

# PATENT SPECIFICATION

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 C07D 207/40



(52) Index at acceptance

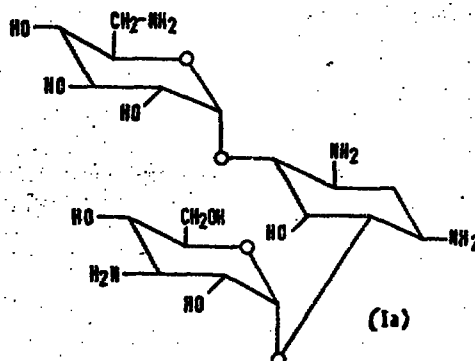
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 627 628 62X 634 635 637 640 643 650 652 660 662  
 670 672 699 70Y 790 79Y KA KB KH LU NK  
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## (54) NEW DERIVATIVES OF KANAMYCIN A AND B AND A PROCESS FOR THE PREPARATION THEREOF

(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, of 345 Park Avenue, New York, State of New York, 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:—

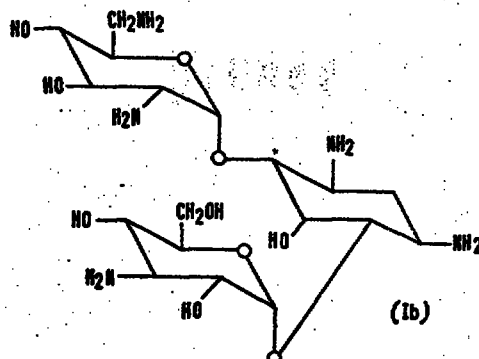
This invention relates to derivatives of kanamycin A and B and to a process for the preparation thereof, and, in particular, to semisynthetic 1 - substituted derivatives of kanamycin A or B. Certain of the kanamycin derivatives of the invention are anti-bacterial agents.

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

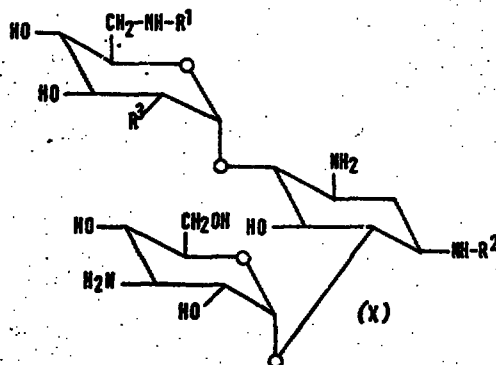


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Kanamycin B is a compound having the formula



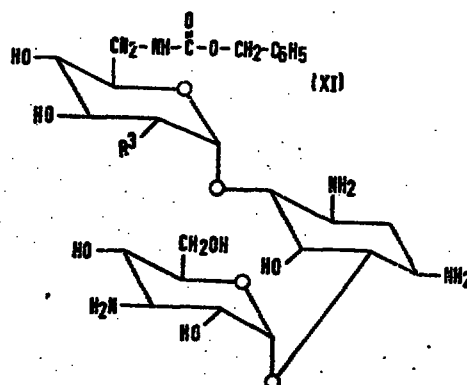
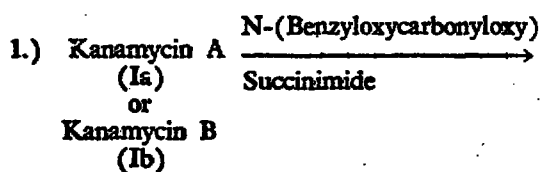
According to the invention there is provided a compound having the formula



wherein  $R^1$  is H or  $C_6H_5-CH_2-O-C(=O)-$ ,  $R^2$  is H or L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl or L - (-) -  $\gamma$  - benzyloxycarbonylamino -  $\alpha$  - hydroxybutyryl,  $R^3$  is OH or  $NH_2$ , and at least one of  $R^1$  and  $R^2$  is other than H, or a nontoxic pharmaceutically acceptable acid addition salt thereof.

For the purpose of this disclosure, the term "nontoxic pharmaceutically acceptable acid addition salt" shall mean a mono, di-, tri- or tetrasalt formed by the interaction of 1 molecule of compound X with 1-4 moles of a nontoxic, pharmaceutically acceptable acid. Included among these acids are acetic, hydrochloric, sulfuric, mandelic, maleic, phosphoric, nitric, hydrobromic, ascorbic, malic and citric acid, and those other acids commonly used to make salts of pharmaceutical compounds containing an amine group.

The compounds of the present invention may be prepared for example by the following preferred reaction sequence:

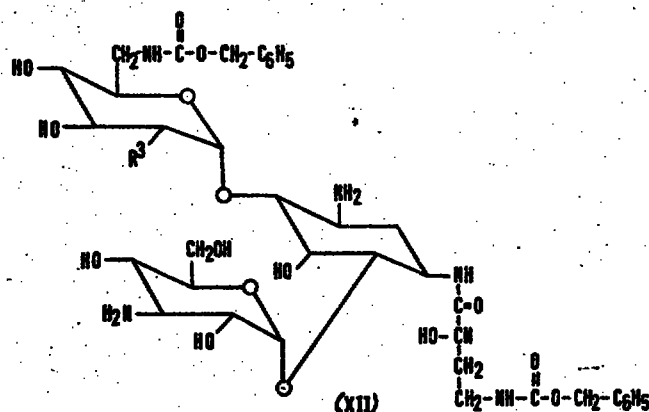


[6'-Monobenzyloxycarbonylkanamycin A or B]

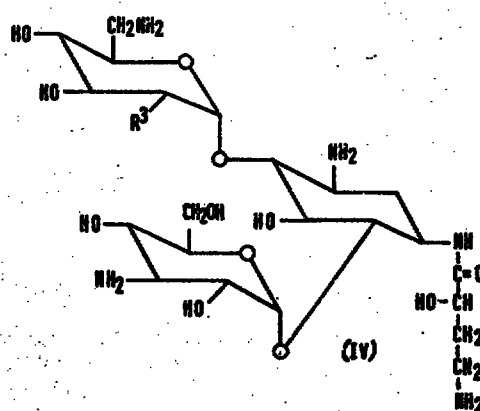
(12)

N-Hydroxysuccinimide ester of L-(-)- $\gamma$ -benzyloxycarbonylamino- $\alpha$ -hydroxybutyric acid

2.) Compound (XI)



3.) Compound (XII)  $\xrightarrow{H_2/Pd/C}$

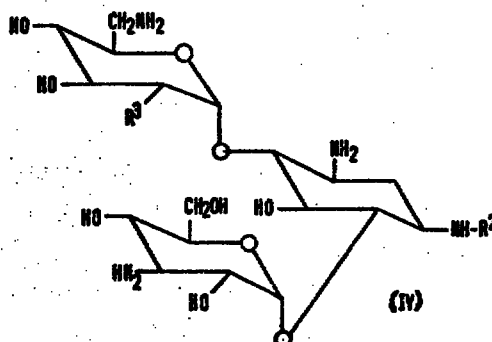


Preferred compounds of the invention are those wherein  $R^2$  is H or L-(-)- $\gamma$ -amino- $\alpha$ -hydroxybutyryl and most preferred compounds are those wherein  $R^1$  is H and  $R^2$  is L-(-)- $\gamma$ -amino- $\alpha$ -hydroxybutyryl, and nontoxic pharmaceutically acceptable acid addition salts thereof.

Preferred salts are the sulfate, hydrochloride, acetate, maleate, mandelate, citrate, ascorbate, nitrate and phosphate salts, in particular the monosulfate and the disulfate salts.

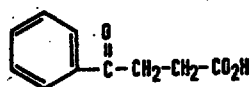
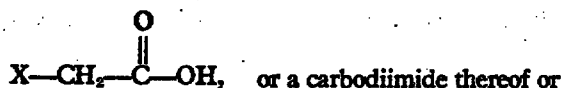
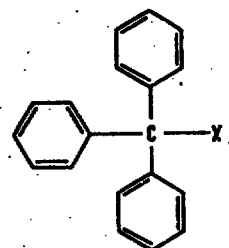
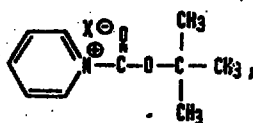
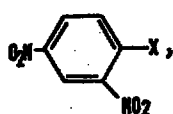
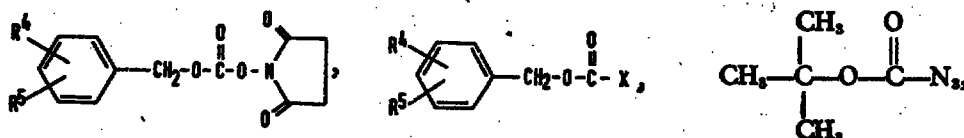
The compounds of the invention may be in their monohydrate form if desired.

