Produced by	S. fradiae S. albogriseolus	S. fradiae S. albogriseolus	S. fradiae S. albogriseolus	S. rimosus f. paromomycinus	S. rimosus f. paromomycinus	Micromonospora echino- spora Micromonospora purpurea
M.P. °C (dec.)	(-H ₂ SO ₄)/250-6					Cl:94-100, C ₂ :107-124
[α] _D + °	(-H ₂ SO ₄)[α] _D ²³ =123 (c 0.7, H ₂ O)	(-H ₂ SO ₄)[α] _D ²³ =58 (c 0.5, H ₂ O)	(-H ₂ SO ₄)[α] _D ²² =82 (c 0.5, H ₂ O)	(-HCl)[α] _D ²⁵ =64 (c 1, H ₂ O)	(-HCl)[α] _D ²⁴ =78	[α] _D ²⁵ =158 c ₂ : [α] _D ²⁵ =160 (H ₂ O) (H ₂ O)
Molecular Formula	C ₁₇ H ₃₆ N ₄ O ₆	C ₂₃ H ₄₀ N ₈ O ₁₃	C ₂₃ H ₄₀ N ₈ O ₁₃	C ₂₃ H ₄₅ N ₅ O ₁₄	C ₂₃ H ₄₅ N ₅ O ₁₄	C ₁₇₋₁₈ H ₃₄₋₃₆ N ₄ O ₇
M.W.	322.36	614.65	614.65	615.63	615.63	
Analysis (%)	C:44.71 H: 8.13 N:17.38	C:44.94 H: 7.52 N:13.67	C:44.94 H: 7.52 N:13.67	C:44.87 H: 7.37 N:11.38	C:44.87 H: 7.37 N:11.38	
(Components of the substance)						
Mannose	-	-	-	-	-	-
Ribose	-	+	+	+	+	-
Deoxystreptamine	+	+	+	+	+	+
Monoamino sugar	-	-	-	+	+	+
Diamino sugar	+	+	+	+	+	-
Biol activity M.I.C. mcg/ml.)	209p	0.8	0.4	0.8		0.8
Staph. aureus				1.56		3.12
S. citreus				0.2		0.8
S. albus						

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TABLE (5)

Name of Antibiotic substance (Biol. activity M.I.C. mcg/ml.)	Neomycin-A	Neomycin-B	Neomycin-C	Paromomycin-I	Paromomycin-II	Gentamicin complex
Strept. faecalis		12.5		25		6.25
Sarcina lutea		1.56		3.12		0.4
Bacillus subtilis		0.8		0.4		0.4
B. cereus		1.56		0.8		0.8
B. anthracis				1.6		0.1
Escherichia coli		1.56		3.12		3.12
Kreb. pneumoniae		6.25		3.12		0.8
Prot. vulgaris		6.25		6.25		1.56
Salmonella typhi		6.25		3.12		0.8
Shigella flexneri		6.25		6.25		3.12
Pseudo. aeruginosa		12.5		>100		0.8
Mycobacterium 607		0.8		1.56		
M. phlei		3.12		0.4		
(Toxicity in mice LD ₅₀ mg/kg)						
Intravenous				90		72
Intraperitoneal						433
Subcutaneously				423		484
Synonym		220	Streptothricin -B ₁₁	Aminosidin, Catenulin, Hydrotomycin, Zygomycin		

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The following Examples illustrate the invention.

EXAMPLE 1

Streptomyces lividus (ATCC 21178 (No. 50 FLT1)) strain was fermented in a medium sterilized and adjusted to a pH of 7.0 which consisted of 0.5% of starch, 2.0% of soybean meal, 0.1% of dipotassium hydrogen phosphate, 0.05% of magnesium sulphate and 0.3% of sodium chloride, and was pre-cultured at 27°C. for about 50 hours. Subsequently, 100—150 ml. of the pre-culture broth was added to 10 liters of the same culture medium, and culturing was effected at 27°C. with stirring at a rate of 250 rpm while passing sterilized air at a rate of 10 liters/min. In forty hours, the pH of the culture broth was 7.2—7.4, and the production of Quintomycin reached a maximum. The culture broth was filtered with the use of Celite as a filtering assistant to give 9.2 liters of a culture filtrate. The filtered broth was stirred for 10 minutes together with 300 ml. of Amberlite IRC—50 (trade name) type H resin, when the Quintomycin was completely adsorbed by the resin. The resin was packed into a column, and washed thoroughly with water. Quintomycin was recovered by elution with a 0.5 N hydrochloric acid. The Quintomycin solution was neutralized with 10 N sodium hydroxide solution to adjust the pH to 7.6—7.8. The Quintomycin was then adsorbed by a column packed with 10 g of activated carbon to purify it and the adsorbant was washed with 0.01% of aqueous ammonia and then with water, and eluted with a 0.02 N hydrochloric acid/methanol (1:1). Methanol was removed by distillation under reduced pressure. Subsequent lyophilising gave 1 g of the hydrochloride of the Quintomycin-A, -B, -C and -D complex.

