

PATENT SPECIFICATION

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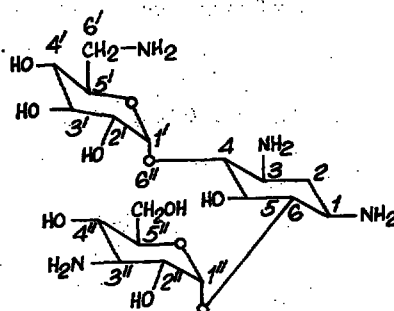


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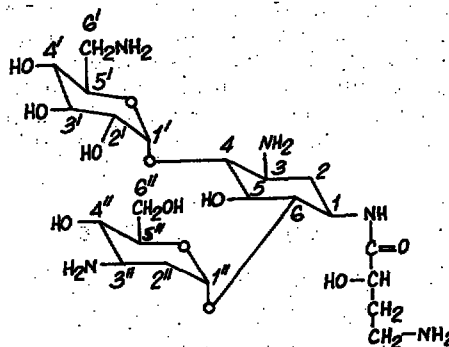
(54) PROCESS FOR THE PRODUCTION OF A KANAMYCIN DERIVATIVE

(71) We, BRISTOL-MYERS COMPANY, incorporated under the laws of the State of Delaware, United States of America, of 345 Park Avenue, New York, N.Y. 10022, United States of America, 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:—

Kanamycin A is a known antibiotic described in Merck Index, 8th Edition, pp. 597—598. Kanamycin A is a compound having the formula



This invention relates to a new and improved process for the preparation of 1-[L-(-)-γ-amino-α-hydroxybutyryl]-kanamycin A (IV), which compound has the formula



Kanamycin A possesses four primary amine functions at the 1, 3, 6' and 3'' positions of the molecule. It has been established that the 6'-amine function is the most reactive and the 1-amine function is second most reactive when treated with an electrophilic agent. Both the 3 and 3'' positions are less reactive than either the 1- or 6'-amine functions but do react to give low percentages of undesired acylated materials.

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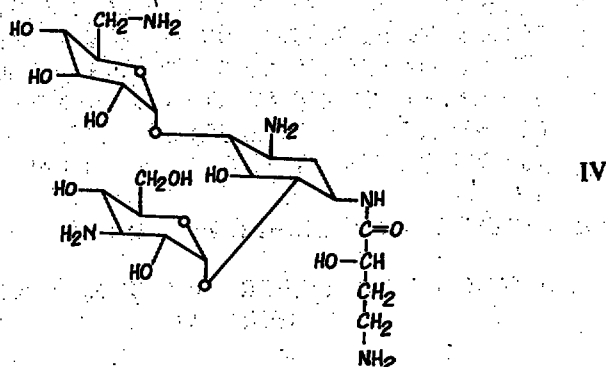
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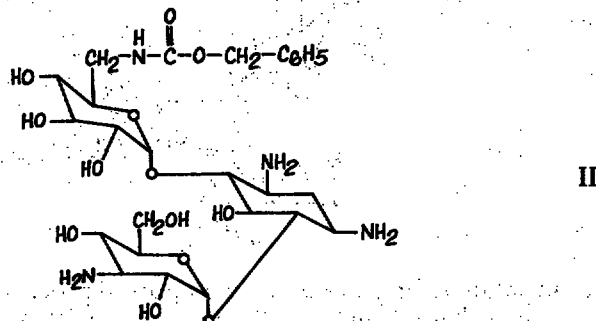
Accordingly, it was the object of the present invention to discover a more satisfactory process than that previously employed and described in our British Patent Specification No. 1,401,221, said process being more selectively directed to acylation of the 1-amine function than to the other amine functions found in the molecule.

