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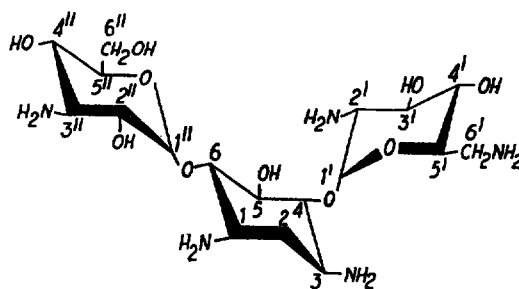
(54) IMPROVED PROCESS FOR THE PRODUCTION OF AN ANTIBIOTIC SUBSTANCE 2'-AMINO-2'-DEOXY-KANAMYCIN

(71) We, MEIJI SEIKA KAISHA, LIMITED, a Japanese Company of No. 8, 2-chome, Kyobashi, Chuo-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 processes for the production of an antibiotic substance kanamycin B, namely 2'-amino-2'-deoxy-kanamycin, in improved yield. More particularly, this invention relates to processes for the production of 2'-amino-2'-deoxy-kanamycin by fermentation and methods for its recovery and purification.

2'-Amino-2'-deoxy-kanamycin is effective in inhibiting the growth of Gram-positive, Gram-negative and acid-fast bacteria such as *Staphylococcus*, *Pseudomonas*, *Salmonella*, *Shigella* and *Mycobacterium* as well as pathogenic bacteria which are resistant to the known antibiotics and synthetic chemotherapeutic agents. This 2'-amino-2'-deoxy-kanamycin is substantially of very low toxicity and particularly of little ototoxicity, and it exhibits a useful therapeutic effect on the infections of Gram-positive, Gram-negative and acid-fast bacteria in human beings and animals.

2'-Amino-2'-deoxy-kanamycin has the empirical formula $C_{18}H_{37}N_5O_{10} \cdot H_2O$ and may be represented by the structural formula:



When the micro-organism *Streptomyces kanamyceticus* is cultivated under aerobic conditions kanamycin A is produced in the culture broth occasionally together with kanamycin B and kanamycin C (British Patents Nos. 817,385 and 925,790). However, it has been found difficult to obtain kanamycin B in sufficient quantities for commercial purposes.

We have now isolated kanamycin B, i.e. 2'-amino-2'-deoxy-kanamycin, in sufficient amounts to investigate its properties and carry out clinical trials. These trials confirmed that 2'-amino-2'-deoxy-kanamycin is a valuable antibiotic in the treatment of infectious diseases.

As a result of our further investigations, we have found that the proportion of 2'-amino-2'-deoxy-kanamycin produced and accumulated in the culture may be improved remarkably and may be recovered therefrom in a high yield, when a strain of *Streptomyces kanamyceticus* (ATCC 21252) is cultured in a conventional manner but in the presence of an amino-glucose, paromamine, deoxystreptamine-O-kanosaminide, neamine, the antibiotic NK—1001 (as hereinafter defined) or the antibiotic NK—1003 (as hereinafter defined).

According to the present invention there is provided a process for the production of 2'-amino-2'-deoxy-kanamycin in improved yield, which comprises cultivating a strain of *Streptomyces Kanamyceticus* (ATCC 21252) under aerobic conditions in a culture medium containing assimilable sources of carbon and nitrogen in the presence of an amino-glucose, paromamine, deoxystreptamine-O-kanosaminide, neamine, the antibiotic NK—1001 (as hereinafter defined) or the antibiotic NK—1003 (as hereinafter defined) until 2'-amino-2'-deoxy-kanamycin accumulates in a significant amount in the culture broth, and then recovering said 2'-amino-2'-deoxy-kanamycin from the culture broth.

