

PATENT SPECIFICATION

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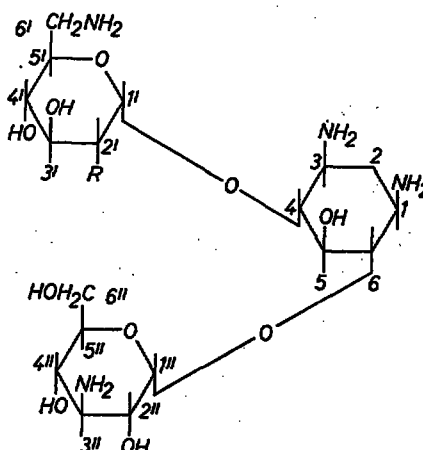


(54) 3'-4'-DIDEOXYKANAMYCIN B ACTIVE AGAINST
 RESISTANT BACTERIA, AND ITS SYNTHESIS

(71) We, ZAIDAN HOJIN BISEIBUTSU KAGAKU KENKYU KAI, a Company organized and existing under the laws of Japan of No. 14-23, 3-chome, Kami-Osaki, Shinagawa-ku, Tokyo, Japan, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to 3',4'-dideoxykanamycin B which is antibacterial and especially active against infection by kanamycin resistant organisms; and to the synthesis thereof. According to one aspect of the present invention there is provided a process for the synthesis of 3',4'-dideoxykanamycin B starting from Kanamycin B which comprises protecting the amino and all or a part of the hydroxyl groups other than 3' and 4'-hydroxyl groups of kanamycin B by a conventional method, sulfonylating the 3' and 4'-hydroxyl groups to afford a derivative having disulfonic ester groups, removing the 3',4' disulfonic esters by known methods, to give a 3',4'-unsaturated compound, reducing the 3',4'-unsaturated compound and removing the residual protecting groups. This invention is based on the findings discovered by a series of researches done by H. Umezawa et al, who demonstrated the mechanism of the drug-inactivation by the resistant bacteria isolated from patients and who then attempted to find, based on the mechanism, new antibiotic derivatives active against drug-resistant bacteria. The invention includes the compound *per se*.

Hamao Umezawa et al (reported in "Science", 157 1559 (1967)) found that drug-resistant gram-negative bacteria carrying R factor of patient-origin, resistant *Staphylococci* and resistant *Pseudomonas aeruginosa* produced enzymes that inactivate kanamycin by transferring phosphate from ATP to 3'-hydroxyl group of kanamycin.

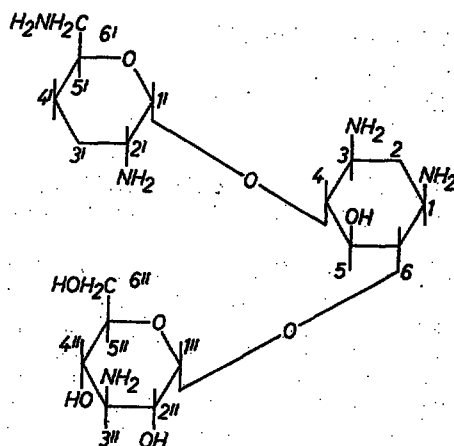


Kanamycin R=OH
 Kanamycin B β R=NH₂

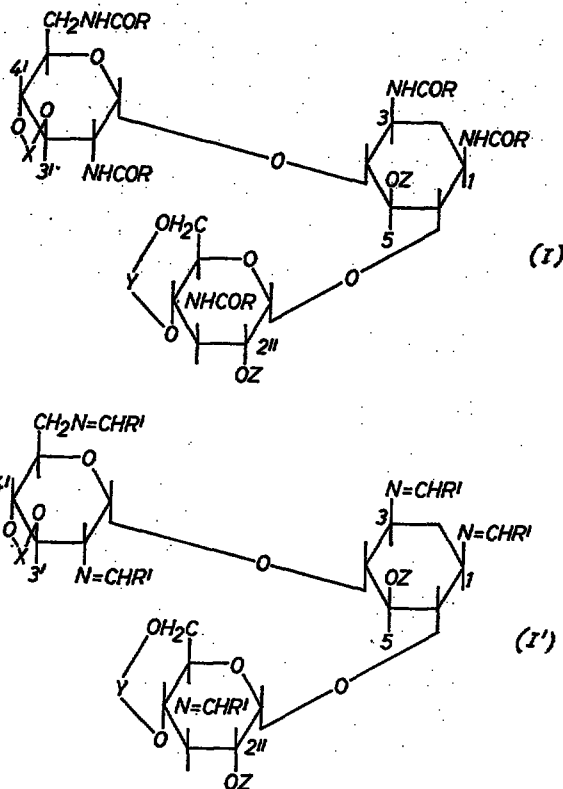
Based on these findings, the inventors first attempted to block the 3'-hydroxyl group and 3'-O-methylkanamycin was synthesized; but the derivative had no antibacterial activity. 3'-Deoxykanamycin was, in the next step, synthesized and it was found that the derivative was active against the above-described resistant bacteria.