The present invention also provides a process for the preparation of a compound having the formula



in which  $R^2$  is L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl and  $R^3$  is OH or  $NH_2$ ; or a nontoxic pharmaceutically acceptable acid addition salt thereof; which process comprises the consecutive steps of

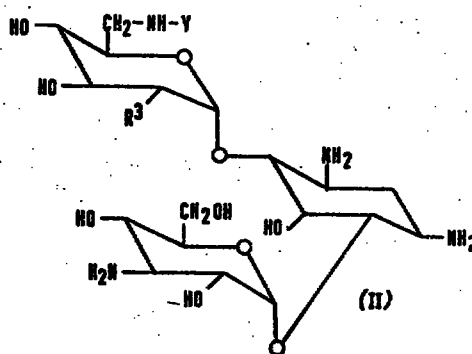
5 A.) acylating kanamycin A or kanamycin B with an acylating agent having the formula 5



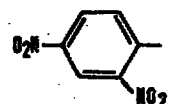
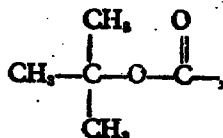
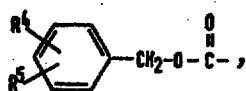
or a carbodiimide thereof,

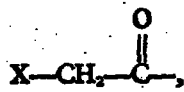
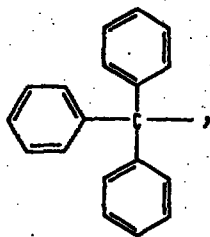
10 in which  $R^4$  and  $R^5$  are alike or different and each is H, F, Cl, Br,  $NO_2$ , OH, (lower) alkyl or (lower) alkoxy, X is chloro, bromo or iodo, in a ratio of 1 mole or less of acylating agent per mole of kanamycin A or B, in a solvent, preferably dimethylformamide, dimethylacetamide, tetrahydrofuran, dioxane, 1,2 - dimethoxyethane, methanol, ethanol, water, acetone, pyridine, N - (lower) alkylpiperidine, or mixtures thereof, but more preferably dimethylformamide, at a temperature below  $50^\circ C$ , and preferably below  $25^\circ C$ , to produce the compound having the formula 10

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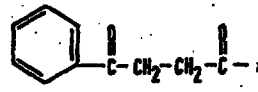


in which  $R^3$  is as defined above and Y is a radical of the formula



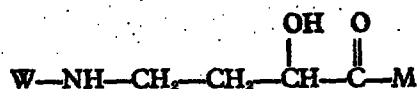


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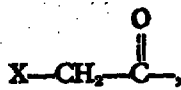
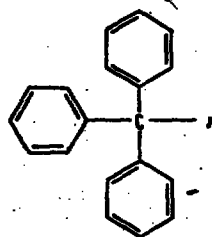
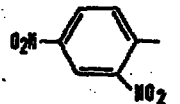
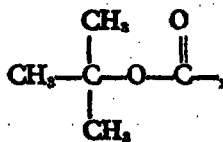
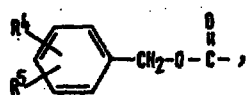


in which  $R^4$  and  $R^5$  are as defined above;

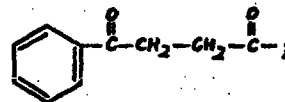
B.) acylating the compound of formula II with an acylating agent having the formula



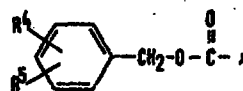
said acylating agent being derived from L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid, in which W is a radical of the formula



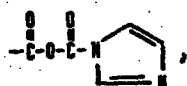
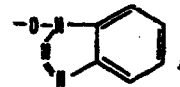
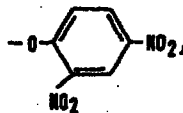
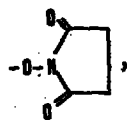
or



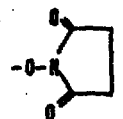
but preferably



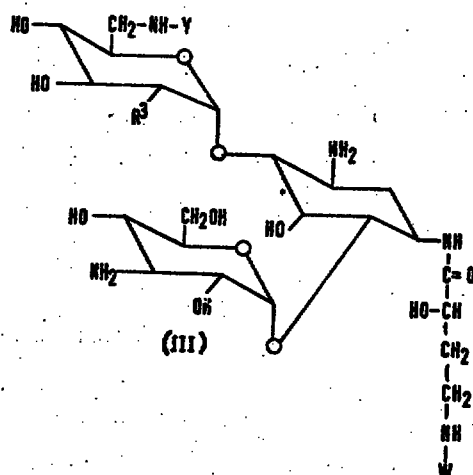
and M is a radical of the formula



but preferably

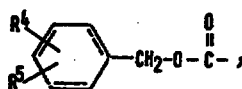


in which  $R^4$  and  $R^5$  are as defined above, in a ratio of at least 0.5 mole of acylating agent per mole of compound II, but preferably in a ratio of about 0.5 to about 1.4, and most preferably in a ratio of about 0.8 to about 1.1, in a solvent, preferably a mixture of water and ethyleneglycol dimethyl ether, dioxane, dimethylacetamide, dimethylformamide, tetrahydrofuran or propyleneglycol dimethyl ether, and most preferably a 1:1 mixture of water and ethylene glycol dimethyl ether, to produce a compound of the formula



in which  $\text{R}^3$ , Y and W are as defined above; and

C.) removing the blocking groups W and Y from compound III by methods commonly known in the art, and preferably when W and Y are radicals of the formula



by hydrogenating compound III with hydrogen in the presence of a metal catalyst, preferably palladium, platinum, Raney nickel, rhodium, ruthenium or nickel, but more preferably palladium, and most preferably palladium on charcoal, in a water-water miscible solvent system, preferably water and dioxane, tetrahydrofuran, ethyleneglycol dimethyl ether and propyleneglycol dimethyl ether but most preferably a 1:1 mixture of water and dioxane, and preferably in the presence of a catalytic amount of glacial acetic acid to produce the compound of formula IV, and, if desired, subsequently converting the product by methods known *per se* into a nontoxic pharmaceutically acceptable acid addition salt thereof.

Acyating agents that produce labile amine blocking groups, are used in the process of the invention, said labile blocking groups being commonly employed in the synthesis of peptides. Examples of said labile blocking groups and their removal can be found in the review of A. Kapoor, J. Pharm. Sciences 59, pp. 1-27 (1970). Functional equivalents as acylating agent for primary amine groups include the corresponding carboxylic chlorides, bromides, acid anhydrides, including mixed anhydrides and particularly the mixed anhydrides prepared from stronger acids such as the lower aliphatic monoesters of carbonic acid, of alkyl and aryl sulfonic acids and of more hindered acids such as diphenylacetic acid. In addition, an acid azide or an active ester of thioester (e.g., with p - nitrophenol, 2,4 - dinitrophenol, thiophenol, thioacetic acid) may be used or the free acid itself may be coupled with the kanamycin derivative (II) after first reacting said free acid with N,N' - dimethylchloroforminium chloride [British Patent Specification No. 1,008,170 and Novak and Weichert, Experientia XXI/6, 360 (1965)] or by the use of enzymes or of an N,N' - carbonyldiimidazole or an N,N' - carbonylditriazole [cf. Sheehan and Hess, J. Amer. Chem. Soc., 77, 1067, (1955)] or of alkynylamine reagent [cf. R. Buijile and H. G. Viehe, Angew. Chem., International Edition 3, 582 (1964)], or of a ketenimine reagent [cf. C. L. Stevens and M. E. Monk, J. Amer. Chem. Soc. 80, 4065 (1958)] or of an isoxazolium salt reagent [cf. R. B. Woodward, R. A. Olofson and H. Mayer, J. Amer. Chem. Soc., 83, 1010 (1961)]. Another equivalent of the acid chloride is a corresponding azolide, i.e., an amide of the corresponding acid whose amide nitrogen is a member of a quasaromatic five membered ring containing at least two nitrogen atoms, e.g., imidazole, pyrazole, the triazoles, benzimidazole, benzotriazole and their substituted derivatives. As an example of the general method for the preparation of an azolide, N,N' - carbonyldiimidazole is reacted with a carboxylic acid in equimolar

proportions at room temperature in tetrahydrofuran, chloroform, dimethylformamide or a similar inert solvent to form the carboxylic acid imidazolidine in practically quantitative yield with liberation of carbon dioxide and one mole of imidazole. Dicarboxylic acids yield diimidazolides. The by-product, imidazole, precipitates and may be separated and the imidazolidine isolated, but this is not essential. These reactions are well-known in the art (cf. U.S. Patent Nos. 3,079,314, 3,117,126 and 3,129,224 and British Patent Nos. 932,644, 957,570 and 959,054).

In preparing the 6' - blocked - amino kanamycin in Step A it is preferred to regulate the molar proportions of reagents so that a ratio of 1 mole or less of acylating agent is used per mole of 6' - blocked - amino kanamycin. Increasing the proportion of acylating agent will result in lower yields of the desired intermediate because of the increase of competing side reactions which produce polyacyl derivative(s) impurities.

As with most chemical reactions, temperatures higher or lower than those specifically described herein may be used. Temperatures substantially higher than those described, i.e. 50° C., however, tend to result in reduced yield due to a greater extent of side reactions.

Compound IVa 1 - (L - (-) -  $\gamma$  - amino -  $\alpha$  - 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 IVa (BB-K8) against a variety of gram-positive and gram-negative bacteria as obtained by the Steers agar - dilution method (Table I) and the two-fold dilution method (Table 2). Mueller-Hinton Agar Medium was used in the study of Table I and Heart Infusion Broth was used in the study of Table 2.

