

PATENT SPECIFICATION

DRAWINGS ATTACHED

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International Classification:—C12d. A61k.

COMPLETE SPECIFICATION

Kanamycin B and its Isolation from a Fermentation Broth

We, HAMAO UMEZAWA, a Japanese Subject, of 23 Kita-4-Chome, Toyotama, Nerima-Ku, Tokyo, Japan, MASAHIRO UEDA, a Japanese Subject, of 355 Seijomachi, Setagaya-Ku, Tokyo, Japan, and KENJI MAEDA, a Japanese Subject, of 258-1 Gotanda, Shinagawa-Ku, Tokyo, 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 the preparation of an improved form of the antibiotic kanamycin described generally in the Specification of our Patent No. 817,385, and more particularly to the preparation of kanamycin B.

Specification No. 817,385 describes and claims a process for the production of a new antibiotic, kanamycin, characterized by the fact that a kanamycin-producing strain of *Streptomyces kanamyceticus* nov. spec., of which the properties are described in the specification, is cultivated under aerobic conditions in an aqueous nutrient medium containing a source of carbon, nitrogen and inorganic salts; until substantial antibiotic activity is imparted to said medium and the antibiotic is recovered from the fermentation broth.

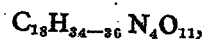
We have now discovered an improved form of kanamycin, herein designated kanamycin B, and a process for preparing this kanamycin B from the kanamycin-producing strain of *Streptomyces Kanamyceticus* described in Specification No. 817,385.

According to the present invention there is provided an improvement in or modification of the process claimed in Specification No. 817,385 which comprises separating from the kanamycin produced by the process claimed in Specification No. 817,385 an antibiotic substance, herein designated kanamycin B, which substance exhibits $[\alpha]_D^{25} + 135^\circ$ ($c=0.63$ in water) contains carbons (44.69%), hydrogen (7.48%) and nitrogen (12.65 to 13.62%) gives a salicylidene derivative which decomposes without melting at 255 to 265°C., fails to give

[Price 4s. 6d.]

a product with an ultra-violet absorption spectrum identical to that of furfural on treatment with 40% sulphuric acid for 100 minutes at 100°C., and exhibits an infra-red absorption spectrum having characteristic absorption maxima at the following wavelengths in microns: 2.96, 3.44, 6.35, 6.48 (shoulder), 6.74, 6.85 (shoulder), 7.25, 7.45, 7.86, 8.08, 8.28, 8.76, 9.55, 9.65, 10.4 and 11.15.

The initial fermentation broth may contain kanamycin A and kanamycin B, each of these kanamycins being a substance which is soluble in water and substantially insoluble in *n*-butanol, ethyl acetate, butyl acetate, ether, chloroform and benzene, which forms salts with acids, which exhibits no absorption of ultra-violet light from 220 $m\mu$ to 400 $m\mu$ which gives a positive reaction to ninhydrin reagent dissolved in pyridine and positive Molisch and Elson-Morgan reactions and negative Tollens, Sakaguchi, Fehling, maltol and Selinwanoff reactions, which contains only the elements carbon, hydrogen, oxygen and nitrogen, which contains free primary amino groups, which is dextro-rotatory in water and which exhibits characteristic absorption bands in the infra-red region of the spectrum when pelleted, in the form of the free base, in potassium bromide at the following wavelengths in microns: 2.96, 6.35, 6.48, 6.85, 7.25, 7.45, 7.86, 8.08, 9.65 and 10.4. Further properties of the kanamycins are: kanamycin A base has the empirical formula



exhibits $[\alpha]_D^{25} + 146^\circ$ ($c=1$ in 0.1 *N* H_2SO_4), gives a salicylidene derivative melting at 272 to 274°C. with decomposition, gives desoxy-streptamine on strong acid hydrolysis, gives a product with an ultra-violet absorption spectrum identical to that of furfural on treatment with 40% sulphuric acid from 100 minutes at 100°C. and exhibits additional characteristic absorption bands in the infra-red region of the spectrum when pelleted, in the