[Price 25p]

Therefore, it was roughly concluded that the replacement of the hydroxyl group, which is phosphorylated by resistant bacteria, with hydrogen will give the antibiotic the necessary character to attack the resistant bacteria. The inventors applied the principle to kanamycin B and highly active 3',4'-dideoxykanamycin B (the formula is shown below) was prepared.



The synthetic route is as follows: Starting material, kanamycin B is transformed to the kanamycin B derivative as shown in the general formula (I) or (I'), in which five aminogroups, 4'',6''-hydroxyl groups and all or a part of 5,3',4' and 2''-hydroxyl groups are protected.



R = H, alkyl, aralkyl, aryl, alkyloxy, aralkyloxy or aryloxy group.
R' = H, alkyl, aralkyl or aryl group.
X = H, or is represented by



(in which A and B are respectively, H, alkyl or aryl group) or



Y is represented by



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(in which A and B are respectively, H, alkyl or aryl group) or

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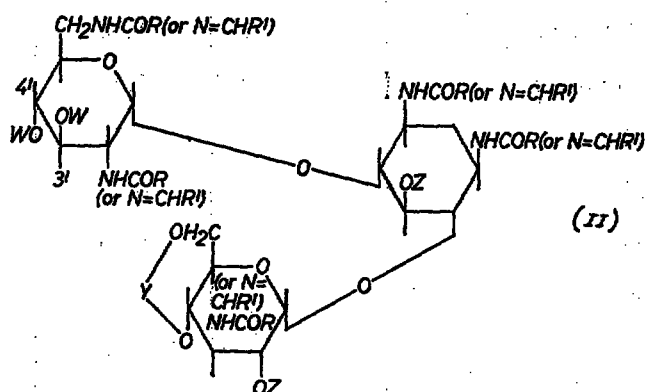


Z=H, acyl, alkyl, aralkyl, aryl group or alkylsulfonyl, aralkylsulfonyl or arylsulfonyl group.

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The derivative (I) or (I') is then transformed to the derivative as shown in the general formula (II) (W=H), in which 3' and 4'-hydroxyl groups are liberated, and the derivative is then transformed to the derivative

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W=H, alkylsulfonyl, aralkylsulfonyl or arylsulfonyl group.

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R, R', Y and Z are the same as described in the general formula (I) or (I').

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as shown in the general formula II (W=SO₂Q, Q: alkyl, aralkyl or aryl group), in which the 3' and 4'-hydroxyl groups are sulfonylated. The derivative (II) (W=SO₂Q) is then treated with iodide ion and a metal powder which acts as a reducing agent such as zinc dust in an appropriate solvent giving the derivative having a 3',4'-unsaturated group. The protecting groups of the resulting derivative, after hydrogenation of the unsaturated group, are removed in a known manner to give the final product, 3',4'-dideoxykanamycin B.

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The synthesized 3',4'-dideoxykanamycin B inhibits the growth of many kinds of bacterial strains; having approximately the same utility as that of kanamycin B, as shown in Table 1. Moreover the said 3',4'-dideoxykanamycin B inhibits the growth of the strains of kanamycin and kanamycin B resistant *Staphylococci*, resistant *Escherichia coli* and resistant *Pseudomonas aeruginosa*. 3',4'-Dideoxykanamycin B shows a therapeutic effect against the infections caused by *Staphylococci*, *Klebsiella pneumoniae*, *Salmonella typhosa* and *Pseudomonas aeruginosa* in mice. 3',4'-Dideoxykanamycin B has low toxicity (LD₅₀, 180 mg/kg mouse, iv.) and shows a high blood level after injection, thus proving the derivative to be a valuable chemotherapeutic agent for treatment of many kinds of infections including those caused by gram-negative and gram-positive resistant bacteria.

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It shows the strong effect in inhibiting microorganisms including pathogenic organisms as shown below.

TABLE 1

Antibacterial spectra of 3',4'-dideoxykanamycin B and kanamycin B

Test Organisms*	Minimal inhibitory concentration (mcg/ml)		
	3',4'-Dideoxykanamycin B		Kanamycin B
	Sample 1	Sample 2	
<i>Staphylococcus aureus</i> FDA 209 P	1.25	0.78	0.78
<i>Escherichia coli</i> NIHJ	2.5	1.56	1.56
K-12 CS-2	2.5	1.56	0.78
K-12 ML 1629	2.5	1.56	>50
K-12 ML 1630	2.5	3.12	>50
K-12 ML 1410		1.56	0.78
<i>Pseudomonas aeruginosa</i> A3	2.5	3.12	50
No. 11		3.12	>50
No. 12		6.25	50
No. 39		6.25	>50
No. 45	1.25	0.78	25
No. 67		6.25	>50
<i>Proteus rettgeri</i>			
GN 311		12.5	3.12
GN 466	2.5	3.12	1.56

* Nutrient agar, 37°C, 18 hours.