TABLE I

(MIC mg./ml.)

| Organism                       | Kanamycin A<br>(6-8198) | Compound IVa<br>[BB-K8]<br>lot No. 4 |
|--------------------------------|-------------------------|--------------------------------------|
| 1. <i>Alk. faecalis</i> A-9423 | 16                      | 8                                    |
| 2. " " A-20648                 | >125                    | >125                                 |
| 3. <i>Ent. cloacae</i> A-9656  | 4                       | 4                                    |
| 4. " species A-20364           | >125                    | 2                                    |
| 5. " <i>hafniae</i> 1 A-20674  | 1                       | 1                                    |
| 6. <i>E. coli</i> A-0636       | 2                       | 1                                    |
| 7. " A-20664                   | 16                      | 4                                    |
| 8. " A-20665                   | >125                    | 1                                    |
| 9. " A-20507                   | 32                      | 2                                    |
| 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                                    |

TABLE I (Continued)

| Organism                                     | (MIC mg./ml.)           |                                      |
|----------------------------------------------|-------------------------|--------------------------------------|
|                                              | Kanamycin A<br>(6-8198) | Compound IVa<br>[BB-K8]<br>lot No. 4 |
| 17. <i>K. pneumoniae</i> A-9867              | 4                       | 4                                    |
| 18. „ <i>species</i> A-20328                 | >125                    | 2                                    |
| 19. „ „ A-20330                              | 32                      | 32                                   |
| 20. „ „ A-20634                              | >125                    | 4                                    |
| 21. „ <i>pneumoniae</i> A-20680              | >125                    | 4                                    |
| 22. „ „ A-9977                               | 1                       | 1                                    |
| 23. <i>Pr. mirabilis</i> A-9900              | 2                       | 2                                    |
| 24. „ <i>morganii</i> A-15153                | 2                       | 2                                    |
| 25. „ <i>vulgaris</i> A-9555                 | 2                       | 1                                    |
| 26. „ <i>rettgeri</i> A-9636                 | 0.25                    | 0.25                                 |
| 27. „ <i>mirabilis</i> A-20645               | 4                       | 4                                    |
| 28. „ <i>mirabilis</i> A-20454               | 2                       | 2                                    |
| 29. <i>Providencia stuartii</i> A-20615      | 2                       | 1                                    |
| 30. <i>Providencia alkalifaciens</i> A-20676 | 1                       | 1                                    |
| 31. <i>Ps. aeruginosa</i> A-20229            | 32                      | 2                                    |
| 32. „ „ A-9843A                              | 125                     | 16                                   |
| 33. „ „ A-20653                              | >125                    | 32                                   |
| 34. „ <i>species</i> A-20601                 | 125, 63                 | 16                                   |
| 35. „ „ A-20621                              | >125                    | >125                                 |
| 36. „ <i>maltophilia</i> A-20620             | 32                      | >125                                 |
| 37. <i>Sal. enteritidis</i> A-9531           | 1                       | 0.5                                  |
| 38. „ <i>derby</i> A-20087                   | >125                    | 1                                    |
| 39. <i>Ser. marcescens</i> A-20019           | 2                       | 4                                    |
| 40. „ „ A-9933                               | 4                       | 8                                    |
| 41. „ „ A-20460                              | >125                    | 4 2                                  |
| 42. „ „ A-20459                              | 4                       | 16                                   |
| 43. <i>Shig. flexneri</i> A9684              | 4                       | 4                                    |
| 44. <i>Aeromonas</i> sp. A-20670             | 2                       | 2                                    |
| 45. <i>Arizona</i> sp. A-20671               | 2                       | 1                                    |

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TABLE I (Continued)

| Organism                              | (MIC mg./ml.)           |                                      |
|---------------------------------------|-------------------------|--------------------------------------|
|                                       | Kanamycin A<br>(6-8198) | Compound IVa<br>[BB-K8]<br>lot No. 4 |
| 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. <i>Str. pyogenes</i> 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<br>(6-8196) | Compound IVa<br>[BB-K8]<br>lot No. 4 |
|-------------------------------------------|-------------------------|--------------------------------------|
| 1. <i>D. pneumoniae</i> +5% serum A-9585. | 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                                  |
| 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 IVa (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 IVa were tested.

Compound IVa and kanamycin A were equally effective in infections of mice caused by kanamycin A-sensitive strains of *E. coli* A15119 and *Staph. aureus* A9537. Although the  $CD_{50}$  values (curative dose in 50% of mice lethally infected) for *Staph. aureus* A9537 suggest that compound IVa is slightly less active than kanamycin A, this small difference is probably not significant because the dose levels were far apart (5X - dilutions).

Against the kanamycin - resistant strain of *E. coli* A20520, kanamycin A as expected was not effective *in vivo*, whereas compound IVa demonstrated a marked

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protective action. Compound IVa 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 IVa and Kanamycin A

| Compounds   | Test Number | Staphylococcus aureus A9537 |                               | Escherichia coli A15119 |                  | Escherichia coli A20520 |                  |
|-------------|-------------|-----------------------------|-------------------------------|-------------------------|------------------|-------------------------|------------------|
|             |             | MIC                         | CD <sub>50</sub> <sup>b</sup> | MIC                     | CD <sub>50</sub> | MIC                     | CD <sub>50</sub> |
| Compound IV | 1           | 1                           | 2.0×2                         | 2                       | 2×2              | 2                       | 66×2             |
|             | 1           | - <sup>c</sup>              | -                             | -                       | -                | -                       | 5×4              |
| Kanamycin A | 1           | 2                           | 0.5×2                         | 4                       | 4×2              | 125                     | 200×2            |
|             | 2           | -                           | -                             | -                       | -                | -                       | 200×4            |

<sup>a</sup>MIC =minimum inhibitory concentration (ug./ml.). Tests conducted as described by Chisholm *et al.*, (Antimicrob. agents and Chemotherapy-1969, p. 244, 1970) using Mueller-Hinten again as the test medium.

<sup>b</sup>CD<sub>50</sub>=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 treatment were given. Other aspects of the test were carried out as described by Price *et al.*, (J. of Antibiotics 22:1. 1969).

<sup>c</sup>- =not tested.

5 The compounds IV are valuable as antibacterial agents, nutritional supplements in animal feeds, therapeutic agents in poultry and animals, including man, and are especially valuable in the treatment of infectious diseases caused by Gram - positive and Gram - negative bacteria. 5

10 The compounds IV when administered orally are useful as an adjunctive treatment for preoperative sterilization of the bowel. Both aerobic and anaerobic flora which are suseptible to these drugs are reduced in the large intestine. When accompanied by adequate mechanical cleansing, they are useful in preparing for colonic surgery. 10

15 The compounds IV are effective in the treatment of systemic bacterial infections 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 compounds are effective when administered at a dosage of about 5.0 to 7.5 mg./kg. of body weight every 12 hours. 15

20 Compound IVb, 1 - [L - (--) - γ - amino - α - hydroxybutyryl] - kannamycin B, possesses excellent antibacterial activity that appears superior to kanamycin A. Illustrated below is Table 4 showing the minimal inhibitory concentration (MIC's) of kanamycin A and compound IVb (BB-K26) against a variety of gram-positive and gram-negative bacteria. The MIC's are obtained by the Steers agar-dilution method (Table 4) on nutrient agar medium. 20

TABLE 4

| Organism                  | Bristol<br>Code No. | Resist<br>to | MIC (mg./ml.) |             |
|---------------------------|---------------------|--------------|---------------|-------------|
|                           |                     |              | BBK-26        | Kanamycin A |
| <i>E. coli</i> N1HJ       |                     |              | 0.4           | 0.8         |
| „ <i>Juhl</i>             | A15119              |              | 1.6           | 0.8         |
| „                         | A15169              |              | 1.6           | 1.6         |
| „                         | A20363              | KM           | 0.8           | 50          |
| „                         | A9844               |              | 0.8           | 0.8         |
| „                         | A20365              | KM           | 0.4           | >50         |
| „ <i>K-12</i>             |                     |              | 0.8           | 0.4         |
| „ <i>K-12</i>             | A20664              | KM           | 0.8           | 3.1         |
| „ <i>K-12</i>             | A20665              | KM           | 0.8           | 50          |
| <i>K. pneumoniae</i> D11  |                     |              | 0.2           | 0.2         |
| „                         | A9678               |              | 0.8           | 0.8         |
| <i>S. marcescens</i>      | A20019              |              | 1.6           | 1.6         |
| <i>Ps. aeruginosa</i> D15 |                     |              | 3.1           | 12.5        |
| „ <i>D113</i>             |                     | KM           | 50            | >50         |
| „                         | A9923               |              | 1.6           | 25          |
| „                         | A9930               |              | 0.8           | 6.3         |
| <i>Ps. aeruginosa</i>     | A15150              |              | 6.3           | 25          |
| „                         | A15194              |              | 1.6           | 12.5        |
| „                         | A20479              | KM           | 1.6           | 50          |
| „                         | A20616              | KM           | 3.1           | 25          |
| „                         | A20653              | KM           | 25            | 50          |
| „                         | A9843               |              | 1.6           | 12.5        |
| <i>Pseudomonas</i> sp.    | A20355              |              | 1.6           | 6.3         |
| „                         | A20358              | KM           | 3.1           | 12.5        |
| „                         | A20368              | GM           | 50            | 25          |
| „                         | A20598              | GM           | 6.3           | 25          |
| „                         | A20600              | GM           | 3.1           | 12.5        |
| „                         | A20603              | GM           | 6.3           | >50         |
| „                         | A20618              | GM           | 50            | 50          |