form of the free base, in potassium bromide at the following wavelengths in microns: 2.86, 2.93, 3.01, 3.03, 3.15, 3.22, 3.67, 6.23, 6.75, 6.93, 7.10, 7.33, 7.57, 7.63, 7.75, 7.82, 7.90, 7.95, 8.19, 8.43, 8.55, 8.68, 8.79, 8.90, 9.00, 9.20, 9.47, 9.85, 10.08, 10.63, 10.95, 11.25, 11.35, 11.50, 11.73, 11.95, 12.28, 12.48, 12.77 and 13.05; and kanamycin B base exhibits $[\alpha]_D + 135^\circ$ ($c=0.63$ in water), gives a salicylidene derivative which decomposes without melting at 255 to 265° C., fails to give a product with an ultra-violet absorption spectrum identical to that of furfural on treatment with 40% sulphuric acid for 100 minutes at 100° C. and exhibits additional characteristic absorption bands in the infra-red region of the spectrum when pelleted in the form of the free base in potassium bromide at the following wavelengths in microns: 3.44, 6.74, 8.28, 8.76, 9.55 and 11.15.

In one process according to the invention the medium to which a substantially antibiotic activity is imparted is treated by adding sodium dodecylbenzene-sulphonate in order to precipitate kanamycin B dodecylbenzene sulphonate, the precipitated sulphonate is separated and dissolved in methanol, sulphuric acid is added thereto, and the resulting kanamycin B sulphate is collected.

In a further process of separating kanamycin B the medium to which a substantially antibiotic activity is imparted is treated with salicylaldehyde to convert kanamycin to its tetrasalicylidene derivative, the salicylidene derivative is isolated, and the salicylidene derivative of kanamycin B is separated from the salicylidene derivative of kanamycin A by counter current distribution techniques using the solvent system methanol-water-chloroform-benzene.

According to another process of the invention kanamycin B is separated from the medium to which a substantially antibiotic activity is imparted by ion exchange resin column chromatography employing a carboxylic acid type cation exchange resin and dilute aqueous ammonia as eluting agent.

Kanamycin A normally predominates in the fermentation broths prepared as described in Specification No. 817,385, e.g. according to the procedures of Examples 5 or 9 of that Specification, but useful amounts of kanamycin B are also present therein. Much larger amounts of kanamycin B were obtained when 80 gallons inoculum of *S. kanamyceticus* (a selected subculture of ATCC 12853 strain) was fermented in 800 gallons of an aqueous medium containing 4.5% of sucrose, 2.25% of soybean meal, 1.7% of Pharmamedia (cottonseed endosperm flour), 0.225% of debittered yeast, 0.57% of $(\text{NH}_4)_2\text{SO}_4$, 1.13% of CaCO_3 , 0.02% of iron chelating agent (Fe^{3+} specific EDTA) and 0.85% of corn oil meal. After 95 hours' fermentation at 82° F. with aeration at 50 cubic feet per minute and stirring at 155 r.p.m.

the broth contained by bioassay 300 mcg. of kanamycin/ml. of which the greater part was kanamycin B.

Pure kanamycin A is prepared by repeated recrystallization of the sulphate from methanol-water at pH 7.8 to 8.2. Pure kanamycin B is obtained by means of the preferential precipitation of its salt formed by reaction with a sodium salt of a sulphonated petroleum (C_{16}) hydrocarbon (Ultrawet, sodium dodecylbenzenesulphonate). The separation is also performed by counter-current distribution (200 transfers) of the salicylidene derivative in the system methanol-water - chloroform - benzene (5:4:2:1 by volume) followed by regeneration. Mixtures of kanamycin A and B are separated by paper-strip chromatography in Peterson's *n*-butanol-water - 2% toluene-*p*-sulphonic acid system on Schleicher and Schuell 589 Blue Ribbon or Whatman No. 1 papers. In this system with S & S 589 Blue Paper, kanamycin A has an R_f of about 0.35 and kanamycin B has an R_f of about 0.6. The presence of impurities or contaminating salts interferes markedly with the paper chromatography of the kanamycins in this system. Kanamycin A, but not kanamycin B, on treatment with 40% sulphuric acid for 100 minutes at 100° C. yields a product with an ultra-violet spectrum identical to that of furfural.