Examples of amino-glucoses which may be used in the process according to the present invention are D-glucosamine, 3-amino-3-deoxy-D-glucose, 6-amino-6-deoxy-D-glucose and 2,6-di-deoxy-2,6-amino-D-glucose. As previously stated additives other than the amino glucoses are paromamine (namely 4-(2-D-glucosaminyl)-alpha-deoxy-streptamine); deoxystreptamine-O-kanosaminide; neamin (namely, 4-(2,6-di-deoxy-2,6-di-amino-glucosyl)-alpha-deoxy-streptamine); antibiotic NK—1001 (a compound of 6-amino-6-deoxy-D-glucose, deoxystreptamine and glucose) and antibiotic NK—1003 [5-O-(6-amino-6-deoxy- α -D-glucosyl)-deoxystreptamine]. (The above-mentioned antibiotics NK—1001 NK—1003 are antibiotic substances described and claimed in our co-pending British patent application Nos. 28147/68 (Serial No. 1,181,623) 28148/68 (Serial No. 1,224,379) respectively.

In a preferred embodiment of the invention 2'-amino-2'-deoxy-kanamycin is recovered from the culture by treating the culture broth or filtrate thereof with a cation-exchange resin to adsorb thereon 2'-amino-2'-deoxy-kanamycin together with kanamycin A, eluting the cation-exchange resin with aqueous ammonia, collecting the active fractions and treating them with an anion-exchange resin, eluting the anion-exchange resin chromatographically with aqueous ammonia, collecting separately the fractions containing 2'-amino-2'-deoxy-kanamycin and the fractions containing kanamycin A, concentrating and freeze-drying the fractions containing 2'-amino-2'-deoxy-kanamycin to form a powder, dissolving the powder in water, treating the resultant aqueous solution with a cation-exchange resin, eluting the second mentioned cation-exchange resin chromatographically with aqueous ammonia, the first fraction containing traces of kanamycin A being discarded, the subsequent fractions containing 2'-amino-2'-deoxy-kanamycin being collected and the 2'-amino-2'-deoxy-kanamycin recovered therefrom.

In another embodiment of the invention 2'-amino-2'-deoxy-kanamycin is recovered from the culture by filtering the culture, adjusting the resultant filtrate to pH 10 by addition of an alkali metal hydroxide, extracting the filtrate with n-butanol containing benzaldehyde, mixing the resulting extract with water, adjusting the mixture to pH² by addition of sulfuric acid, agitating the mixture to extract the water-soluble basic antibiotics into the aqueous phase, separating the aqueous phase, treating the aqueous phase with an anion-exchange resin to adsorb 2'-amino-2'-deoxy-kanamycin and kanamycin A, eluting the anion-exchange resin chromatographically with aqueous ammonia, collecting separately the fractions containing 2'-amino-2'-deoxy-kanamycin and the fractions containing kanamycin A, concentrating and freeze-drying the fractions containing 2'-amino-2'-deoxy-kanamycin to form a powder, dissolving the powder in water, treating the resultant aqueous solution with a cation-exchange resin, eluting the cation-exchange resin chromatographically with aqueous ammonia, the first fraction containing traces of kanamycin A being discarded, the subsequent fractions containing 2'-amino-2'-deoxy-kanamycin being collected and the 2'-amino-2'-deoxy-kanamycin recovered therefrom.

In the process according to the present invention, the aforesaid additives may be added to the culture medium before the cultivation of the *Streptomyces* is started, or the additives may be fed into the culture medium during the cultivation. The proportion of the additive present in the culture medium is not critical but may be 0.05 to 1.0% by weight, and preferably 0.1 to 0.5% by weight of the culture medium. It has been found that the quantity of the anti-biotic 2'-amino-2'-deoxy-kanamycin produced and accumulating in the culture reaches maximum at the end of 3 to 7 days after the beginning of the cultivation.

In addition to the above-mentioned fact that the strain of *Streptomyces kanamyceticus* ATCC 21252 exhibits a higher capacity of producing 2'-amino-2'-deoxy-kanamycin when cultured in the presence of the aforesaid additives, we have further discovered as described in our copending Application No. 3492/71 (Serial No. 1,244,042) that some new mutants of *Streptomyces kanamyceticus* which we have derived from the strain ATCC 21252 of *Streptomyces kanamyceticus* can exhibit a high ability to produce 2'-amino-2'-deoxy-kanamycin when they are cultivated in a conventional culture medium in the absence of the aforesaid additives.