EXAMPLE 2

A culture broth obtained in the same manner as in Example 1 was passed through Amberlite IRC—50 (trade name) (type NH₄) resin packed into a column, and the Quintomycin was adsorbed completely by the resin. The resin was thoroughly washed with water, and eluted with a 1.0 N aqueous ammonia. The resulting solution was treated with a 2N hydrochloric acid to adjust its pH to 6—7.8. The Quintomycin obtained was purified by the procedure of Example 1.

EXAMPLE 3

Streptomyces lividus strain (ATCC 21178 (No. 50 FLT1)) was fermented in a medium sterilized and adjusted to a pH of 6.8 which consisted of 0.5% of starch, 0.05% of glucose, 2.0% of soybean meal, 0.2% of peptone, 0.05% of magnesium sulphate, 0.1% of dipotassium hydrogen phosphate and 0.3% of sodium chloride, and was pre-cultured at 35°C. for about 50 hours. Subsequently, 300—400 ml. of the pre-culture broth was added

to 10 liters of the same medium, and culture-rate of 200 rpm while passing sterilized air at 35°C. with stirring at a rate of 14 liter/min. In 96 hours, the pH of the culture broth was 7.8—8.2, and the production of Quintomycin reached a maximum. The culture broth was stirred for 10 minutes together with 100 ml. of a weakly acidic ion exchange resin, Amberlite IRC—84 (trade name) (NH₄ type), when the Quintomycin was completely adsorbed by the resin. After removal of the mycelium and waste broth by fractional filtration, the resin having the Quintomycin adsorbed therein was packed into a column, and was thoroughly washed with water, followed by elution with aqueous 2.0 N ammonia. The Quintomycin solution was collected and concentrated, and then passed through a column having a size of 30 × 2400 mm and packed with a weakly acidic cation exchange resin for use in chromatography. A fraction was eluted first with 0.08 N aqueous ammonia and then with 0.1 N aqueous ammonia. The first elution yielded Quintomycin-A, and the second Quintomycin-B. Subsequent elution with 0.15 N aqueous ammonia produced Quintomycin-C, after which on elution with 0.3 N aqueous ammonia Quintomycin-D came out.

Concentrating and lyophilising procedures gave pure Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D as free bases in yields of 650 mg, 4200 mg, 40 mg and 120 mg, respectively.

EXAMPLE 4

One hundred and fifty milligrams of the Quintomycin complex hydrochloride obtained in Example 1 or 2 was dissolved in about 40 ml. of distilled water, and the solution was passed through a column having a diameter of 1 cm packed with 30 ml. of carboxymethyl Sephadex C—25 (trade name) (type NH₄), which was then water-washed. Using first 200 ml. of a 0.05 N aqueous ammonia and then 200 ml. of 1.0 N aqueous ammonia, elution was effected by a gradient method at a rate of 25 ml./hr. The eluate was recovered as successive fractions each of 4 ml. Quintomycin-A was contained in fractions 45—50; Quintomycin-B in fractions 53—58; and Quintomycin-C in fractions 88—90. Continuing the elution with aqueous ammonia of a concentration 1.0% or more resulted in the elution of Quintomycin-D. Lyophilising procedure gave pure Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D as free bases in yields of 31 mg, 49 mg, trace and 18 mg, respectively.

EXAMPLE 5

One hundred and fifty grams of the Quintomycin complex hydrochloride obtained in Example 1 or 2 was dissolved in 40 ml. of distilled water, and the solution was passed through a column with a diameter of 1 cm

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Aminocidin,
Catenulin,
Hydroxymycin,
Zygomycin

Streptothricin
-B₁

220

Framycetin
Streptothricin
-B₁₁

Subcutaneously
Synonym

packed with 30 ml. of carboxymethyl Sephadex C-25 (trade name) (type NH_4) buffered with ammonium formate, followed by water-washing. Using first 200 ml. of 0.5 molar ammonium formate solution and then 200 ml. of 3.0 molar ammonium formate solution, elution was effected by a gradient method at a rate of 25 ml./hr. The eluate was recovered in successive fractions each amounting to 3 ml. Quintomycin-A was contained in fractions 29—37; Quintomycin-B in fractions 40—45; and Quintomycin-C in fractions 78—80. Continuing the elution with 3.0 molar ammonium formate solution gave Quintomycin-D. Lyophilising procedure gave pure Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D as free bases in yields of 35 mg, 56 mg, trace and 19 mg, respectively.