The objective of the present invention has been achieved by the process of preparing the compound having the formula

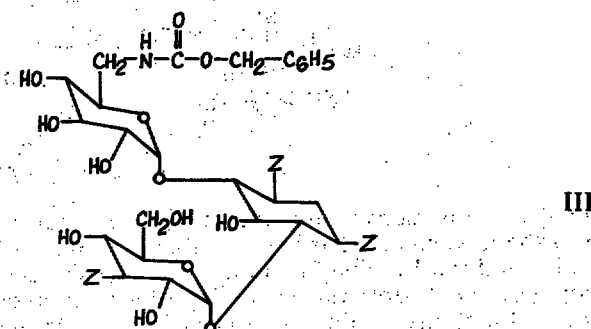


which process comprises the consecutive steps of

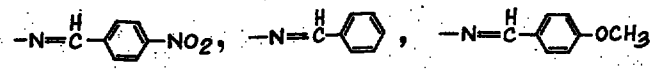
A.) acylating one mole of kanamycin A with one mole or less of N-(benzyloxy-carbonyloxy)-succinimide, but preferably about one mole, in a solvent, preferably selected from dimethylformamide, dimethylacetamide, tetrahydrofuran, dioxane, 1,2-dimethoxyethane, methanol, ethanol, water, acetone, pyridine, an N-(lower)-alkylpiperidine, and mixtures thereof, but preferably dimethylformamide, at a temperature below 50°C., and preferably below 25°C. to produce the compound having the formula



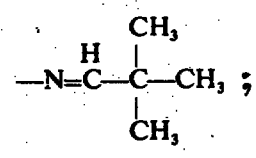
B.) treating one mole of compound II with at least three moles of an aldehyde selected from 4-nitrobenzaldehyde, benzaldehyde, 4-methoxybenzaldehyde and pivaldehyde, in a (lower) alkanol such as absolute ethanol, methanol, n-propanol, isopropanol, n-butanol, sec-butanol, tert-butanol, or mixtures thereof, at elevated temperatures, e.g. at about reflux temperature, for a period of at least two, preferably two to five, hours, to produce the compound having the formula



in which Z is



or



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and

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C.) treating one mole of compound III with at least one mole of L-(-)-p-benzyloxycarbonylamino- α -hydroxybutyric acid N-hydroxy-succinimide ester, but preferably in a ratio of about one to two moles of ester per mole of compound III, and most preferably at a molar ratio of about 1.0 to 1.3:1, in an organic solvent preferably selected from dimethylformamide, tetrahydrofuran, dimethylacetamide, propyleneglycol dimethyl ether, ethylene glycol dimethyl ether and dioxane, but preferably dimethylformamide, at a temperature in the range of 0°C. to 50°C. but preferably at about room temperature, preferably for a period of time till the acylation is complete, but preferably for at least five hours; then removing the solvent and hydrogenating the residue, e.g. *in situ* in the presence of a metal catalyst preferably selected from palladium, platinum, Raney nickel, rhodium, ruthenium and nickel, but preferably palladium, and more preferably palladium on charcoal, in a water/water-miscible solvent system, preferably selected from water and dioxane, tetrahydrofuran, ethyleneglycol dimethyl ether and propyleneglycol dimethyl ether, but preferably 1:1 water-dioxane, and preferably in the presence of a catalytic amount of glacial acetic acid, to produce the compound of formula IV.

The invention also includes Compound IV when prepared by this process and pharmaceutical compositions comprising compound IV when so prepared together with an inert pharmacologically-acceptable carrier.

Compound IV, 1-[L-(-)- γ -amino- α -hydroxybutyryl]-kanamycin A, possesses excellent antibacterial activity that appears superior to kanamycin A itself. Illustrated below are two tables showing the minimal inhibitory concentrations (MIC's) of kanamycin A and compound IV (BB-K8) against a variety of gram-positive and gram-negative bacteria as obtained by the Steers agar-dilution method (Table 1) and the two-fold dilution method (Table 2). Mueller-Hinton Agar Medium was used in the study of Table 1 and Heart Infusion Broth was used in the study of Table 2.

TABLE I (MIC mg./ml.)