In the process according to the present invention, cultivation may generally be carried out in a usual manner as in the known methods of cultivating actinomycetes. The culture medium containing known kinds of nutritional sources for actinomycetes are useful for the production of 2'-amino-2'-deoxy-kanamycin. Thus, the culture medium may contain sources of carbon such as starch, sucrose, maltose, glucose and glycerol and sources of nitrogen such as soybean meal, soluble vegetable proteins, corn steep liquor, peptone, meat extract, nitrates and ammonium salts. The culture medium may also contain other appropriate inorganic salts such as NaCl, MgSO₄ and other materials which will promote the growth of *Streptomyces kanamyceticus*, if necessary.

For the production of 2'-amino-2'-deoxy-kanamycin, cultivation on solid medium is possible, but for its production in large quantity it is preferred to carry out the cultivation in a liquid medium under aerobic conditions. For the production of the antibiotic on a large scale it is much more preferable to employ a method of deep aerated submerged cultivation. It is possible to use shake-cultivation or aerated agitated cultivation in a liquid medium. The cultivation temperature usually may be between 25° and 35° C. and preferably at a temperature of 28° C. or in the vicinity thereof. It has been found that the amount of 2'-amino-2'-deoxy-kanamycin accumulating in the culture becomes maximum at the end of 3 to 7 days after the beginning of the cultivation.

2'-Amino-2'-deoxy-kanamycin which has accumulated in the culture may be recovered therefrom by any of the known methods e.g. using ion-exchange resins, extraction with organic solvents, or the use of a precipitating agent suitable for the purification of water-soluble, basic antibiotics. For the commercial production of 2'-amino-2'-deoxy-kanamycin, it is preferred to recover this by adsorption on and elution from an ion-exchanger, preferably a carboxylic acid type cation-exchange resin, or by extraction with an organic solvent containing an appropriate carrier such as para-toluene sulfonic acid or lauric acid. For instance, 2'-amino-2'-deoxy-kanamycin may be recovered by filtering off the solids such as mycelium cake from the fermented broth containing 2'-amino-2'-deoxy-kanamycin, subjecting the filtrate to an adsorption treatment with "Amberlite" (Registered Trade Mark) IRC-50, eluting with aqueous ammonia, collecting and concentrating the active fractions, subjecting the concentrated fraction to chromatographic separation with "Dowex" (Registered Trade Mark) 1 × 2, and collecting and concentrating the active fractions. The concentrated fraction is again subjected to a chromatographic separation by adsorbing on "Amberlite" CG-50 and gradient-eluting with water-aqueous ammonia. A single fraction containing the active compound, 2'-amino-2'-deoxy-kanamycin is thus obtained. This fraction is freeze-dried and the resulting powder is crystallised from water-dimethyl formamide to yield crystals of 2'-amino-2'-deoxy-kanamycin.

2'-Amino-2'-deoxy-kanamycin may be obtained in the form of the base as colorless needle-like crystals having a melting point of 183—186° C. soluble in water, aqueous methanol, aqueous ethanol, and aqueous acetone but insoluble in absolute methanol, absolute ethanol, ethyl acetate, butyl acetate, ethyl ether, chloroform and benzene, giving positive reaction to ninhydrin, Elson-Morgan and Molisch reagents but negative reaction to Sakaguchi, Tollens, Fehling, Millon and Benedict reagents. An aqueous 1% solution of this substance shows a specific rotation $[\alpha]_D^{21} = +118^\circ$. An N-acetylate obtained by acetylating 2'-amino-2'-deoxy-kanamycin with methanol-acetic anhydride has the empirical formula $C_{18}H_{32}N_5O_{10} \cdot 5(CH_3CO)_2O \cdot H_2O$, melts at 290—300° C. with decomposition and gives a specific rotation of $[\alpha]_D^{21} = +118^\circ$.