TABLE 4 (Continued)

| Organism                     | Bristol<br>Code No. | Resist.<br>to | MIC (mg./ml.) |             |
|------------------------------|---------------------|---------------|---------------|-------------|
|                              |                     |               | BBK-26        | Kanamycin A |
| <i>Pr. vulgaris</i>          | A9436               |               | 1.6           | 0.8         |
| "                            | A9526               |               | 0.8           | 0.8         |
| <i>Pr. mirabilis</i>         | A9554               |               | 1.6           | 1.6         |
| "                            | A9900               |               | 1.6           | 1.6         |
| <i>Pr. morganii</i>          | A9553               |               | 1.6           | 1.6         |
| "                            | A20031              |               | 6.3           | 3.1         |
| <i>Pr. rettgeri</i>          | A15167              |               | 0.8           | 0.8         |
| <i>S. aureus</i> 209P        |                     |               | 1.6           | 0.8         |
| " Smith                      |                     |               | 0.8           | 0.4         |
| " 209P                       |                     |               | 1.6           | 0.8         |
| "                            | A20239              | KM            | 1.6           | 25          |
| <i>S. lutea</i> PCI-1001     |                     |               | 1.6           | 3.1         |
| <i>M. flavus</i>             |                     |               | 0.4           | 0.8         |
| <i>B. subtilis</i> PCI-219   |                     |               | 0.1           | 0.1         |
| <i>St. pyogenes</i> S-23     |                     |               | 25            | 25          |
| " Dick                       |                     |               | 1.6           | 1.6         |
| <i>St. pyogenes</i> Digonnet | A9604               |               | 12.5          | 25          |
| "                            | A20065              |               | 25            | 25          |
| <i>D. pneumoniae</i>         |                     |               | 50            | 25          |
| <i>Mycobacterium</i> 607     |                     |               | 6.3           | 0.8         |
| " 607 D105                   |                     | KM            | >100          | >100        |
| " 607 D107                   |                     | KM            | >100          | >100        |
| " <i>phlei</i>               |                     |               | 1.6           | 1.6         |
| " <i>rauae</i>               |                     |               | 6.3           | 1.6         |
| " <i>H<sub>37</sub>Rv*</i>   |                     |               | 0.8           | 0.4         |

\*determined by tube dilution method.

KM is kanamycin A; GM is gentamycin.

The above MIC data show that compound IVb (BB-K26) is superior to kanamycin A in activity, particularly against kanamycin A-resistant organisms.

An *in vivo* evaluation of compound IVb was carried out against both kanamycin B-sensitive and resistant strains of *E. coli* and *Ps. aeruginosa*.

The organisms are designated as follows:

|                            | Code No. | Kanamycin Sensitivity              |
|----------------------------|----------|------------------------------------|
| <i>E. coli</i> Juhl        | A15119   | sensitive                          |
| <i>E. coli</i> ML—1630     | A20363   | resistant (phosphorylation)        |
| <i>Ps. aeruginosa</i> D15  | —        | moderately resistant               |
| <i>Ps. aeruginosa</i> D113 | —        | highly resistant (phosphorylation) |

Compound IVb (BB—K26) and kanamycin B were equally effective against *E. coli* Juhl A15119 at a dose of 6.3 mg./kg. in mice. Compound IBb was most effective against kanamycin B resistant *E. coli* ML—1630 at a dose of 6.3 mg./kg. vs. 100—400 mg./kg. for kanamycin B.

Compound IVb showed slightly better activity against moderately resistant *Ps. aeruginosa* D15 than kanamycin B. Compound IVb showed substantially better activity against highly resistant *Ps. aeruginosa* D—113 than kanamycin B (see Table 5 below).

TABLE 5

| dose (sc)                  | <i>E. coli</i> Juhl A15119 |       | <i>E. coli</i> ML—1630 |        |
|----------------------------|----------------------------|-------|------------------------|--------|
|                            | BB—K26                     | KM—B* | BB—K26                 | KM—B   |
| 400 mg./kg.                | —                          | —     | —                      | 2/5    |
| 100                        | 5/5                        | 5/5   | 5/5                    | 2/5    |
| 25                         | 5/5                        | 5/5   | 5/5                    | 0/5    |
| 6.3                        | 5/5                        | 5/5   | 5/5                    | 0/5    |
| 1.6                        | 0/5                        | 0/5   | 2/5                    | —      |
| CD <sub>50</sub> (mg./kg.) | 3.1                        | 3.1   | 1.8                    | ca 300 |

| dose (sc)                  | <i>Ps. aeruginosa</i> D15 |      | doses (sc)                 | <i>Ps. aeruginosa</i> D-113 |      |
|----------------------------|---------------------------|------|----------------------------|-----------------------------|------|
|                            | BB—K26                    | KM—B |                            | BB—K26                      | KM—B |
| 100 mg./kg.                | 5/5                       | 5/5  | 400 mg./kg.                | —                           | 0/5  |
| 25                         | 1/5                       | 0/5  | 200                        | 3/5                         | —    |
| 6.3                        | 1/5                       | 0/5  | 50                         | 1/5                         | —    |
| 1.6                        | 0/5                       | 0/5  | 12.5                       | 0/5                         | —    |
|                            |                           |      | 3.1                        | 0/5                         | —    |
| CD <sub>50</sub> (mg./kg.) | 38                        | 50   | CD <sub>50</sub> (mg./kg.) | 145                         | >400 |

\* KM—B is kanamycin B. The figures shown as 0/5, 1/5, 5/5, etc., indicate the number of surviving animals per five animals challenged; for example, 5/5 indicates 5 of 5 animals surviving the lethal dose of challenge organism when treated with kanamycin B or BB—K26.

The invention includes a pharmaceutical composition, which composition comprises a compound of the above formula IV or a nontoxic pharmaceutically acceptable acid addition salt thereof and a pharmaceutical diluent or carrier.

The invention also includes a method of treating animals other than human beings to cure them of diseases caused by Gram-positive and Gram-negative bacteria, which method comprises administering to the animal an effective dose of a compound of the above formula IV, a nontoxic pharmaceutically acceptable acid addition salt thereof or a composition as defined above.

Following is a description by way of example of the preparation of compounds in accordance with the invention. Examples 1, 2, 5, 7 and 11 relate to the preparation of starting materials.

#### Example 1.

Preparation of L - (-) -  $\gamma$  - Benzyloxycarbonylamino -  $\alpha$  - hydroxybutyric acid (VI).  
L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid (7.4 g., 0.062 mole) was added to a solution of 5.2 g. (0.13 mole) of sodium hydroxide in 50 ml. of water. To the stirred solution was added dropwise at 0—5° C. over a period of 0.5 hour, 11.7 g. (0.068 mole) of carbobenzoxy chloride and the mixture was continued to stir for one hour at the same temperature. The reaction mixture was washed with 50 ml. of ether, adjusted to pH 2 with dilute hydrochloric acid and extracted with four 80-ml. portions of ether. The ethereal extracts were combined, washed with a small amount of saturated sodium chloride solution, dried with anhydrous sodium sulfate and filtered. The filtrate was evaporated *in vacuo* and the resulting residue was crystallized from benzene to give 11.6 g (74%) of colorless plates; melting point 78.5—79.5° C.,  $[\alpha]_D^{25} = 4.5$  (c=2, CH<sub>3</sub>OH). Infrared (IR) [KBr]: IR(KBr)  $\gamma$ C=O 1740, 1690 cm<sup>-1</sup>. Nuclear Magnetic Resonance (NMR) (acetone-d<sub>6</sub>)  $\delta$ (in ppm from TMS) 2.0 (2H,m), 3.29 (2H,d-d, J=6.7 and 12 Hz), 4.16 (1H,d-d, J=4.5 and 8 Hz), 4.99 (2H,s), 6.2 (2H, broad), 7.21 (5H,s).

Anal. calc'd for C<sub>13</sub>H<sub>15</sub>NO<sub>5</sub>: C, 56.91; H, 5.97; N, 5.53.  
Found: C, 56.66; H, 5.97; N, 5.47.

#### Example 2.

N - Hydroxysuccinimide ester of L - (-) -  $\gamma$  - benzyloxycarbonylamino -  $\alpha$  - hydroxybutyric acid (VII).