Organism		Kanamycin A (6-8198)	Compound IV [BB-K8]
1.	Alk. faecalis A-9423	16	8
2.	" " A-20668	>125	>125
3.	Ent. cloacae A-0656	4	4
4.	" species A-20364	>125	2
5.	" hafniae I A-20674	1	1
6.	E. coli A-0636	2	1
7.	" " A-20664	16	4
8.	" " A-20665	>125	1
9.	" " A-20507	32	2

TABLE I (Continued)

Organism	Kanamycin A (6—8198)	Compound IV [BB—K8]
10. „ „ A—20520	>125	4
11. „ „ A—20365	>125	1
12. „ „ A—20684	2	2
13. „ „ A—20682	>125	2
14. „ „ A—20683	>125	8
15. „ „ A—20681	>125	2
16. „ „ A—15119	4	4
17. <i>K. pneumoniae</i> A—0967	4	4
18. „ species A—20328	>125	2
19. „ „ A—20330	32	32
20. „ „ A—20634	>125	4
21. „ pneumoniae A—20680	>125	4
22. „ „ A—0077	1	1
23. <i>Pr. mirabilis</i> A—9900	2	2
24. <i>Pr. morganii</i> A—15153	2	2
25. „ vulgaris A—9555	2	1
26. „ rettgeri A—9636	0.25	0.25
27. „ mirabilis A—20645	4	4
28. <i>Pr. mirabilis</i> A—20454	2	2
29. <i>Providencia stuartii</i> A—20615	2	1
30. „ alkalifaciens A—20676	1	1
31. <i>Ps. aeruginosa</i> A—20229	32	2
32. „ „ A—0943A	125	16
33. „ „ A—20653	>125	32
34. „ species A—20601	125, 63	16
35. „ „ A—20621	>125	>125
36. „ maltophilia A—20620	32	>125
37. <i>Sal. enteritidis</i> A—0531	1	0.5
38. „ derby A—20087	>125	1
39. <i>Ser. marcescens</i> A—20019	2	4

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TABLE I (Continued)

Organism	Kanamycin A (6—8198)	Compound IV [BB—K8]
40. „ „ A—9933	4	8
41. „ „ A—20460	>125	4, 2
42. „ „ A—20459	4	16
43. <i>Shig. flexneri</i> A—9684	4	4
44. <i>Aeromonas</i> sp. A—20670	2	2
45. <i>Arizona</i> sp. A—20671	2	2
46. <i>Citrobacter</i> sp. A—20673	4	4
47. <i>Edwardsiella</i> sp. A—20678	4	4
48. <i>Staph. aureus</i> A—9606	1	1
49. „ „ A—4749	0.5	1
50. „ „ A—9537	2	1
51. „ „ A—20610	>125	2
52. „ „ A—20240	>125	8
53. „ „ A—15197	1	2
Mueller-Hinton Medium +4% sheep blood		
54. <i>Str. faecalis</i> A—9854	63	63
55. „ „ A—9575	125	>125
56. <i>Str. pyogenes</i> A—20200	32, 16	32
57. „ „ A—9604	125	125
58. „ „ A—15040	125	125
59. „ „ A—20065	125	125
60. <i>D. pneumoniae</i> A—9585	63, 32	63
61. „ „ A—20159	125	>125

TABLE 2 (MIC mg./ml.)

Organism	Kanamycin A (6—8196)	Compound IV [BB—K8]
1. <i>D. pneumoniae</i> ±5% serum A—0585	63	63
2. <i>Str. pyogenes</i> ±5% serum A—9604	125	125
3. <i>Staph. aureus</i> Smith A—9537	0.5	0.5

TABLE 2 (Continued)