Isolation and Purification of Kanamycin A

Kanamycin A Sulphate. Kanamycin A was isolated from fermentation broths by adsorption on "Amberlite IRC-50" resin in the sodium cycle and elution with aqueous hydrochloric acid. The eluate was neutralized, diluted and reabsorbed on "Amberlite IRC-50" which has been regenerated with ammonium hydroxide. The column was eluted with 0.2 N NH_4OH , the eluate was concentrated *in vacuo* to between 50 and 100 mg./ml. of kanamycin activity, diluted with 0.8 to 1 volume of methanol and adjusted with H_2SO_4 to pH 8.0 to 8.2. Kanamycin A sulphate crystallized slowly in small irregular pale yellow prismatic crystals.

The crystalline kanamycin A sulphate was purified by repeated recrystallization from methanol-water at pH 7.8 to 8.2 to give white irregular prismatic crystals. This preparation contains absorbed moisture which is removed with difficulty. Samples for analytical determinations were dried to constant at 170° C. *in vacuo*.

Calcd. for $\text{C}_{18}\text{H}_{36}\text{N}_4\text{O}_{11} \cdot \text{H}_2\text{SO}_4$: C, 37.13; H, 6.58; N, 9.62; S, 5.50; SO_4 , 16.6%; neutral eq., 145.6.

Found: C, 37.3, 37.4, 37.3; H, 6.8, 6.6, 6.3; N, 9.3, 9.6; S, 5.5; SO_4 , 16.8%; neutral eq., 146.6.

The sulphate is soluble in water, insoluble in the common alcohols and nonpolar solvents. It shows no melting point, decomposing over a wide range above 250° C. It gave $[\alpha]_D +$

70

75

80

85

90

95

100

105

110

115

120

125

130

125° ($c=1$ in 0.1 N H_2SO_4) on an anhydrous basis.

Following is a description by way of example of methods of carrying the invention into effect.

EXAMPLE

Isolation and Purification of Kanamycin B

A solution (1500 ml.) at pH 6 of kanamycin was obtained by using two absorptions on "Amberlite IRC-50" resin in the sodium form followed by elution with sulphuric acid. To this solution there was added 45 g. of sodium dodecylbenzenesulphonate (Ultrawet K) to precipitate kanamycin B dodecylbenzenesulphonate, which was collected by filtration, washed with water and dissolved in methanol. The methanol solution was then acidified with sulphuric acid, leaving dodecylbenzenesulphonic acid in solution and precipitating more than 8.5 g. of kanamycin B sulphate which was substantially free of kanamycin A, as indicated by its bioassay of 732 u./mgm. and its furfural ultra-violet assay of 45 u./mgm.

In another experiment 50 g. of solid kanamycin (456 u./mgm. by bioassay; 131 u./mgm. by furfural ultra-violet assay; ratio = 0.287 for the two assays) was dissolved at pH 6 in 2 l. of water and 60 g. of sodium dodecylbenzenesulphonate (Ultrawet) was added. The precipitate was collected, water-washed and dissolved in methanol and the methanol solution was acidified with sulphuric acid to precipitate purified kanamycin B sulphate. After repetition of this process, there was obtained 9.5 g. of solid kanamycin B sulphate (630 u./mgm. by bioassay; 51 u./mgm. by furfural ultra-violet assay; ratio = 0.081 for the two assays).

Both kanamycin A and kanamycin B were obtained in pure form by way of counter-current distribution of the salicylidene derivatives in the system methanol-water-chloroform-benzene in the ratio by volume of 5:4:2:1. Thus kanamycin sulphate (10 g.) was dissolved in water and treated with a solution of barium hydroxide to convert it to the base by precipitating barium sulphate. The filtrate was then shaken with a solution of 8 g. of salicylaldehyde in *n*-butanol and the precipitation of the Schiff's base (tetra-salicylidene derivative of kanamycin) was completed by the addition of 2 N sodium hydroxide. The *n*-butanol phase was separated, combined with additional *n*-butanol used to extract the aqueous phase, washed with aqueous sodium bicarbonate and distilled *in vacuo* to leave the Schiff's base as residue.