A solution of 10.6 g. (0.042 mole) of VI and 4.8 g. (0.042 mole) of N - hydroxysuccinimide in 200 ml. of ethyl acetate was cooled to 0° C. and then 8.6 g. (0.042 mole) of dicyclohexylcarbodiimide was added. The mixture was kept overnight in a refrigerator. The dicyclohexylurea which separated was filtered off and the filtrate was concentrated to about 50 ml. under reduced pressure to give colorless crystals of VII which were collected by filtration; 6.4 g., M.p. 121—122.5° C. The filtrate was evaporated to dryness *in vacuo* and the crystalline residue was washed with 20 ml. of a benzene - n - hexane mixture to give an additional amount of VII. The total yield was 13.4 g. (92%).  $[\alpha]_D^{25} = 1.5^\circ$  (c=2, CHCl<sub>3</sub>). IR(KBr)  $\gamma$ C=O 1810, 1755, 1740, 1680 cm<sup>-1</sup>. NMR (acetone - d<sub>6</sub>)  $\delta$ (in ppm from TMS) 2.0 (2H,m), 2.83 (4H,s), 3.37 (2H,d-d, J=6.5 and 12.5 Hz), 4.56 (1H,m), 4.99 (2H,s), 6.3 (2H, broad), 7.23 (5H,s).

Anal. calc'd for C<sub>18</sub>H<sub>19</sub>N<sub>2</sub>O<sub>7</sub>: C, 54.85; H, 5.18; N, 8.00.  
Found: C, 54.79, 54.70; H, 5.21, 5.20; N, 8.14, 8.12.

1. G. W. Anderson et al., J. Am. Chem. Soc., 86, 1839 (1964).

#### Example 3.

Preparation of 1 - [L - (-) -  $\gamma$  - benzyloxycarbonylamino -  $\alpha$  - hydroxybutyryl] - 6' - carbobenzoxykanamycin A (IIIa).

A solution of 1.6 g. (4.6 mmoles) of VII in 40 ml. of ethyleneglycol dimethyl ether (DME) was added dropwise to a stirred solution of 2.6 g. (4.2 mmoles) of 6'-monobenzyloxycarbonylkanamycin A (II) in 40 ml. of 50% aqueous ethyleneglycol dimethyl ether and the mixture was stirred overnight. The reaction mixture was evaporated under reduced pressure to give a brown residue of IIIa which was used for the next reaction without further purification.

#### Example 4.

Preparation of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl] - kanamycin A (IVa).

The crude product IIIa from Example 3 was dissolved in 40 ml. of 50% aqueous dioxane and a small amount of insoluble material was removed by filtration. To the filtrate was added 0.8 ml. of glacial acetic acid and 1 g. of 10% - palladium-on-charcoal and the mixture was hydrogenated at room temperature for 24 hours in a Parr hydrogenation apparatus. The reaction mixture was filtered to remove the palladium catalyst and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 30 ml. of water and chromatographed on a column of CG-50 ion

exchange resin ( $\text{NH}_4^+$  type, 50 cm  $\times$  1.8 cm). The column was washed with 200 ml. of water and then eluted with 800 ml. of 0.1 N  $\text{NH}_4\text{OH}$ , 500 ml. of 0.2 N  $\text{NH}_4\text{OH}$  and finally 500 ml. of 0.5 N  $\text{NH}_4\text{OH}$ . Ten-milliliter fractions were collected and fractions 146 to 154 contained 552 mg. (22%, based on carbobenzoxy kanamycin A, II) of the product IV which was designated BB-K8 lot 2. M.p. 187° (dec.). Relative potency against *B. subtilis* (agar plate) = 560 mcg./mg. (standard: Kanamycin A free base).

A solution of 250 mg of BB-K8 lot 2 in 10 ml. of water was subjected to chromatography on a column of CG-50 ( $\text{NH}_4^+$  type, 30 cm  $\times$  0.9 cm). The column was washed with 50 ml. of water and then eluted with 0.2 N  $\text{NH}_4\text{OH}$ . Ten milliliter fractions were collected. Fractions 50 to 63 were combined and evaporated to dryness under reduced pressure to give 98 mg. of the pure product, K8 lot 2-1. M.p. 194° (dec.)  $[\alpha]_D^{21} +85^\circ$  ( $c=2$ ,  $\text{H}_2\text{O}$ ). Relative potency against *B. subtilis* (agar plate) = 960 mcg./mg. (standard: kanamycin A free base).

Anal. calc'd for  $\text{C}_{22}\text{H}_{43}\text{N}_5\text{O}_{13}\cdot 2\text{H}_2\text{CO}_3$ : C, 40.62; H, 6.68; N, 9.87.  
Found: C, 40.21, 39.79; H, 6.96, 6.87; N, 9.37, 9.49.

#### Example 5.

##### Preparation of N - (Benzyloxycarbonyloxy)succinimide.

N - Hydroxysuccinimide<sup>1</sup> (23 g., 0.2 mole) was dissolved in a solution of 9 g. (0.22 mole) of sodium hydroxide in 200 ml. of water. To the stirred solution was added dropwise 34 g. (0.2 mole) of carbobenzoxy chloride with water-cooling and then the mixture was stirred at room temperature overnight to separate the carbobenzoxy derivative which was collected by filtration, washed with water and air-dried. Yield 41.1 g. (82%). Recrystallization from benzene - n - hexane (10:1) gave colorless prisms melting at 78-79° C.

1. G. W. Anderson et al., J. Am. Chem. Soc., 86, 1839 (1964).

#### Example 6.

##### Preparation of 6' - Carbobenzoxykanamycin A.

A solution of 42.5 g. (90 mmoles) of kanamycin A free base in 450 ml. of water and 500 ml. of dimethylformamide (DMF) was cooled below 0° C. and stirred vigorously. To the solution was added dropwise over a period of about two hours a solution of 22.4 g. (90 mmole) of N - (benzyloxycarbonyloxy)succinimide in 500 ml. of DMF. The mixture was stirred at -10° to 0° C. overnight and then at room temperature for one day. The reaction mixture was evaporated under reduced pressure below about 50° C. The oily residue was dissolved in a mixture of 500 ml. water and 500 ml. butanol, the mixture being filtered to remove insoluble material and separated into two layers. The butanol and aqueous layers were treated with butanol-saturated water (500 ml.  $\times$  2) and water-saturated butanol (500 ml.  $\times$  2), respectively, using a technique similar to counter current distribution. The three aqueous layers were combined and evaporated to dryness under reduced pressure to give an oily residue, a part of which crystallized on standing at room temperature. To the residue including the crystals was added about 100 ml. of methanol, which dissolved the oil and separated it from the crystals. After adding about 300 ml. of ethanol, the mixture was kept at room temperature overnight to give a crystalline mass which was collected by filtration. It weighed 44 g. The product contained a small amount of kanamycin A as indicated by thin layer chromatography using n - propanol - pyridine - acetic acid - water (15:10:3:12) as the solvent system and ninhydrin as the spray reagent.

The crude product was dissolved in 300 ml. of water and chromatographed on a column (30 mm. diameter) of CG-50 ion - exchange resin ( $\text{NH}_4^+$  type, 500 ml.). The column was irrigated with 0.1 N ammonium hydroxide solution and the eluate was collected in 10-ml. fraction. The desired product was contained in tube numbers 10-100, while kanamycin R recovered from slower-moving fractions and the position isomer(s) of the product seemed to be contained in the faster-moving fractions. The fractions 10-110 were combined and evaporated to dryness under reduced pressure to give 24.6 g. (45%) of a colorless product 6 - carbobenzoxykanamycin A (II) [6' - Cbz - kanamycin A], which began to melt and color at 204° C. and decomposed at 212° C. with gas evolution.  $[\alpha]_D +106^\circ$  ( $c=2$ ,  $\text{H}_2\text{O}$ ).

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| TLC (silica gel F <sub>254</sub> ; ninhydrin)<br>Solvent System            | Rf value           |       |             |       |
|----------------------------------------------------------------------------|--------------------|-------|-------------|-------|
|                                                                            | 6'-Coz-Kanamycin A |       | Kanamycin A |       |
| n-PrOH-Pyridine-AcOH-H <sub>2</sub> O<br>(15:10:3:12)                      | 0.42               | 0.33  | 0.15        | 0.04  |
|                                                                            | (main)             |       | minor       |       |
| Acetone-AcOH-H <sub>2</sub> O<br>(20:6:74)                                 |                    | 0.24  |             | 0.14  |
| CHCl <sub>3</sub> -MeOH-c.NH <sub>4</sub> OH-H <sub>2</sub> O<br>(1:4:2:1) |                    | 0.76  |             | 0.50  |
| AcOMe-n-PrOH-c.NH <sub>4</sub> OH<br>(45:105:60)                           |                    | 0.22* |             | 0.04* |

\* Detected by anthrone-sulfuric acid.

The final product was found to be accompanied by two minor components by TLC with one of the solvent systems tested. However, the final product was used without further purification for the preparation of BB-K8 (I).

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#### Example 7.

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Preparation of L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid from ambutyrosin A or B or mixtures thereof.

Ambutyrosin A (5.0 gm.) [U.S. Patent No. 3,541,078, issued November 17, 1970] was refluxed with 160 ml. of 0.5 N sodium hydroxide for one hour. The hydrolysate was neutralized with 6N HCl and chromatographed on a column of CG-50 (NH<sub>4</sub><sup>+</sup> type). The desired L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid was isolated by developing the column with water and removing the water by freeze drying. The L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid is characterized as a crystalline material having a m.p. of 212.5—214.5° C. [Column 2, lines 31—38, U.S. Patent No. 3,541,078].

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#### Example 8.

Preparation of 6' - Carbobenzoxycanamycin B.