	Organism	Kanamycin A (6-8196)	Compound IV [BB-K8]
4.	<i>Staph. aureus</i> A-9497	0.5	0.5
5.	<i>Staph. aureus</i> A-20239	125	4
6.	<i>Staph. aureus</i> A-20240	125	4
7.	<i>Enter. cloacae</i> A-0656	2	2
8.	<i>Enter. species</i> A-20364	125	2
9.	<i>K. pneumoniae</i> A-9867	2	4
10.	<i>E. coli</i> K-12 ML1410 A-20361	2	4
11.	<i>E. coli</i> K-12 ML1630 A-20363	125	2
12.	<i>E. coli</i> K-12 A-9632	2	1
13.	<i>E. coli</i> A-20664	32	8
14.	<i>E. coli</i> A-20665	125	8
15.	<i>Pr. mirabilis</i> A-9900	2	16
16.	<i>Pr. morganii</i> A-15153	4	16
17.	<i>Pr. vulgaris</i> A-9436	1	2
18.	<i>Ps. aeruginosa</i> A-20227	4	1
19.	<i>Ps. species</i> A-20499	63	4
20.	<i>Ps. aeruginosa</i> A-20653	125	4
21.	<i>Ps. species</i> A-20621	125	125
22.	<i>Ser. marcescens</i> A-20019	2	4
23.	<i>Ser. marcescens</i> A-20141	16	16

The above MIC data show that compound IV (BB-K8) is superior to kanamycin A in activity, particularly against kanamycin A-resistant organisms.

The MIC data also correlate well with the *in vivo* results for all three organisms against which kanamycin A and compound IV were tested.

Compound IV and kanamycin A were equally effective against infections of mice caused by kanamycin A-sensitive strains of *E. coli* A-15119 and *Staph. aureus* A-9537. Although the CD_{50} values (curative dose in 50% of mice lethally infected) for *Staph. aureus* A-9537 suggest that compound IV is slightly less active than kanamycin A; this small difference is probably not significant because the dose levels were far apart (5X dilution).

Against the kanamycin-resistant strain of *E. coli* A-20520, kanamycin A, as expected, was not very effective *in vivo*, whereas compound IV demonstrated a marked protective action. Compound IV was approximately 10 times more active against this *E. coli* strain when administered in a 4-treatment regimen rather than in a 2-treatment one.

TABLE 3

A comparison of *In Vitro* and *In Vivo* Activities of Compound IV and Kanamycin A

Compounds	Test Number	<i>Staphylococcus aureus</i> A-9537		<i>Escherichia coli</i> A-15119		<i>Escherichia coli</i> A-20520	
		MIC	CD ₅₀ ^b	MIC	CD ₅₀	MIC	CD ₅₀
Compound IV	1	1	2.0×2	2	2×2	2	66×2
	2	— ^c	—	—	—	—	5×4
Kanamycin A	1	2	0.5×2	4	4×2	125	200×2
	2	—	—	—	—	—	200×4

^aMIC = minimum inhibitory concentration (ug./ml.). Tests conducted as described by Chisholm et al., (Antimicrob. Agents and Chemotherapy-1969, p. 244, 1970) using Mueller-Hinton agar as the test medium.

^bCD₅₀ = curative dose, 50% (mg./kg./treated × number of treatments). Mice were treated subcutaneously at 1 and 4 hours post-infection when 2 treatments were administered and at 0, 2, 4 and 6 hours post-infection when 4 treatments were given. Other aspects of the test were carried out as described by Price et al. (J. of Antibiotics 22:1, 1969).

^c— = not tested.

Compound IV is valuable as an antibacterial agent, nutritional supplement in animal feeds and therapeutic agent in poultry and animals, including man, and is especially valuable in the treatment of infectious diseases caused by Gram-positive and Gram-negative bacteria.

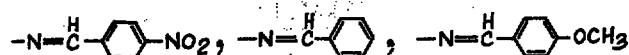
Compound IV, when administered orally, is useful as an adjunctive treatment for preoperative sterilization of the bowel. Both aerobic and anaerobic flora which are susceptible to this drug are reduced in the large intestine. When accompanied by adequate mechanical cleansing, it is useful in preparing for colonic surgery.

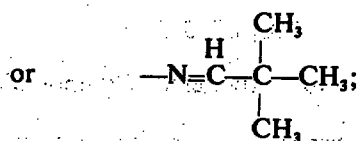
Compound IV is effective in the treatment of systemic bacterial infections in man when administered parenterally in the dosage range of about 250 mg. to about 3000 mg. per day in divided doses three or four times a day. Generally the compound is effective when administered at a dosage of about 5.0 to 7.5 mg./kg. of body weight every 12 hours.