EXAMPLE 6

One hundred and fifty grams of the Quintomycin complex hydrochloride obtained in Example 1 or 2 was dissolved in 40 ml. of distilled water. The solution was adjusted to a pH of 7.0 with 0.1 N aqueous ammonia, and the Quintomycin therein was adsorbed completely by 20 ml. of Amberlite CG-50 (trade name) (type NH_4) packed into a column having a diameter of 1 cm. Using first 200 ml. of 0.02 N aqueous ammonia and then 200 ml. of 1.0 N aqueous ammonia, elution was effected by a gradient method at a rate of 25 ml./hr. The eluate was recovered successively in fractions each amounting to 3 ml. Quintomycin-A was contained in fractions 29—35; Quintomycin-B in fractions 40—50; and Quintomycin-C in fractions 88—92. Continuing the elution with a 1.0 N aqueous ammonia resulted in the elution of Quintomycin-D. Lyophilising procedure gave pure Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D as free bases in yields of 33 mg, 52 mg, trace and 16 mg. respectively.

EXAMPLE 7

Free bases of Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D obtained in Example 3, 4, 5 or 6 were each dissolved in methanol. Addition of 2 N sulphuric acid to the resulting methanol solution led to the precipitation of a sulphate of each Quintomycin. The precipitate was thoroughly washed with methanol/acetone, and a pure sulphate of each Quintomycin was obtained.

Free bases of the Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D were dissolved into methanol and acidified with a 2 N hydrochloric acid. Addition of acetone to each of the resulting methanol solution led to the precipitation of a hydrochloride of each Quintomycin. The precipitate was washed thoroughly with acetone-ether, and a pure pentahydrochloride of each Quintomycin was obtained.

WHAT WE CLAIM IS:—

1. Antibiotic Quintomycin as hereinbefore defined, belonging to the aminocyclitol group which inhibits the growth of gram-positive bacteria, gram-negative bacteria and acid-fast bacteria and contains at least one of the following compounds:

(A) a compound of empirical formula $\text{C}_{29}\text{H}_{55}\text{N}_5\text{O}_{10}$ (the pentahydrochloride of which has a specific optical rotation $[\alpha]_{\text{D}}^{22}$ of $+59^\circ$)

(B) a compound of empirical formula $\text{C}_{29}\text{H}_{55}\text{N}_5\text{O}_{18}$ (the pentahydrochloride of which has a specific optical rotation $[\alpha]_{\text{D}}^{22}$ of $+50^\circ$) and

(D) a compound of empirical formula $\text{C}_{29}\text{H}_{45}\text{N}_5\text{O}_{13}$ (the pentahydrochloride of which has a specific optical rotation $[\alpha]_{\text{D}}^{20}$ of $+36^\circ$), with or without the further compound known as paromycin.

2. Antibiotic Quintomycin-A containing in the molecule residues of mannose, ribose, deoxystreptamine, monoaminosugar and diaminosugar and having an R_f value (as hereinbefore defined) of 0.23, which inhibits the growth of gram-positive bacteria, gram-negative bacteria and acid-fast bacteria and has the empirical formula $\text{C}_{29}\text{H}_{55}\text{N}_5\text{O}_{19}$ and whose pentahydrochloride has a specific optical rotation $[\alpha]_{\text{D}}^{22}$ of $+59^\circ$ and a decomposition point of $190-200^\circ\text{C}$.

3. Antibiotic Quintomycin-B containing in the molecule residues of mannose, ribose, deoxystreptamine, monoaminosugar and diaminosugar and having an R_f value (as hereinbefore defined) of 0.50, which inhibits the growth of gram-positive bacteria, gram-negative bacteria and acid-fast bacteria and has the empirical formula $\text{C}_{29}\text{H}_{55}\text{N}_5\text{O}_{18}$ and whose pentahydrochloride has a specific optical rotation $[\alpha]_{\text{D}}^{22}$ of $+50^\circ$ and a decomposition point of $185-195^\circ\text{C}$.