To a chilled solution of 8.1 g. (0.0168 mole) of kanamycin B in 120 ml. of water and 80 ml. of 1,2 - dimethoxyethane was added dropwise with stirring a solution of 4.2 g. (0.0168 mole) of N - (benzyloxycarbonyloxy)succinimide in 40 ml. of 1,2-dimethoxyethane. The reaction mixture was stirred overnight and evaporated under reduced pressure. The residue was dissolved in 100 ml. of water and shaken twice with 50 ml. of water-saturated n - butanol. The aqueous layer was separated and adsorbed on a column of 100 ml. of CG-50 (NH<sub>4</sub><sup>+</sup> type). The column was washed with 200 ml. of water, eluted with 0.05 N NH<sub>4</sub>OH. The eluate was collected in 10-ml. fraction. Fractions 121 to 180 were collected, evaporated and freeze-dried to give 1.58 g. (15%) of the desired product. Fractions 1 to 120 were evaporated and re-chromatographed on CG-50 (NH<sub>4</sub><sup>+</sup>) to give 1.21 g. (12%) of the product. M.p. 151—152° C. (dec.).  $[\alpha]_D^{24} +104^\circ$  (C. 2.5, H<sub>2</sub>O).  $\gamma_{0=0}$  1710 cm.<sup>-1</sup>.

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Anal. calc'd. for C<sub>26</sub>H<sub>43</sub>N<sub>4</sub>O<sub>12</sub>: C, 50.56; H, 7.02; N, 11.34.

Found: C, 50.71; H, 7.38; N, 11.48.

TLC (silica gel F254), Rf 0.03 in n - PrOH - AcOH - H<sub>2</sub>O (15:10:3:12); Rf 0.16 in acetone - AcOH - H<sub>2</sub>O (20:6:74).

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#### Example 9.

Preparation of 1 - [L - (-) -  $\gamma$  - benzyloxycarbonylamino -  $\alpha$  - hydroxybutyryl]-6' - carbobenzoxycanamycin B (IIIb).

To a stirred solution of 1.85 g. (3.0 m. moles) of 6' - carbobenzoxycanamycin B in 40 ml. of H<sub>2</sub>O and 50 ml. of 1,2 - dimethoxyethane was added all at once at 5° C. 1.1 g. (3.1 m. moles) of N - (L -  $\gamma$  - carbobenzoxycarbonyl -  $\alpha$  - hydroxybutyryl-oxy)succinimide. The reaction mixture was stirred overnight at room temperature and subjected to hydrogenolysis without isolating the carbobenzoxycarbonyl derivative (IIIb). TLC (silica gel F254), Rf 0.06 (starting material), 0.41, 0.57 (n - PrOH - pyridine-AcOH - H<sub>2</sub>O=15:10:3:12), Rf 0.11 (starting material) 0.21, 0.34, 0.46 (Acetone-AcOH - H<sub>2</sub>O=20:6:74).

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## Example 10.

Preparation of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl] - kanamycin B (IVb).

To the solution obtained in Example 9 was added 0.2 g. of 10% palladium on charcoal. After the mixture had been hydrogenated under atmospheric pressure for five hours, an additional amount of 10% palladium on charcoal (0.1 g.) and 10 ml. of water were added. Hydrogenation was continued overnight. The reaction mixture was filtered, the filtrate was evaporated under reduced pressure, the residue was dissolved in 50 ml. of water and chromatographed on a column of CG-50 ( $\text{NH}_4^+$ , 1.2 cm.  $\times$  50 cm.). The column was washed with 200 ml. of water and then eluted with 500 ml. of 0.1 N  $\text{NH}_4\text{OH}$ , 500 ml. of 0.2 N  $\text{NH}_4\text{OH}$ , 900 ml. of 0.5 N  $\text{NH}_4\text{OH}$  and 500 ml. of 1 N  $\text{NH}_4\text{OH}$ . The effluents were collected in 10-ml. fraction. Kanamycin B was recovered from fractions 60 to 76 in 32% recovery (459 mg.). Fractions 128-138 were collected, evaporated under reduced pressure and freeze-dried to give 318 mg. (17%, based on carbobenzoxy kanamycin B) of BB-K26 (IVb). M.p. 186-187° C. (dec.).  $[\alpha]_D^{25} + 78^\circ$  (C. 1.15,  $\text{H}_2\text{O}$ ).  $\gamma_{\text{C=O}}$  1640  $\text{cm}^{-1}$ .

Anal. calc'd for  $\text{C}_{22}\text{H}_{44}\text{N}_6\text{O}_{12}\cdot\text{H}_2\text{CO}_3$ : C, 42.72; H, 7.17; N, 13.00.

Found: C, 42.23; H, 7.19; N, 12.37.

TLC (silica gel F254, ninhydrin), Rf 0.11 in acetone - AcOH -  $\text{H}_2\text{O}$  (20:6:74); Rf 0.19 in  $\text{CHCl}_3$  - MeOH - concentrate  $\text{NH}_4\text{OH}$  -  $\text{H}_2\text{O}$  (1:4:2:1).

Fractions 201 to 222 were combined, evaporated under reduced pressure and freeze-dried to give 209 mg. (12%) of another active component which was designated BB-K27. M.p. 183-184° C. (dec.)  $\gamma_{\text{C=O}}$  1750  $\text{cm}^{-1}$ .

Anal. calc'd. for  $\text{C}_{22}\text{H}_{44}\text{N}_6\text{O}_{12}\cdot\text{H}_2\text{CO}_3$ : C, 42.72; H, 7.17; N, 13.00.

Found: C, 42.25; H, 6.93; N, 12.18.

TLC (silica gel F254), Rf 0.15 in acetone - AcOH -  $\text{H}_2\text{O}$  (20:6:74); Rf 0.07 in  $\text{CHCl}_3$  - MeOH - concentrate  $\text{NH}_4\text{OH}$  -  $\text{H}_2\text{O}$  (1:5:2:1).

## Example 11.

Preparation of L(-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid from DL -  $\alpha$  - hydroxy -  $\gamma$  - phthalimidobutyric acid.

(A.) *Dehydroabietylammmonium* L -  $\alpha$  - hydroxy -  $\gamma$  - phthalimidobutyrate: To a solution of 25 g. (0.1 mole) of 2 - hydroxy -  $\gamma$  - phthalimidobutyric acid<sup>1</sup> in 200 ml of ethanol was added a solution of 29g. (0.1 mole) of dehydroabietylamine in 130 ml. of ethanol. The solution was shaken vigorously for a minute and stood at room temperature for five hours during which time fine needles crystallized out. The crystals were collected by filtration, washed with 50 ml. of ethanol and air-dried to obtain 30.1 g. (56%) of a diastereomer of the dehydroabietylamine salt. M.p. 93-94° C.  $[\alpha]_D^{24} + 15^\circ$  (C. 2.5, MeOH). Recrystallization from 300 ml. of ethanol gave 23.2 g. (43%) of the pure product. M.p. 94-95° C.  $[\alpha]_D^{24} + 10.8^\circ$  (C. 2.5, MeOH). Further recrystallization did not change the melting point and the specific rotation.

Anal. calc'd for  $\text{C}_{32}\text{H}_{42}\text{N}_2\text{O}_5\cdot\text{H}_2\text{O}$ : C, 69.54; H, 8.02; N, 5.07.

Found: C, 69.58; H, 8.02; N, 5.07.

1. Y. Saito et al., Tetrahedron Letters, 1970, 4863.

(B.) L - (-) -  $\gamma$  - Amino -  $\alpha$  - hydroxybutyric acid: To a solution of 1.5 g. (0.014 mole) of sodium carbonate in 40 ml. of water were added 5.3 g. (0.01 mole) of dehydroabietylammmonium - L -  $\alpha$  - hydroxy -  $\gamma$  - phthalimidobutyrate and 60 ml. of ether. The mixture was shaken vigorously until all of the solid had dissolved. The ether layer was separated. The aqueous solution was washed twice with 20-ml. portions of ether and evaporated to 15 ml. under reduced pressure. To the concentrate was added 10 ml. of concentrated hydrochloric acid and the mixture was refluxed for ten hours. After cooling, separated phthalic acid was removed by filtration. The filtrate was evaporated under reduced pressure. The residue was dissolved in 10 ml. of water and the solution was evaporated to dryness. This operation was repeated twice to remove excess hydrochloric acid. The residual syrup was dissolved in 10 ml. of water and filtered to remove a small amount of insoluble phthalic acid. The filtrate was absorbed in a column of IR-120 ( $\text{H}^+$ , 1 cm.  $\times$  35 cm.), the column was washed with 300 ml. of water and eluted with 1 N ammonium hydroxide solution. The eluate was collected in 15-ml. fraction. The ninhydrin positive fractions 10 to 16 were combined and evaporated under reduced pressure to give a syrup which crystal-

lized gradually. The crystals were triturated with ethanol, filtered and dried in a vacuum desiccator to give 0.78 g. (66%) of L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid M.p. 206—207° C.  $[\alpha]_D^{25}$  -29° (C. 2.5, H<sub>2</sub>O). The IR spectrum was identical with the authentic sample which was obtained from ambutyrosin.

#### Example 12.

Preparation of the Monosulfate Salt of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl]kanamycin A or B.