A preferred embodiment of the process for the preparation of compound IV comprises the consecutive steps of

A.) acylating one mole of kanamycin A with about one mole of N-(benzyloxy-carbonyloxy)-succinimide; in a solvent selected from dimethylformamide, dimethylacetamide, tetrahydrofuran, dioxane, 1,2-dimethoxyethane, methanol, ethanol, water, acetone, pyridine, an N-(lower)alkylpiperidine, and mixtures thereof, at a temperature below 25°C., to produce compound II;

B.) treating one mole of compound II with 3 to 6 moles of an aldehyde selected from 4-nitrobenzaldehyde, benzaldehyde, 4-methoxybenzaldehyde and pivaldehyde, in a (lower)alkanol selected from absolute ethanol, methanol, n-propanol, isopropanol, n-butanol, sec-butanol, tert-butanol, and mixtures thereof, at temperatures in the range of 50°C. to reflux temperature of the system, for a period of 2 to 10 hours, to produce compound III in which Z is a radical of the formula





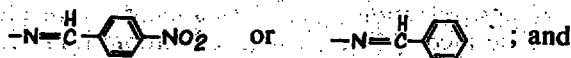
and

C.) treating one mole of compound III with 1 to 1.3 moles of L-(α)-p-benzyloxycarbonylamino- α -hydroxybutyric acid N-hydroxysuccinimide ester, in a solvent selected from dimethylformamide, tetrahydrofuran, dimethylacetamide, propyleneglycol dimethyl ether, ethyleneglycol dimethyl ether and dioxane, at a temperature in the range of 10°C. to 35°C.; removing the solvent *in vacuo* and hydrogenating the residue *in situ* in the presence of a metal catalyst selected from palladium, platinum, rhodium, Raney nickel, ruthenium and nickel, in a water/water-miscible solvent system selected from water and dioxane, tetrahydrofuran, ethyleneglycol dimethyl ether and propyleneglycol dimethyl ether, in the presence of a catalytic amount of glacial acetic acid to produce compound IV.

An even more preferred embodiment is the process for the preparation of compound IV which comprises the consecutive steps of

A.) acylating one mole of kanamycin A with about one mole of N-(benzyloxycarbonyloxy)succinimide in dimethylformamide at a temperature in the range of -20°C. to 10°C. to produce compound II;

B.) treating one mole of compound II with 3.5 to 4.5 moles of p-nitrobenzaldehyde or benzaldehyde in absolute ethanol, methanol, n-propanol or isopropanol at about reflux temperature for a period of 2 to 4 hours to produce compound III in which Z is a radical of the formula

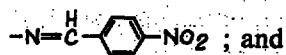


C.) treating one mole of compound III with 1.1 to 1.3 moles of L-(α)-p-benzyloxycarbonylamino- α -hydroxybutyric acid N-hydroxysuccinimide ester in dimethylformamide at about room temperature for at least five hours; then removing the solvent *in vacuo* and hydrogenating the residue *in situ* in the presence of palladium on charcoal in a 1:1 mixture of dioxane and water in the presence of a catalytic amount of glacial acetic acid to produce compound IV.

The most preferred embodiment is the process for the preparation of compound IV which comprises the consecutive steps of

A.) acylating one mole of kanamycin A with about one mole of N-(benzyloxycarbonyloxy)succinimide, in dimethylformamide at a temperature in the range of -20°C. to 10°C. to produce compound II;

B.) treating one mole of compound II with about four moles of p-nitrobenzaldehyde in absolute ethanol at about reflux temperature for a period of 2 to 4 hours to produce compound III in which Z is



C.) treating one mole of compound III with about 1.2 moles of L-(α)-p-benzyloxycarbonylamino- α -hydroxybutyric acid N-hydroxysuccinimide ester in dimethylformamide, at about room temperature for at least five hours; then removing the solvent *in vacuo* and hydrogenating the residue *in situ* in the presence of palladium on charcoal in a 1:1 mixture of dioxane and water in the presence of a catalytic amount of glacial acetic acid to produce compound IV.