The antibacterial activity of 2'-amino-2'-deoxy-kanamycin is heat-stable. When a solution of the 2'-amino-2'-deoxy-kanamycin in 6N-hydrochloric acid has been degraded by heating at 100° C. for 40 minutes, the antibacterial activity against *Bacillus subtilis* of the degraded solution was 60—80% of that of the initial solution. When degraded solution is applied in the form of a spot on a sheet of 40 cm. × 40 cm. of Toyo filter paper No. 50 and then developed upwards with a solvent consisting

of butanol-acetic acid-water (4:2:1) there occur two spots which give a positive reaction to ninhydrin and one of the spots is also positive in the reaction to ammoniacal silver nitrate.

5 2'-Amino-2'-deoxy-kanamycin exhibits high and useful antibacterial activity against Gram-positive, Gram-negative and acid-fast bacteria as well as against such bacteria and strains which are resistant to kanamycin A, other known antibiotics and chemotherapeutic agents. Toxicity of 2'-amino-2'-deoxy-kanamycin is very low, as its LD₅₀ is only 190 mg/kg. in mice (intravenous injection).

10 The antibacterial spectrum of 2'-amino-2'-deoxy-kanamycin is tabulated in Table 1 below.

TABLE 1

	Micro-organisms tested	Minimum concentration for complete inhibition in mcg/ml.	
15	<i>Aerobacter aerogenes</i> IAM 1102	0.15	15
	<i>Alcaligenes faecalis</i>	0.031	
	<i>Bacillus agri</i>	0.078	
	<i>Bacillus subtilis</i> ATCC 6633	0.039	
	<i>Bacillus subtilis</i> PCI 219	0.078	
20	<i>Diplococcus pneumoniae</i> Type 311D	0.31	20
	<i>Escherichia coli</i> IAM 1253	1.25	
	<i>Klebsiella pneumoniae</i> 607	0.31	
	<i>Lactobacillus arabinosus</i> ATCC 8014	1.56	
	<i>Lactobacillus fermenti</i> ATCC 9338	0.15	
25	<i>Staphylococcus albus</i> PCI 1200A	0.039	25
	<i>Staphylococcus aureus</i> 209P	0.31	
	<i>Staphylococcus aureus</i> Terashima	0.015	
	<i>Mycobacterium phlei</i> No. 56	0.62	
	<i>Mycobacterium phlei</i> Sekiguchi	0.31	
30	<i>Mycobacterium</i> 607	0.78	30
	<i>Proteus vulgaris</i>	7.8	
	<i>Pseudomonas aeruginosa</i> IAM 1007	0.1	
	<i>Pseudomonas aeruginosa</i> Takasaki	0.78	
	<i>Pseudomonas aeruginosa</i> Masakuro	0.39	
35	<i>Salmonella paratyphi</i> A	0.62	35
	<i>Salmonella paratyphi</i> B	0.62	
	<i>Salmonella paratyphi</i> C	1.25	
	<i>Sarcina lutea</i> PCI 1001	0.31	
	<i>Saccharomyces cerevisiae</i> IAM 4626	500	
40	<i>Shigella flexneri</i> EW 10 2a	0.039	40
	<i>Streptococcus faecalis</i> ATCC 8043	15.6	
	<i>Staphylococcus aureus</i> 209P (resistant to streptomycin)	2.5	
45	<i>Staphylococcus aureus</i> 209P (resistant to erythromycin)	0.31	45
	<i>Escherichia coli</i> K-12 CS-2	0.31	
	<i>Escherichia coli</i> K-12 CS-2 (resistant to antibiotics)	1.56	

In the attached drawings:

50 Figure 1 shows a curve of the ultra-violet absorption spectrum of 2'-amino-2'-deoxy-kanamycin dissolved in water at a concentration of 1 mg/ml. of water.

Figure 2 shows a curve of the infra-red absorption spectrum of 2'-amino-2'-deoxy-kanamycin in Nujol.

55 Referring to Figures 1 and 2, it is noted that 2'-amino-2'-deoxy-kanamycin does not exhibit absorption of ultra-violet light from 220-millimicrons to 340 milli-microns but exhibits characteristic infra-red absorption bands at the following wave numbers in cm⁻¹: 3600, 3000, 1650, 1600, 1570, 1140, 1110, 1095, 1090, 1060, 1035, 960, 900, 895, 880, 845, 815, 790, 780, 765 and 725.