One mole of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl]kanamycin A or B is dissolved in 1 to 3 liters of water. The solution is filtered to remove any undissolved solids. To the chilled and stirred solution is added one mole of sulfuric acid dissolved in 500 ml. of water. The mixture is allowed to stir for 30 minutes, following which cold ethanol is added to the mixture till precipitation occurs. The solids are collected by filtration and are determined to be the desired monosulfate salt.

#### Example 13.

Preparation of the Disulfate Salt of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl]kanamycin A (BB—K8.2H<sub>2</sub>SO<sub>4</sub>).

Thirty-five grams of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl] - kanamycin A (is the monobicarbonate trihydrate) was dissolved in 125 ml. of deionized water. A pH of approximately 9.0 was noted. The pH was lowered to 7—7.5 with 50% V/V sulfuric acid.

Eight and one half grams of Darco G—60 (activated charcoal) was added and the mixture was slurried at ambient room temperature for 0.5 hour. The carbon was removed by suitable filtration and washed with 40 ml. of water. The water wash was added to the filtrate. (DARCO is a Registered Trade Mark).

The combined filtrate-wash above was adjusted to pH 2—2.6 with 50% V/V sulfuric acid. A large amount of carbon dioxide was evolved. The solution was left with stirring for 20 minutes to expel remaining carbon dioxide.

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 room 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-wash was 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 5 minutes at pH 1—1.3, passed through a 100 mesh screen, stirred for 2 minutes and allowed to settle for 5 minutes. Most of the supernatant was decanted. The remaining slurry was suitably filtered, washed with 200 ml. methanol and vacuum dried at 50° C. for 24 hours. The yield of amorphous BB—K8 (dihydrogen sulfate), was 32—34 grams;  $[\alpha]_D^{25}$  H<sub>2</sub>O = +74.75, decomposition at 220—230° C.

#### Elemental Analysis (On Dry Basis\*)

|       | Found                | Theory |
|-------|----------------------|--------|
| % C   | 32.7, 35.5, 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. A compound having the formula

lized gradually. The crystals were triturated with ethanol, filtered and dried in a vacuum desiccator to give 0.78 g. (66%) of L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyric acid M.p. 206—207° C.  $[\alpha]_D^{24}$  -29° (C. 2.5, H<sub>2</sub>O). The IR spectrum was identical with the authentic sample which was obtained from ambutyrosin.

Example 12.

Preparation of the Monosulfate Salt of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl]kanamycin A or B.

One mole of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl]kanamycin A or B is dissolved in 1 to 3 liters of water. The solution is filtered to remove any undissolved solids. To the chilled and stirred solution is added one mole of sulfuric acid dissolved in 500 ml. of water. The mixture is allowed to stir for 30 minutes, following which cold ethanol is added to the mixture till precipitation occurs. The solids are collected by filtration and are determined to be the desired monosulfate salt.

Example 13.

Preparation of the Disulfate Salt of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl]kanamycin A (BB—K8.2H<sub>2</sub>SO<sub>4</sub>).

Thirty-five grams of 1 - [L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl] - kanamycin A (is the monobicarbonate trihydrate) was dissolved in 125 ml. of deionized water. A pH of approximately 9.0 was noted. The pH was lowered to 7—7.5 with 50% V/V sulfuric acid.

Eight and one half grams of Darco G—60 (activated charcoal) was added and the mixture was slurried at ambient room temperature for 0.5 hour. The carbon was removed by suitable filtration and washed with 40 ml. of water. The water wash was added to the filtrate. (DARCO is a Registered Trade Mark).

The combined filtrate-wash above was adjusted to pH 2—2.6 with 50% V/V sulfuric acid. A large amount of carbon dioxide was evolved. The solution was left with stirring for 20 minutes to expel remaining carbon dioxide.

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 room 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-wash was 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 5 minutes at pH 1—1.3, passed through a 100 mesh screen, stirred for 2 minutes and allowed to settle for 5 minutes. Most of the supernatant was decanted. The remaining slurry was suitably filtered, washed with 200 ml. methanol and vacuum dried at 50° C. for 24 hours. The yield of amorphous BB—K8 (dihydrogen sulfate)<sub>2</sub> was 32—34 grams;  $[\alpha]_D^{22}$  H<sub>2</sub>O = +74.75, decomposition at 220—230° C.

Elemental Analysis (On Dry Basis\*)

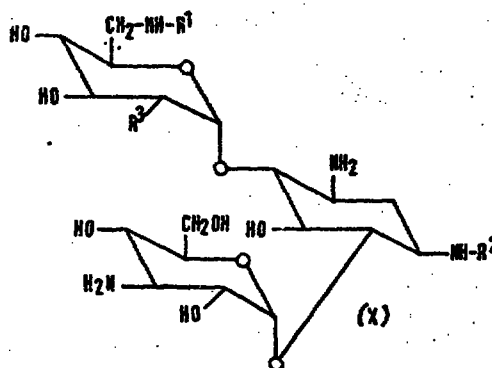
|       | Found                | Theory |
|-------|----------------------|--------|
| % C   | 32.7, 35.5, 32.3     | 33.5   |
| % N   | 8.78, 8.7, 8.2, 8.8  | 8.97   |
| % S   | 8.75, 8.9, 7.8, 8.85 | 8.2    |
| % Ash | nil                  | —      |

\*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. A compound having the formula





wherein  $R^1$  is H or  $C_6H_5-CH_2-O-C(=O)-$ ,  $R^2$  is H or L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl or L - (-) -  $\gamma$  - benzyloxycarbonylamino -  $\alpha$  - hydroxybutyryl,  $R^3$  is OH or  $NH_2$ , and at least one of  $R^1$  and  $R^2$  is other than H.

5

2. A compound as claimed in claim 1, wherein  $R^1$  is  $C_6H_5-CH_2-O-C(=O)-$ ,  $R^2$  is H and  $R^3$  is  $NH_2$ .

5

3. A compound as claimed in claim 1, wherein  $R^1$  is  $C_6H_5-CH_2-O-C(=O)-$ ,  $R^2$  is H and  $R^3$  is OH.

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4. A compound as claimed in claim 1, wherein  $R^1$  is  $C_6H_5-CH_2-O-C(=O)-$ ,  $R^2$  is L - (-) -  $\gamma$  - benzyloxycarbonylamino -  $\alpha$  - hydroxybutyryl and  $R^3$  is OH.

10

5. A compound as claimed in claim 1, wherein  $R^1$  is  $C_6H_5-CH_2-O-C(=O)-$ ,  $R^2$  is L - (-) -  $\gamma$  - benzyloxycarbonylamino -  $\alpha$  - hydroxybutyryl and  $R^3$  is  $NH_2$ .

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6. A compound as claimed in claim 1, wherein  $R^1$  is H,  $R^2$  is L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl and  $R^3$  is OH.

7. A compound as claimed in claim 1, wherein  $R^1$  is H,  $R^2$  is L - (-) -  $\gamma$  - amino -  $\alpha$  - hydroxybutyryl and  $R^3$  is  $NH_2$ .

15

8. A nontoxic pharmaceutically acceptable acid addition salt of a compound as claimed in any one of the preceding claims.

20

9. A salt as claimed in claim 8 with acetic, hydrochloric, sulfuric, mandelic, maleic, phosphoric, nitric, hydrobromic, ascorbic, malic or citric acid.

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10. A monosulfate or disulfate salt of a compound as claimed in claim 6 or claim 7.

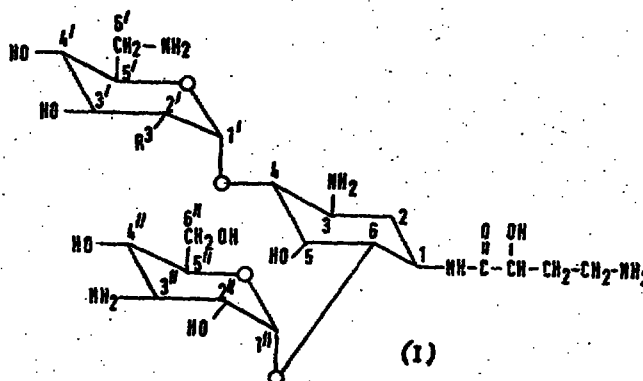
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11. A compound as claimed in claim 6 or claim 7 or a salt as claimed in claim 10 in its monohydrate form.