Throughout the specification the term "(lower)alkyl" is an alkyl residue, having a straight or branched chain, of 1 to 6 carbon atoms. "(lower)alkanol" is a saturated alcohol, having a straight or branched chain, of 1 to 6 carbon atoms, containing one OH function.

EXAMPLES

Example 1.

Preparation of L-(α)-p-Benzyloxycarbonylamino- α -hydroxybutyric acid (VI). L-(α)-p-amino- α -hydroxybutyric acid (7.4 g., 0.062 mole) was added to a

Eight and one half grams of Darco G-60 was added to the degassed solution. The mixture was slurried for 0.5 hour at ambient temperature. The carbon was removed by suitable filtration and washed with 35 ml. of deionized water. The water was added to the filtrate.

The combined filtrate-washings were adjusted to pH 1-1.3 with 50% V/V sulfuric acid. This solution was added with rapid stirring over a 10 minute period to 600-800 ml. of methanol (3-4 volumes of methanol). The mixture was stirred for five minutes at pH 1-1.3, passed through a 100 mesh screen, stirred for two minutes and allowed to settle for five minutes. Most of the supernatant liquid was decanted. The remaining slurry was suitably filtered, washed with 200 ml. of methanol and vacuum-dried at 50°C. for 24 hours. The yield of amorphous BB-K8 (H_2SO_4)₂ was 32-34 grams; $[\alpha]_D^{22} H_2O = +74.75$, decomposition at 220-23°C.

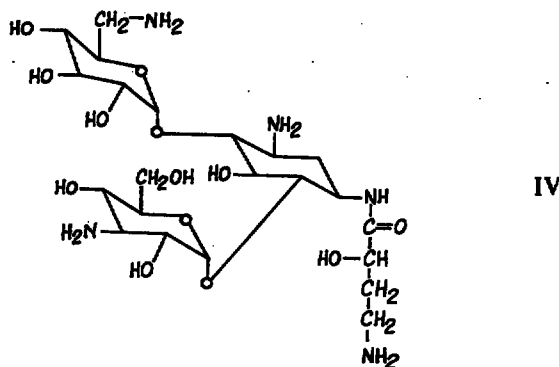
Elemental Analysis (On Dry Basis*).

	Found	Theory
% C	32.7, 33.5, 32.3	33.5
% N	8.78, 8.7, 8.2, 8.8	8.97
% S	8.75, 8.9, 7.8, 8.85	8.2
% Ash	nil	—

*Karl Fisher water content: 2.33, 1.79, 2.87% (theory for monohydrate is 2.25% water). This salt is hygroscopic but not deliquescent. After storage of an aliquot in air at room temperature for 18 hours the water content increased to 9.55, 9.89% (theory for a pentahydrate is 10.33% water).

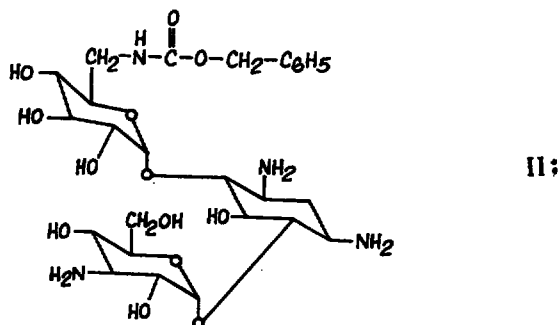
WHAT WE CLAIM IS:—

1. The process for the preparation of the compound having the formula



which comprises the consecutive steps of

- A.) acylating one mole of kanamycin A with one mole or less of N-(benzyloxycarbonyloxy)-succinimide, in a solvent, at a temperature below 50°C., to produce the compound having the formula



Eight and one half grams of Darco G-60 was added to the degassed solution. The mixture was slurried for 0.5 hour at ambient temperature. The carbon was removed by suitable filtration and washed with 35 ml. of deionized water. The water was added to the filtrate.