60 The process of the present invention is now illustrated with reference to the following Examples.

EXAMPLE 1:

A liquid culture medium (15 l.) containing 3.0% starch, 2.0% maltose, 2.5% soybean meal, 1.2% sodium nitrate and 0.5% sodium chloride was inoculated with *Streptomyces kanamyceticus* (ATCC 21252) and incubated at 28° C. under aeration and agitation. After 22 hours incubation, 50 g. of 3-amino-glucose were added to the culture medium and the incubation was continued. The quantity of 2'-amino-2'-deoxy-kanamycin produced in the culture reached maximum at the end of 150 hours.

The culture liquid was then filtered and the filtrate was treated with 4 l. of "Amberlite" IRC-50 (ammonium type). The antibiotics adsorbed were then eluted from the resin using 0.5 N aqueous ammonia. The active fractions were collected and concentrated to a syrup. The syrup was dissolved in 500 ml. of water and the solution was decolorised by treating with 6 g. of active carbon. The solution was filtered to remove the active carbon, and the filtrate was subjected to chromatographic elution from 600 ml. of "Dowex" 1 × 2 (OH type). The fractions containing 2'-amino-2'-deoxy-kanamycin were eluted first before the separation of kanamycin A. The fractions containing high concentration of 2'-amino-2'-deoxy-kanamycin were collected, concentrated and freeze-dried to give 2.2 g. of a crude powder of 2'-amino-2'-deoxy-kanamycin. This crude powder (2.2 g.) was dissolved in 50 ml. of water and the solution was treated with "Amberlite" CG-50 (ammonia type). The resin was eluted chromatographically with 0.1 N to 0.5 N aqueous ammonia and the co-existing small amount of kanamycin A being eluted out first. 2'-Amino-2'-deoxy-kanamycin was thereafter eluted separately as a single fraction which was then concentrated and freeze-dried to give a crude powder. Recrystallisation of the powder from water-dimethylformamide gave 1.1 g. of 2'-amino-2'-deoxy-kanamycin in the form of colorless needle-like crystals. An elementary analysis showed that this product contained 42.62% C; 7.52% H and 12.30% N (found values). The molecular weight of this product was found to be 485 when determined.

EXAMPLE 2:

A liquid culture medium (15 l.) having the same composition as in Example 1 was inoculated with the same strain of *Streptomyces kanamyceticus* used in Example 1 and then incubated at 28° C. under aeration and agitation. After 22 hours incubation, 50 g. of 6-amino-glucose were added to the culture medium and the incubation was continued. The quantity of 2'-amino-2'-deoxy-kanamycin accumulating in the culture reached maximum at the end of 145 hours after the beginning of incubation.

The culture liquid was then treated and the crude powder obtained was purified in the same manner as in Example 1 to yield 1.0 g. of a crystalline form of 2'-amino-2'-deoxy-kanamycin.

EXAMPLE 3

A liquid culture medium (15 l.) containing 3.0% starch, 2.5% soybean meal, 1.2% sodium nitrate and 0.35% sodium chloride was inoculated with the same strain of *Streptomyces kanamyceticus* used in Example 1 and then incubated at 28° C. under aeration and agitation. After 20 hours incubation, 30 g. of the antibiotic NK-1001 were added to the culture medium and the incubation was further continued. The production of 2'-amino-2'-deoxy-kanamycin reached maximum at the end of 140 hours of incubation.

The culture liquid was then treated and the crude powder obtained was purified similarly to Example 1 to give 2.5 g. of a crystalline form of 2'-amino-2'-deoxy-kanamycin.

EXAMPLE 4

A liquid culture medium (15 l.) containing 3.0% starch, 2.5% soya meal, 1.2% sodium nitrate and 0.35% sodium chloride was inoculated with the same strain of *Streptomyces kanamyceticus* as one used in Example 1 and then incubated at 28° C. under aeration and agitation. After 20 hours incubation, 30 g. of the antibiotic NK-1003 were added to the culture medium and the incubation was continued. The quantity of 2'-amino-2'-deoxy-kanamycin accumulating in the culture reached maximum at the end of 140 hours of incubation.