4. Antibiotic Quintomycin-D containing in the molecule residues of ribose, deoxystreptamine, monoaminosugar and diaminosugar and having an R_f value (as hereinbefore defined) of 0.75, which inhibits the growth of gram-positive bacteria, gram-positive bacteria and acid-fast bacteria and has the empirical formula $\text{C}_{29}\text{H}_{45}\text{N}_5\text{O}_{13}$ and whose pentahydrochloride has a specific optical rotation $[\alpha]_{\text{D}}^{20}$ of $+36^\circ$ and a decomposition point of $198-208^\circ\text{C}$.

5. A process for preparation of antibiotic Quintomycin, which comprises culturing *Streptomyces lividus* ATCC 21178 (No. 50 F.L.T.I.) or a Quintomycin-producing mutant, under aerobic conditions in a medium containing an assimilable carbon source and an assimilable nitrogen source, and recovering antibiotic Quintomycin from the culture broth.

6. A process according to claim 5 wherein said medium contains a mineral substance besides the carbon and nitrogen sources.



packed with 30 ml. of carboxymethyl Sephadex C-25 (trade name) (type NH₄) buffered with ammonium formate, followed by water-washing. Using first 200 ml. of 0.5 molar ammonium formate solution and then 200 ml. of 3.0 molar ammonium formate solution, elution was effected by a gradient method at a rate of 25 ml/hr. The eluate was recovered in successive fractions each amounting to 3 ml. Quintomycin-A was contained in fractions 29—37; Quintomycin-B in fractions 40—45; and Quintomycin-C in fractions 78—80. Continuing the elution with 3.0 molar ammonium formate solution gave Quintomycin-D. Lyophilising procedure gave pure Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D as free bases in yields of 35 mg, 56 mg, trace and 19 mg, respectively.

EXAMPLE 6

One hundred and fifty grams of the Quintomycin complex hydrochloride obtained in Example 1 or 2 was dissolved in 40 ml. of distilled water. The solution was adjusted to a pH of 7.0 with 0.1 N aqueous ammonia, and the Quintomycin therein was adsorbed completely by 20 ml. of Amberlite CG-50 (trade name) (type NH₄) packed into a column having a diameter of 1 cm. Using first 200 ml. of 0.02 N aqueous ammonia and then 200 ml. of 1.0 N aqueous ammonia, elution was effected by a gradient method at a rate of 25 ml./hr. The eluate was recovered successively in fractions each amounting to 3 ml. Quintomycin-A was contained in fractions 29—35; Quintomycin-B in fractions 40—50; and Quintomycin-C in fractions 88—92. Continuing the elution with a 1.0 N aqueous ammonia resulted in the elution of Quintomycin-D. Lyophilising procedure gave pure Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D as free bases in yields of 33 mg, 52 mg, trace and 16 mg. respectively.

EXAMPLE 7

Free bases of Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D obtained in Example 3, 4, 5 or 6 were each dissolved in methanol. Addition of 2 N sulphuric acid to the resulting methanol solution led to the precipitation of a sulphate of each Quintomycin. The precipitate was thoroughly washed with methanol/acetone, and a pure sulphate of each Quintomycin was obtained.

Free bases of the Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D were dissolved into methanol and acidified with a 2 N hydrochloric acid. Addition of acetone to each of the resulting methanol solution led to the precipitation of a hydrochloride of each Quintomycin. The precipitate was washed thoroughly with acetone-ether, and a pure pentahydrochloride of each Quintomycin was obtained.

WHAT WE CLAIM IS:—

1. Antibiotic Quintomycin as hereinbefore defined, belonging to the aminocyclitol group which inhibits the growth of gram-positive bacteria, gram-negative bacteria and acid-fast bacteria and contains at least one of the following compounds:

(A) a compound of empirical formula $C_{29}H_{38}N_5O_{19}$ (the pentahydrochloride of which has a specific optical rotation $[\alpha]_D^{22}$ of + 59°)

(B) a compound of empirical formula $C_{29}H_{38}N_5O_{18}$ (the pentahydrochloride of which has a specific optical rotation $[\alpha]_D^{22}$ of + 50°) and

(D) a compound of empirical formula $C_{29}H_{38}N_5O_{13}$ (the pentahydrochloride of which has a specific optical rotation $[\alpha]_D^{20}$ of + 36°), with or without the further compound known as paromycin.

2. Antibiotic Quintomycin-A containing in the molecule residues of mannose, ribose, deoxystreptamine, monoaminosugar and diaminosugar and having an R_f value (as hereinbefore defined) of 0.23, which inhibits the growth of gram-positive bacteria, gram-negative bacteria and acid-fast bacteria and has the empirical formula $C_{29}H_{38}N_5O_{19}$ and whose pentahydrochloride has a specific optical rotation $[\alpha]_D^{22}$ of + 59° and a decomposition point of 190—200°C.