The combined filtrate-washings were adjusted to pH 1-1.3 with 50% V/V sulfuric acid. This solution was added with rapid stirring over a 10 minute period to 600-800 ml. of methanol (3-4 volumes of methanol). The mixture was stirred for five minutes at pH 1-1.3, passed through a 100 mesh screen, stirred for two minutes and allowed to settle for five minutes. Most of the supernatant liquid was decanted. The remaining slurry was suitably filtered, washed with 200 ml. of methanol and vacuum-dried at 50°C. for 24 hours. The yield of amorphous BB-K8 (H_2SO_4)₂ was 32-34 grams; $[\alpha]_D^{25} H_2O = +74.75$, decomposition at 220-23°C.

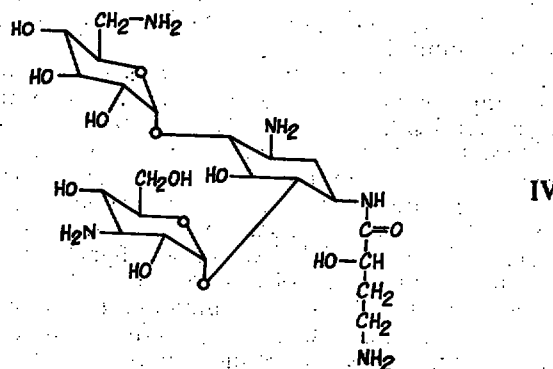
Elemental Analysis (On Dry Basis*).

	Found	Theory
% C	32.7, 33.5, 32.3	33.5
% N	8.78, 8.7, 8.2, 8.8	8.97
% S	8.75, 8.9, 7.8, 8.85	8.2
% Ash	nil	—

*Karl Fisher water content: 2.33, 1.79, 2.87% (theory for monohydrate is 2.25% water). This salt is hygroscopic but not deliquescent. After storage of an aliquot in air at room temperature for 18 hours the water content increased to 9.55, 9.89% (theory for a pentahydrate is 10.33% water).

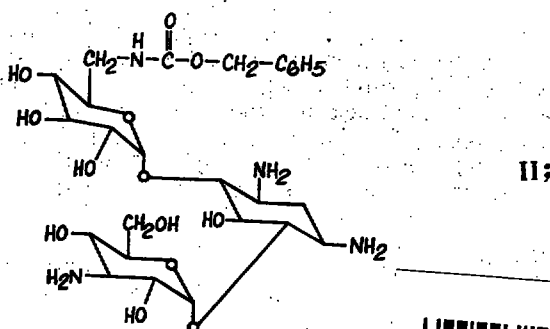
WHAT WE CLAIM IS:—

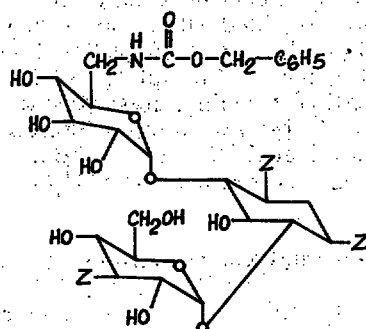
1. The process for the preparation of the compound having the formula



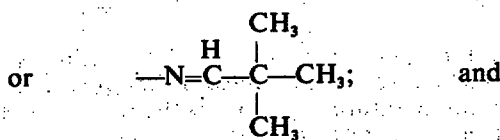
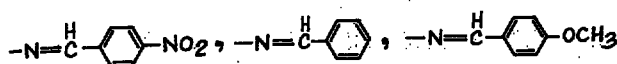
which comprises the consecutive steps of

- A.) acylating one mole of kanamycin A with one mole or less of N-(benzyloxycarbonyloxy)-succinimide, in a solvent, at a temperature below 50°C., to produce the compound having the formula





in which Z is



C.) treating one mole of compound III with at least one mole of L(-)-benzyloxycarbonylamino- α -hydroxybutyric acid N-hydroxysuccinimide ester, in an organic solvent at a temperature in the range of 0°C. to 50°C.; then removing the solvent and hydrogenating the residue in a water/water-miscible solvent system to produce compound IV.