The culture liquid was then treated and the crude powder obtained was purified in the same manner as in Example 1 to yield 1.5 g. of a crystalline form of 2'-amino-2'-deoxy-kanamycin.

EXAMPLE 5

A liquid culture medium (15 l.) having the same initial composition as used in Example 3 was inoculated with the same strain of *S. kanamyceticus* used as in Example 1 and then incubated at 28° C. under aeration and agitation. After 20 hour incubation, 45 g. of neamin were added to the culture medium and the incubation was further continued. The quantity of 2'-amino-2'-deoxy-kanamycin accumulating in the culture reached maximum at the end of 140 hours of incubation.

The culture broth was then treated and the crude powder obtained was purified similarly to Example 1 to yield 1.2 g. of a crystalline form of 2'-amino-2'-deoxy-kanamycin.

EXAMPLE 6

A liquid culture medium (15 l.) containing 3.0% starch, 2.5% soybean meal, 1.2% sodium nitrate and 0.35% sodium chloride was sterilised, cooled and admixed with a sterilised and cooled solution of 30 g. of the antibiotic NK—1001 in water. The resulting mixture was inoculated with the same strain of *S. kanamyceticus* as used in Example 1 and then incubated at 28° C. under aeration and agitation. The quantity of 2'-amino-2'-deoxy-kanamycin accumulating in the culture reached maximum at the end of 140 hours of incubation.

The culture broth was then treated and the crude powder obtained was purified in the same manner as in Example 1 to give about 2.2 g. of a crystalline form of 2'-amino-2'-deoxy-kanamycin.

EXAMPLE 7

The cultivation of *Streptomyces kanamyceticus* (ATCC 21252) was carried out in the presence of the antibiotic NK—1001 in the same way as in Example 3. The resulting culture liquid was filtered and the filtrate (14 l.) was adjusted to pH 10 by addition of sodium hydroxide. The filtrate was then extracted with one fourth volume of n-butanol which contained 2 g. of benzaldehyde per one gram of the estimated quantity of water-soluble basic antibiotics present in the filtrate. The n-butanol extract was removed and mixed with one tenth volume of water. When the resulting mixture was adjusted to pH 2.0 by addition of sulphuric acid and agitated, the water-soluble basic antibiotics were extracted into the water phase. The resultant solution of the antibiotics in water was decolorised with active carbon and subjected to chromatography with "Dowex" 1 × 2 (OH type) and to chromatography with Amberlite CG—50 (ammonium type) in a similar way to Example 1. When the crude powder obtained was recrystallised from water and dimethyl-formamide, 1.1 g. of 2'-amino-2'-deoxy-kanamycin was obtained in the form of colorless, needle-like crystals.

EXAMPLE 8 (COMPARATIVE)

In this Example, a series of tests was carried out in order to demonstrate the unexpected and advantageous effect of addition of the amino-glucoses and their deoxy-streptamine compounds on the production of 2'-amino-2'-deoxy-kanamycin according to the present invention, as compared to its production when no such additives are provided.

In these tests, the cultivation of *Streptomyces kanamyceticus* (ATCC 21252) was made at 28°C. for 150 hours in the same way as in Example 1 using similar fermentation vessels and culture media. After various periods of time as indicated below had elapsed from the beginning of the cultivation, different amino-glucoses and their deoxystreptamine compounds as indicated below were added to the culture media. After the cultivation had proceeded for a total of 150 hours, the culture broths were treated in the same way as in Example 1, so that as much 2'-amino-2'-deoxy-kanamycin and kanamycin A as possible could be recovered therefrom. Recovered amounts of the products and details of tests are shown in Table 2 below.