12. A compound as claimed in claim 1 and substantially as hereinbefore described in any one of the specific Examples.

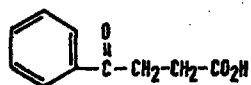
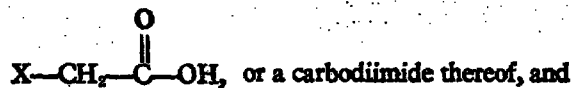
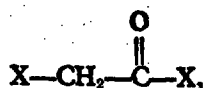
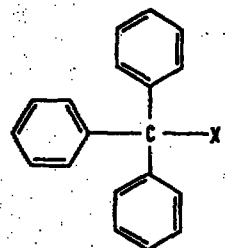
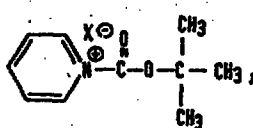
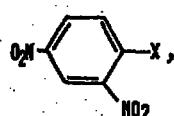
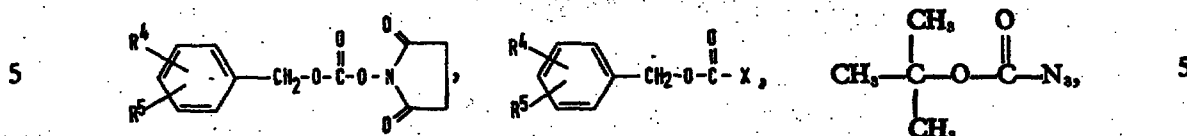
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13. A process for the preparation of a compound having the formula



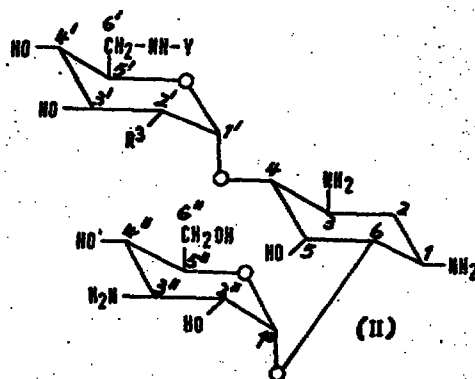
in which  $R^3$  is OH or  $NH_2$ ; or a nontoxic pharmaceutically acceptable acid addition salt thereof; which process comprises the consecutive steps of

A) acylating kanamycin A or kanamycin B with an acylating agent having one of the formulae:

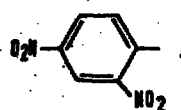
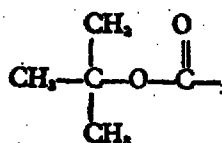
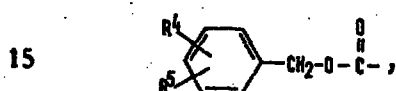


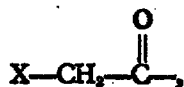
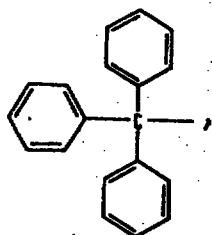
or a carbodiimide thereof,

in which  $R^4$  and  $R^5$  are alike or different and each is H, F, Cl, Br,  $NO_2$ , OH, (lower)-alkyl or (lower)alkoxy and X is chloro, bromo or iodo, in a ratio of 1 mole or less of acylating agent per mole of kanamycin A or B, in a solvent, at a temperature below  $50^\circ C$ . to produce the compound having the formula

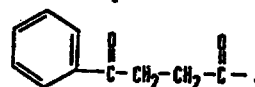


in which  $R^3$  is as defined above and  $\gamma$  is a radical of one of the formulae:





and

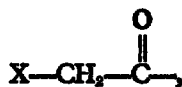
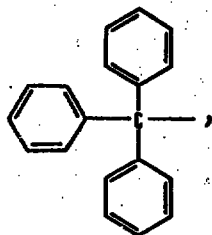
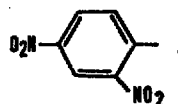
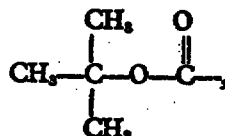
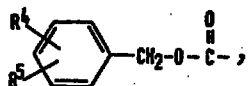


in which R<sup>4</sup> and R<sup>5</sup> are as defined above:

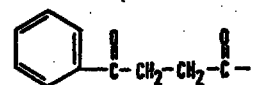
B) acylating the compound of formula II with an acylating agent having the formula



5 said acylating agent being derived from L - (—) - γ - amino - α - hydroxybutyric acid, in which W is a radical of one of the formulae: 5

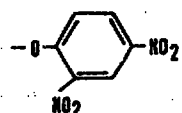
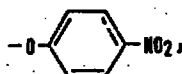
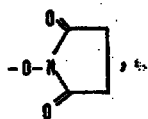


and

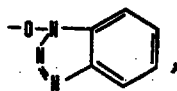


and M is a radical of one of the formulae:

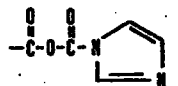
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and



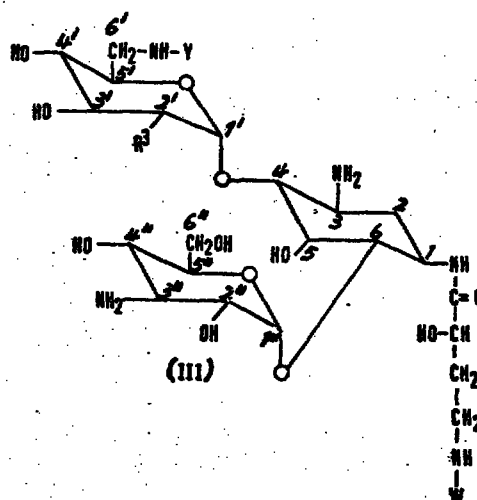
in which R<sup>4</sup> and R<sup>5</sup> are as defined above, in a ratio of at least 0.5 mole of acylating agent per mole of the compound II, in a solvent, to produce a compound of the formula





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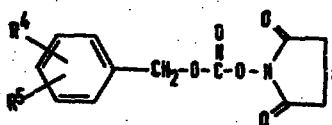
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in which  $R^3$ ,  $\gamma$  and  $W$  are as defined above; and

C) removing the blocking groups  $W$  and  $Y$  to produce the compound of formula I and, if desired, subsequently converting the product into a nontoxic pharmaceutically acceptable acid addition salt thereof.

14. A process as claimed in claim 13 wherein in step A the kanamycin A or B is reacted with an acylating agent of the formula

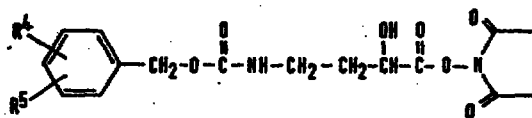


wherein  $R^4$  and  $R^5$  are as defined in claim 13.

15. A process as claimed in claim 13 or claim 14, wherein the solvent employed in step A is dimethylformamide, dimethylacetamide, tetrahydrofuran, dioxane, 1,2-dimethoxyethane, methanol, ethanol, water, acetone, pyridine, an N - (lower) alkyl-piperidine, or mixtures thereof.

16. A process as claimed in any one of claims 13 to 15 wherein step A is carried out at a temperature below 25° C.

17. A process as claimed in any one of claims 13 to 16, wherein in step B the acylating agent has the formula



wherein  $R^4$  and  $R^5$  are as defined in claim 13.

18. A process as claimed in any one of claims 13 to 17, wherein in step B there are employed from 0.5 to 1.4 moles of acylating agent per mole of compound II.

19. A process as claimed in claim 18, wherein in step B there are employed from 0.8 to 1.1 moles of acylating agent per mole of compound II.

20. A process as claimed in any one of claims 13 to 19, wherein in step B the solvent is a mixture of water and ethyleneglycol dimethyl ether, dioxane, dimethylacetamide, dimethylformamide, tetrahydrofuran or propyleneglycol dimethyl ether.

21. A process as claimed in claim 20, wherein in step B the solvent is a 1:1 mixture of water and ethylene glycol dimethyl ether.

22. A process as claimed in any one of claims 13 to 21, wherein in step C the blocking  $W$  and  $Y$  are removed by hydrogenating compound III with hydrogen in the presence of a metal catalyst in a water-water miscible solvent system.

23. A process as claimed in claim 22, wherein the metal catalyst is palladium, platinum, Raney nickel, rhodium, ruthenium or nickel.



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24. A process as claimed in claim 23, wherein the catalyst is palladium on charcoal.

25. A process as claimed in any one of claims 22 to 24, wherein the solvent system is water and dioxane, tetrahydrofuran, ethylene glycol dimethyl ether, or propylene glycol dimethyl ether.

26. A process as claimed in claim 25, wherein the solvent is a 1:1 mixture of water and dioxane.

27. A process as claimed in any one of claims 22 to 26, wherein the hydrogenation is carried out in the presence of a catalytic amount of glacial acetic acid.

28. A process as claimed in claim 13 and substantially as hereinbefore described with reference to any one of Examples 3, 4, 6, 8 to 10, 12 or 13.

29. A compound of the formula I or a nontoxic pharmaceutically acceptable acid addition salt thereof when prepared by a process as claimed in any one of claims 13 to 27.

30. A pharmaceutical composition, which composition comprises a compound as claimed in claim 6, claim 7, claim 11 or claim 29, or a salt as claimed in claim 8 or claim 9 when appendent to claim 6 or claim 7, or a salt as claimed in claim 10 and a pharmaceutical diluent or carrier.

31. A method of treating animals other than human beings to cure them of diseases caused by Gram-positive or Gram-negative bacteria, which method comprises administering to the animal an effective dose of a compound as claimed in claim 6, claim 7, claim 11 or claim 29, or a salt as claimed in claim 8 or claim 9 when appendent to claim 6 or claim 7, or a salt as claimed in claim 10 or a composition as claimed in claim 31.

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## ERRATA

SPECIFICATION No. 1,401,221

Page 18, line 26, for (1:5:2:1). read  
(1:4:2:1).

Page 18, line 41, for H, 8.02; read H, 8.08;

THE PATENT OFFICE  
14th November, 1975