The tetra-salicylidene derivative of Kanamycin is described and claimed in British Patent Specification No. 833,851. Processes for isolating kanamycin from a fermentation broth as its tetrasalicylidene derivative are described and claimed in British Specification No. 823,516.

In one case of separation by counter-current techniques, distribution by using 200 transfers gave peak concentrations of the tetra-salicylidene-kanamycin A at about tube 65 and the tetra-salicylidene-kanamycin B at about tube 40. The contents of the tubes near these peaks were combined and the derivative therein was converted to sulphate by dissolving it in acetone and adding dilute aqueous sulphuric acid to decompose the Schiff's base and precipitate kanamycin sulphate which was then redissolved in water and precipitated by the addition of methanol-acetone (1:1) to give to kanamycin A sulphate (423 u./mgm. by bioassay; 470 u./mgm. by furfural ultra-violet assay) and kanamycin B sulphate (936 u./mgm. by bioassay; 25 u./mgm. by furfural ultra-violet assay). These sulphates were converted to the respective free bases by dissolving them in water, raising the pH of 3-4 to 10-10.5 with a basic anionic exchange resin, e.g. "Amberlite IR-410," filtering off the resin and then lyophilizing the filtrate. These kanamycin A and B bases were demonstrated by paper-strip chromatography to be free of kanamycin B and A, respectively.

In another case, 10 g. of kanamycin (700 u./mgm. by bioassay; 110 u./mgm. by furfural ultra-violet assay) was converted to the salicylidene derivative and put through 300 transfers. The peak concentration of the tetra-salicylidene-kanamycin B was found in tube 63; found by analysis after drying at 100° C.: C, 61.95, 62.12; H, 6.02, 5.87%. The tetra-salicylidene-kanamycin B, which decomposed at 255 to 265° C., was converted to kanamycin B sulphate and then to kanamycin B base according to the previous procedure. The base was then recrystallized from 95% ethanol (1000 mcg./mgm. by bioassay; 0 mcg./mgm. by furfural ultra-violet assay).

Kanamycin B base decomposes at a considerably lower temperature than kanamycin A base; thus kanamycin B base darkens at 170° C. (corr.) and forms a dark gum on drying *in vacuo* at the boiling point of cymene (176° C.). Kanamycin B base exhibited $[\alpha]_D + 135^\circ$ ($c=0.63$ in water) and contained by analysis 44.69% of carbon and 7.48% of hydrogen and 12.65 to 13.62% of nitrogen after correction for the 10.3% loss in weight found on drying a sample at 100° C.

The wavelengths in microns of characteristic infra-red absorption maxima for kanamycin B base, as shown in the accompanying drawing, when pelleted in potassium bromide after drying at 137° C. for 15 hours *in vacuo* are as follows: 2.96, 3.44, 6.35, 6.48 (shoulder), 6.74, 6.85 (shoulder), 7.25, 7.45, 7.86, 8.08, 8.28, 8.76, 9.55, 9.65, 10.4 and 11.15.

About 2 g. of kanamycin B base was treated with 4 ml. of acetic anhydride in 20 ml. of methanol. After the mixture had stood overnight, crystals of product separated. Precipitation was completed by the addition of ether



anion

and the product, tetra-N-acetyl-kanamycin B, was washed with more ether, recrystallized from aqueous ethanol and found to darken at 180° C. but not to melt below 280° C. Found by analysis: C, 48.64, 48.66; H, 6.64, 6.73; N, 9.82, 9.79%.