2. The process of claim 1 which comprises the consecutive steps of

2.) acylating one mole of kanamycin A with about one mole of N-(benzyloxy-carbonyloxy)succinimide, in a solvent selected from dimethylformamide, dimethylacetamide, tetrahydrofuran, dioxane, 1,2-dimethoxyethane, methanol, ethanol, water, acetone, pyridine, an N-(lower)-alkylpiperidine, and mixtures thereof, at a temperature below 25°C., to produce compound II;

B.) treating one mole of compound II with 3 to 6 moles of an aldehyde selected from 4-nitrobenzaldehyde, benzaldehyde, 4-methoxybenzaldehyde and pivaldehyde, in a (lower)alkanol selected from absolute ethanol, methanol, n-propanol, isopropanol, n-butanol, sec-butanol, tert-butanol, and mixtures thereof, at temperatures in the range of 50°C. to reflux temperature of the system, for a period of two to ten hours, to produce compound III; and

C.) treating one mole of compound III with 1 to 1.3 moles of L-(γ -benzyloxy-carbonyl-amino- α -hydroxybutyric acid N-hydroxysuccinimide ester, in a solvent selected from dimethylformamide, tetrahydrofuran, dimethylacetamide, propyleneglycol dimethyl ether, ethylene-glycol dimethyl ether and dioxane, at a temperature in the range of 10°C. to 35°C.; then removing the solvent *in vacuo* and hydrogenating the residue *in situ* in the presence of a metal catalyst selected from palladium, platinum, rhodium, Raney nickel, ruthenium and nickel, in a water/water-miscible solvent system selected from water and dioxane, tetrahydrofuran, ethyleneglycol dimethyl ether, and propylene-glycol dimethyl ether, in the presence of a catalytic amount of glacial acetic acid to produce compound IV.

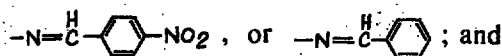
3. The process of claim 2 which comprises the consecutive steps of

A). acylating one mole of kanamycin A with about 1 mole of N-(benzyloxy-carbonyloxy)succinimide, in dimethylformamide at a temperature in the range of -20°C . to 10°C . to produce compound II;

B.) treating one mole of compound II with 3.5 to 4.5 moles of *p*-nitrobenzaldehyde or benzaldehyde in absolute ethanol, methanol, *n*-propanol or isopropanol at about reflux temperature for a period of 2 to 4 hours to produce compound III in which Z is _____



(27)

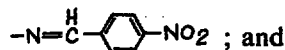


C.) treating one mole of compound III with 1.1 to 1.3 moles of L-(−)-p-benzyloxycarbonylamino-α-hydroxybutyric acid N-hydroxysuccinimide ester in dimethylformamide at about room temperature for at least five hours; then removing the solvent *in vacuo* and hydrogenating the residue *in situ* in the presence of palladium on charcoal in a 1:1 mixture of dioxane and water in the presence of a catalytic amount of glacial acetic acid to produce compound IV.

4. The process of claim 3 which comprises the consecutive steps of

A.) acylating one mole of kanamycin A with about one mole of N-(benzyloxycarbonyloxy)succinimide, in dimethylformamide at a temperature in the range of −20°C. to 10°C. to produce compound II;

B.) treating one mole of compound II with about four moles of p-nitrobenzaldehyde in absolute ethanol at about reflux temperature for a period of 2 to 4 hours to produce compound III in which Z is



C.) treating one mole of compound III with about 1.2 moles of L-(−)-p-benzyloxycarbonylamino-α-hydroxybutyric acid N-hydroxysuccinimide ester in dimethylformamide, at about room temperature, for at least five hours; removing the solvent *in vacuo* and hydrogenating the residue *in situ* in the presence of palladium on charcoal in a 1:1 mixture of dioxane and water in the presence of a catalytic amount of glacial acetic acid to produce compound IV.

5. A process for the preparation of the compound of formula IV given in claim 1 substantially as hereinbefore described in the Examples.

6. A compound of formula IV given in claim 1 when prepared by the process claimed in any of claims 1 to 5.

7. A pharmaceutical composition which comprises a compound as claimed in claim 6 together with an inert pharmacologically-acceptable carrier.

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