3. Antibiotic Quintomycin-B containing in the molecule residues of mannose, ribose, deoxystreptamine, monoaminosugar and diaminosugar and having an R_f value (as hereinbefore defined) of 0.50, which inhibits the growth of gram-positive bacteria, gram-negative bacteria and acid-fast bacteria and has the empirical formula $C_{29}H_{38}N_5O_{18}$ and whose pentahydrochloride has a specific optical rotation $[\alpha]_D^{22}$ of + 50° and a decomposition point of 185—195°C.

4. Antibiotic Quintomycin-D containing in the molecule residues of ribose, deoxystreptamine, monoaminosugar and diaminosugar and having an R_f value (as hereinbefore defined) of 0.75, which inhibits the growth of gram-positive bacteria, gram-positive bacteria and acid-fast bacteria and has the empirical formula $C_{29}H_{38}N_5O_{13}$ and whose pentahydrochloride has a specific optical rotation $[\alpha]_D^{20}$ of + 36° and a decomposition point of 198—208°C.

5. A process for preparation of antibiotic Quintomycin, which comprises culturing *Streptomyces lividus* ATCC 21178 (No. 50 F.L.T.I.) or a Quintomycin-producing mutant, under aerobic conditions in a medium containing an assimilable carbon source and an assimilable nitrogen source, and recovering antibiotic Quintomycin from the culture broth.

6. A process according to claim 5 wherein said medium contains a mineral substance besides the carbon and nitrogen sources.

7. A process according to claim 5 or 6 wherein the culturing temperature is 20—39°C.
8. A process according to any one of claims 5—7 wherein the pH of the culture medium is 6.6—7.5.
9. A process according to claim 5 or 6 wherein the culturing temperature is 25—37°C and the pH of the culture medium is 7.0 ± 0.3.
10. A process according to any one of claims 5—9 wherein the culturing is effected for 2 days to 2 weeks.
11. A process according to any one of claims 5—10 wherein the carbon source is one or more of starch, glucose, glycerol, maltose, inositol, fructose, dextrin, sucrose and galactose.
12. A process according to any one of claims 5—11 wherein the nitrogen source is one or more of yeast extract, polypeptone, dry yeast, meat extract, cornsteep liquor, soybean meal, inorganic nitrate and ammonium salt.
13. A process according to any one of claims 5—12 wherein the culture medium contains as a mineral substance one or more of sodium chloride, dipotassium hydrogen phosphate, magnesium sulphate, iron sulphate, calcium chloride, calcium carbonate, calcium hydroxide, iron chloride, zinc sulphate and cobalt chloride.
14. A process according to any one of claims 5—13 wherein the Quintomycin is recovered by adsorption and elution.
15. A process according to any one of claims 5—13 wherein the Quintomycin is recovered by solvent extraction.
16. A process according to claim 14 wherein the Quintomycin is adsorbed on a cation exchanger and is eluted by an aqueous solution of an acid or an alkali-reacting substance.
17. A process according to claim 16 wherein the cation exchanger is a weakly acidic cation exchange resin or carboxymethyl cellulose.
18. A process according to claim 14 wherein the Quintomycin is adsorbed on activated carbon and is eluted by an aqueous solution of an acid, an acidic lower aliphatic alcohol, or acidic acetone.
19. A process according to claim 15 wherein the Quintomycin is extracted with a water-immiscible aliphatic alcohol.
20. A process according to claim 19 wherein the aliphatic alcohol is n-butanol, iso-butanol or amyl alcohol.
21. A process according to any one of claims 5—14, 16 and 17 wherein the Quintomycin is adsorbed by a cation exchange resin and eluted with an aqueous solution of an alkaline-reacting substance, and one or more of Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D is separately recovered.
22. A process according to claim 14 wherein the Quintomycin is adsorbed by an anion exchange resin and eluted with water, and one or more of Quintomycin-A, Quintomycin-B, Quintomycin-C and Quintomycin-D is separately recovered.
23. A process according to claim 21 or 22 wherein the elution is carried out by a gradient method or stepwise.
24. A method of preparing Quintomycin according to claim 5 substantially as hereinbefore described.
25. A Quintomycin complex or Quintomycin-A, -B, -C or -D individually, when obtained by a process claimed in any one of claims 5—24.

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