Kanamycin A and kanamycin B were also separated and prepared in pure form by ion exchange resin column chromatography. A column 35 mm. in diameter and 870 mm. high was filled with 800 ml. of "Amberlite IRC-50" (200 mesh), a carboxylic acid type cation exchange resin, in the ammonium form and brought to equilibrium with 0.075 N

NH₄OH. A solution of 6 g. of kanamycin base (bioassaying 580 mcg./mgm.) in 120 ml. of water was passed slowly through the column followed by 0.075 N NH₄OH as the eluting agent. The eluate was collected at the rate of 250 to 300 ml. per 24 hours in test tubes holding 17 to 18 ml. The contents of each test tube were bioassayed on pH 8 trays. Consecutive trays having comparable activity were combined into "fractions," concentrated by distillation *in vacuo* and lyophilized to give solid kanamycin base, which was weighed and assayed. The results may be summarized as follows:

Fraction Number	Tube Numbers	Weight in mgm.	Potency in mcg./mgm.	
			By Bioassay	By furfural Ultra-violet Assay
I	29—44	19	30	184
II	971—1070	13	140	274
III	1071—1200	22	180	368
IV	1201—1500	39	350	512
V	1501—1700	43	450	654
VI	1701—2000	531	660	734
VII	2001—2300	16	370	594
VIII	2301—2600	36	320	204
IX	2601—3000	47	330	212
X	3001—3400	42	640	144
XI	3401—3600	55	1250	52
XII	3601—3940	548	1350	48
XIII	3941—4200	405	1150	46
XIV	4201—4500	175	1200	72
XV	4501— 3800	85	720	64
XVI	4801—5200	35	500	132
XVII	5201—5550	53	190	136

Thus many of the early fractions, and particularly Fraction VI, were kanamycin A base free of kanamycin B. Later fractions, and particularly Fractions XI, XII, XIII and XIV, were pure kanamycin B base. The absence of

kanamycin A from Fraction XII was confirmed by paper-strip chromatography.

The two forms of kanamycin react as follows in the indicated qualitative tests:

Test	A	B
Concentrated sulphuric acid	Colourless	Colourless
Molisch	Slow formation of colour	Rapid formation of colour
Elson-Morgan	Weakly positive	Weakly positive
Seliwanoff	Negative	—
Folin and Ciocalteu	Weakly positive	Weakly positive
Fishbach and Levin (HCl, Me ₂ CO)	Negative	Negative
Fishbach and Levin (HCl, H ₂ O)	Netative	—
Ninhydrin	Positive	
Reducing Sugar (ammoniacal silver nitrate)	Negative	
Sakaguchi	Negative	
Maltol	Negative	
Acid hydrolysis to "furfural"	Positive	Negative

Included within the scope of the present invention are acid addition salts of kanamycin B with organic and inorganic acids such as hydrochloric acid, sulphuric acid, hydrobromic acid, hydriodic acid, phosphoric acid, nitric acid, citric acid, maleic acid, malic acid, tartaric acid, benzoic acid, cinnamic acid, ascorbic acid, acetic acid, pantothenic acid, picric acid, phytic acid, *laevopimaric-6:8α-cis-endosuccinic* acid, sulphamic acid, glycolic acid and mandelic acid. For therapeutic purposes use is made of salts of non-toxic acids but salts of toxic acids, e.g. picric acid, are useful in isolation procedures, e.g. as precipitants from aqueous solutions, and for disinfectant purposes where toxicity is not important.

When desired for specific purposes and provided they are pharmaceutically compatible with Kanamycin B, there may be admixed with the compounds of the present invention other medicaments such as antihistamines, sulpha drugs (e.g. sulphadiazine, sulphabenzamide, sulphacetamide, sulphanilamide, sulphapyridine, sulphathiazole, sulphapyrazine, sulphaguanidine, sulphathalidine, sulphasuxidine, sulphisoxazole, sulphamylon, phthalylsulphacetamide, N¹-3:4-dimethylbenzoyl-sulphanilamide, benzylsulphanilamide and N¹-2-(2-quinoxalyl)sulphanilamide), lipotropic agents (particularly methionine, choline, inositol and beta-sitosterol and mixtures thereof), stimulants of the central nervous system (e.g. caffeine, amphetamines), local anaesthetics,