TABLE 2

Experiment No.	Additive	Quantity of additive added (g)	Time of addition from the start (hr.)	Final Content in culture of 2'-amino-2'-deoxy-kanamycin (mcg/ml.)	Yield of 2'-amino-2'-deoxy-kanamycin (g)	Final Content in culture of kanamycin A (mcg/ml.)	Yield of kanamycin A (g)
1	No addition	—	—	26	0.12	2790	26.8
2	3-Amino-glucose	50	22	240	1.10	1480	12.7
3	6-Amino-glucose	50	22	225	1.00	1730	15.2
4	Antibiotic NK—1001	30	20	575	2.50	1240	11.2
5	Antibiotic NK—1001	30	0	480	2.20	1450	13.6
6	Antibiotic NK—1003	30	20	345	1.50	1700	16.0
7	Neamine	45	20	260	1.20	1390	12.8

As will be seen from the results as shown in Table 2, the addition of the amino-glucoses and their deoxystreptamine compounds to the culture medium clearly leads to a remarkable improvement in the yield of 2'-amino-2'-deoxy-kanamycin, which, in turn, indicates that the amount of 2'-amino-2'-deoxy-kanamycin as produced and accumulated in the culture is increased considerably according to the present invention.

WHAT WE CLAIM IS:—

1. A process for the production of 2'-amino-2'-deoxy-kanamycin in improved yield, which comprises cultivating a strain of *Streptomyces kanamyceticus* (ATCC 21252) under aerobic conditions in a culture medium containing assimilable sources of carbon and nitrogen, in the presence of an amino-glucose, paromamine, deoxy-streptamine-O-kanosaminide, neamine, the antibiotic, NK—1001 as hereinbefore defined or the antibiotic NK—1003 as hereinbefore defined, until 2'-amino-2'-deoxy-kanamycin accumulates in a significant amount in the culture broth, and then recovering 2'-amino-2'-deoxy-kanamycin from the culture broth.
2. A process as claimed in Claim 1 in which the amino-glucose is 3-amino-glucose or 6-amino-glucose.
3. A process as claimed in Claim 1 or Claim 2, wherein 2'-amino-2'-deoxy-kanamycin is recovered by treating the culture broth or filtrate thereof with a cation-exchange resin to adsorb thereon 2'-amino-2'-deoxy-kanamycin together with kanamycin A, eluting the cation-exchange resin with aqueous ammonia, collecting the active fractions and treating them with an anion-exchange resin, eluting the anion-exchange resin chromatographically with aqueous ammonia, collecting separately the fractions containing 2'-amino-2'-deoxy-kanamycin and the fractions containing kanamycin A, concentrating and freeze-drying the fractions containing 2'-amino-2'-deoxy-kanamycin to form a powder, dissolving the powder in water, treating the resultant aqueous solution with a cation-exchange resin, eluting the second mentioned cation-exchange resin chromatographically with aqueous ammonia, the first fraction containing traces of kanamycin A being discarded, the subsequent fractions containing 2'-amino-2'-deoxy-kanamycin being collected and the 2'-amino-2'-deoxy-kanamycin recovered therefrom.
4. A process as claimed in claim 1 or claim 2, wherein 2'-amino-2'-deoxy-kanamycin is recovered from the culture by filtering the culture, adjusting the resultant filtrate to pH 10 by addition of an alkali metal hydroxide, extracting the filtrate with *n*-butanol containing benzaldehyde, mixing the resulting extract with water, adjusting the mixture to pH 2 by addition of sulfuric acid, agitating the mixture to extract the water-soluble basic antibiotics into the aqueous phase, separating the aqueous phase, treating the aqueous phase with an anion-exchange resin to adsorb 2'-amino-2'-deoxy-kanamycin and kanamycin A, eluting the anion-exchange resin chromatographically with aqueous ammonia, collecting separately the fractions containing 2'-amino-2'-deoxy-kanamycin and the fractions containing kanamycin A, concentrating and freeze-drying the fractions containing 2'-amino-2'-deoxy-kanamycin to form a powder, dissolving the powder in water, treating the resultant aqueous solution with a cation-exchange resin, eluting the cation-exchange resin chromatographically with aqueous ammonia, the first fraction containing traces of kanamycin A being discarded, the subsequent fractions containing 2'-amino-2'-deoxy-kanamycin being collected and the 2'-amino-2'-deoxy-kanamycin recovered therefrom.
5. A process for the production of 2'-amino-2'-deoxy-kanamycin substantially as hereinbefore described with reference to any one of Examples 1 to 7.
6. 2'-amino-2'-deoxy-kanamycin when produced by a process as claimed in any preceding claim.

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