analgesics (e.g. aspirin, salicylamide, sodium gentisate, *p*-acetyl-aminophenol, phenacetin, codeine), laxatives (e.g. phenolphthalein), sedatives (e.g. barbiturates, bromides), salts of penicillin (e.g. potassium penicillin G, procaine penicillin G, 1-phenamine penicillin G, dibenzylamine penicillin G, other salts disclosed by U.S. Patent Specification No. 2,627,491; these combinations are particularly useful to enable variation of the pattern of blood levels obtained), phenoxymethylpenicillin and salts thereof, other antibiotic agents (e.g. streptomycin, dihydrostreptomycin, bacitracin, polymyxin, tyrothricin, erythromycin, chlorotetracycline, oxytetracycline, tetracycline, oleandomycin, chloramphenicol, Magnamycin (Registered Trade Mark), novobiocin, cycloserine, neomycin; in some cases such combinations attack a wider range of organisms or show synergistic efficacy or provide decreased toxicity with equal efficacy), vitamins (e.g. vitamins A, A₁, B₁, B₂, B₆, B₁₂ and members of that family, folic acid and members of that family, vitamins C, D₂, D₃ and E), hormones (e.g. cortisone, hydrocortisone, 9-α-fluorocortisone, 9-α-fluorohydrocortisone, prednisone and prednisolone), anabolic agents (e.g. 11:17-dihydroxy-9-α-fluoro-17-α-methyl-4-androsten-3-one; 17-α-ethyl-19-nortestosterone) and antifungal agents (e.g. mycostatin).

WHAT WE CLAIM IS:—

1. An improvement in or modification of the process claimed in Specification No.



italics



Vita -

Vitamin





Test	A	B
Concentrated sulphuric acid	Colourless	Colourless
Molisch	Slow formation of colour	Rapid formation of colour
Elson-Morgan	Weakly positive	Weakly positive
Seliwanoff	Negative	—
Folin and Ciocalteu	Weakly positive	Weakly positive
Fishbach and Levin (HCl, Me ₂ CO)	Negative	Negative
Fishbach and Levin (HCl, H ₂ O)	Netative	—
Ninhydrin	Positive	—
Reducing Sugar (ammoniacal silver nitrate)	Negative	—
Sakaguchi	Negative	—
Maltol	Negative	—
Acid hydrolysis to "furfural"	Positive	Negative

Included within the scope of the present invention are acid addition salts of kanamycin B with organic and inorganic acids such as hydrochloric acid, sulphuric acid, hydrobromic acid, hydriodic acid, phosphoric acid, nitric acid, citric acid, maleic acid, malic acid, tartaric acid, benzoic acid, cinnamic acid, ascorbic acid, acetic acid, pantothenic acid, picric acid, phytic acid, laevopimaric-6:8-*cis*-endosuccinic acid, sulphamic acid, glycolic acid and mandelic acid. For therapeutic purposes use is made of salts of non-toxic acids but salts of toxic acids, e.g. picric acid, are useful in isolation procedures, e.g. as precipitants from aqueous solutions, and for disinfectant purposes where toxicity is not important.

When desired for specific purposes and provided they are pharmaceutically compatible with Kanamycin B, there may be admixed with the compounds of the present invention other medicaments such as antihistamines, sulpha drugs (e.g. sulphadiazine, sulphabenzamide, sulphacetamide, sulphanilamide, sulphapyridine, sulphathiazole, sulphapyrazine, sulphaguanidine, sulphathalidine, sulphasuxidine, sulphisoxazole, sulphamylon, phthalylsulphacetamide, N¹-3:4-dimethylbenzoyl-sulphanilamide, benzylsulphanilamide and N¹-2-(2-quinoxalyl)sulphanilamide), lipotropic agents (particularly methionine, choline, inositol and beta-sitosterol and mixtures thereof), stimulants of the central nervous system (e.g. caffeine, amphetamines), local anaesthetics,

analgesics (e.g. aspirin, salicylamide, sodium gentisate, *p*-acetyl-aminophenol, phenacetin, codeine), laxatives (e.g. phenolphthalein), sedatives (e.g. barbiturates, bromides), salts of penicillin (e.g. potassium penicillin G, procaine penicillin G, 1-ephenamine penicillin G, dibenzylamine penicillin G, other salts disclosed by U.S. Patent Specification No. 2,627,491; these combinations are particularly useful to enable variation of the pattern of blood levels obtained), phenoxymethylpenicillin and salts thereof, other antibiotic agents (e.g. streptomycin, dihydrostreptomycin, bacitracin, polymyxin, tyrothricin, erythromycin, chlorotetracycline, oxytetracycline, tetracycline, oleandomycin, chloramphenicol, Magnamycin (Registered Trade Mark), novobiocin, cycloserine, neomycin; in some cases such combinations attack a wider range of organisms or show synergistic efficacy or provide decreased toxicity with equal efficacy), vitamins (e.g. vitamins A, A₁, B₁, B₂, B₆, B₁₂ and members of that family, folic acid and members of that family, vitamins C, D₂, D₃ and E), hormones (e.g. cortisone, hydrocortisone, 9- α -fluorocortisone, 9- α -fluorohydrocortisone, prednisone and prednisolone), anabolic agents (e.g. 11:17-dihydroxy-9- α -fluoro-17- α -methyl-4-androsten-3-one; 17- α -ethyl-19-nortestosterone) and antifungal agents (e.g. mycostatin).

WHAT WE CLAIM IS:—

1. An improvement in or modification of the process claimed in Specification No.



italics



Vita-

Vitamins



- 817,385 which comprises separating from the kanamycin produced by the process claimed in Specification No. 817,385 an antibiotic substance, herein designated kanamycin B, which
- 5 substance exhibits $[\alpha]_D + 135^\circ$ ($c=0.63$ in water) contains carbon (44.69%), hydrogen (7.48%) and nitrogen (12.65 to 13.62%) gives a salicylidene derivative which decomposes without melting at 255 to 265°C., fails to give
- 10 a product with an ultra-violet absorption spectrum identical to that of furfural on treatment with 40% sulphuric acid for 100 minutes at 100°C. and exhibits an infra-red absorption spectrum having characteristic absorption
- 15 maxima at the following wavelengths in microns: 2.96, 3.44, 6.35, 6.48 (shoulder), 6.74, 6.85 (shoulder), 7.25, 7.45, 7.86, 8.08, 8.28, 8.76, 9.55, 9.65, 10.4 and 11.15.
2. A process as claimed in claim 1 wherein
- 20 the medium to which a substantially antibiotic activity is imparted is treated by adding sodium dodecylbenzenesulphonate in order to precipitate kanamycin B dodecylbenzenesulphonate, the precipitated sulphonate is separated and dissolved in methanol, sulphuric
- 25 acid is added thereto, and the resulting kanamycin B sulphate is collected.
3. A process as claimed in claim 1 wherein the medium to which a substantially antibiotic
- 30 activity is imparted is treated with salicylaldehyde to convert kanamycin to its tetra-salicylidene derivative, the salicylidene derivative is isolated, and the salicylidene derivative of kanamycin B is separated from the salicyli-
- 35 dene derivative of kanamycin A by counter current distribution techniques using the

solvent system methanol-water-chloroform-benzene.

4. A process as claimed in claim 1 wherein Kanamycin B is separated from the medium to which a substantially antibiotic activity is imparted by ion exchange resin column chromatography employing a carboxylic acid type cation exchange resin and dilute aqueous ammonia as eluting agent.

5. The antibiotic substance as herein designated kanamycin B, which exhibits $[\alpha]_D + 135^\circ$ ($c=0.63$ in water) contains carbon (44.69%), hydrogen (7.48%) and nitrogen (12.65 to 13.62%) gives a salicylidene derivative which decomposes without melting at 255 to 265°C., fails to give a product with an ultra-violet absorption spectrum identical to that of furfural on treatment with 40% sulphuric acid for 100 minutes at 100°C. and exhibits an infra-red absorption spectrum having characteristic absorption maxima at the following wavelengths in microns: 2.96, 3.44, 6.35, 6.48 (shoulder), 6.74, 6.85 (shoulder), 7.25, 7.45, 7.86, 8.08, 8.28, 8.76, 9.55, 9.65, 10.4 and 11.15.

6. An acid addition salt of kanamycin B.

7. Kanamycin B sulphate.

8. A process for the production of a new antibiotic herein designated kanamycin B substantially as hereinbefore described with reference to any one of the procedures of the Example.

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Published by The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.



* C L M S *