In the next, examples of the preparation of 3',4'-dideoxykanamycin B are shown. Since 3',4'-dideoxykanamycin B synthesized was proved effective for the treatment of a wide variety of infections, this invention is not limited to the preparations of 3',4'-dideoxykanamycin B as shown in the examples, but includes all modified procedures as claimed.

Example 1.

The synthesis of penta-N-ethoxycarbonylkanamycin B. To a stirred mixture of kanamycin B free base (1.0 g) and anhydrous sodium carbonate (0.9 g) in aqueous acetone (1:1, 20 ml), ethoxycarbonyl chloride (1.05 ml) was added and the stirring was continued for 5 hours at room temperature. The resulting precipitates were filtered, washed with water and dried to give a solid (1.46 g), mp 305° (decomp.), $[\alpha]_D^{25} + 88^\circ$ (c, 0.5; dimethylformamide).

Example 2.

The synthesis of penta-N-ethoxycarbonyl-3',4'; 4'',6''-di-O-isopropylidene-kanamycin B [in general formula (I), $R = OC_2H_5$, $X = Y = CH(CH_3)_2$, $Z = H$].

The penta-N-ethoxycarbonylkanamycin B (13.7 g) prepared by the procedure described in example 1 was dissolved in dimethylformamide (70 ml) and the solution, after addition of 2,2-dimethoxypropane (18.8 g) and anhydrous *p*-toluenesulfonic acid

(0.35 g), was heated at 65° C. for 1 hour. The resulting solution was concentrated to approximately 50 ml and, after addition of 2,2-dimethoxypropane (30 g), the solution was again heated at 65° C. for 1 hour. Triethylamine (6 ml) was added and the solution was poured into a stirred mixture of benzene (500 ml) and water (500 ml), whereupon a solid precipitated (Solid A). After removal of the Solid A by filtration, the benzene layer was allowed to stand. The precipitates obtained were filtered, washed with benzene and water and dried to give a solid, 3.5 g, mp 236—237° C., $[\alpha]_D^{20} + 87^\circ$ (c=1, dimethylformamide).

The Solid A obtained (8.1 g) was proved to be penta-N-ethoxycarbonyl-4'',6''-O-isopropylidenekanamycin B and the compound could be converted to penta-N-ethoxycarbonyl-3',4',6''-di-O-isopropylidenekanamycin B by the same treatment as described above.

Example 3.

The synthesis of 2''-O-benzoyl-penta-N-ethoxycarbonyl-3',4',4'',6''-di-O-isopropylidenekanamycin B [in general formula (I), R=OC₂H₅, X=Y=CH(CH₃)₂, Z at C—2'' is COC₂H₅ and Z at C—5 is H].

The kanamycin B derivative (3.24 g) prepared by the procedure described in example 2 was dissolved in pyridine (48 ml) and to the solution, benzoyl chloride (2 g) was added. After allowing to stand at room temperature for 1 hour, the solution was evaporated and the residue was dissolved in chloroform. The solution was washed with water, dried over sodium sulfate and concentrated to approximately 20 ml. Addition of n-hexane gave crystals, 3.35 g, mp 205—209° C., $[\alpha]_D^{21} + 114^\circ$ (c=1, dimethylformamide).

Example 4.

The synthesis of 2''-O-benzoyl-penta-N-ethoxycarbonyl-4'',6''-O-isopropylidenekanamycin B.

The kanamycin B derivative (2.82 g) prepared by the procedure described in example 3 was dissolved in aqueous acetic acid (1:3 by volume 40 ml) and the solution was heated at 95° C. for 30 minutes. The resulting solution was evaporated and the deacetonated product obtained (2.58 g) was dissolved in dimethylformamide (15 ml) and, after addition of 2,2-dimethoxypropane (0.65 g) and anhydrous p-toluenesulfonic acid (55 mg), the solution was allowed to stand at room temperature for 1 hour. The solution was poured into water and the resulting precipitates were filtered, washed with water and treated with benzene to give a benzene-insoluble solid, 2.01 g, mp 285—287° C., $[\alpha]_D^{21} + 105^\circ$ (c 1, dimethylformamide); NMR spectrum (in dimethylsulfoxide-d₆): ν 8.72 and 8.60 (each 3-proton singlet assignable to isopropylidene protons), ν 1.5—2.8 (5-proton multiplet assignable to benzoyl protons).

Example 5.

The synthesis of 2''-O-benzoyl-penta-N-ethoxycarbonyl-4'',6''-O-isopropylidene-3',4'-di-O-methylkanamycin B [in general formula II, R=OC₂H₅, Y=CH(CH₃)₂, W=SO₂CH₃, Z at C—2'' is COC₂H₅ and Z at C—5 is H].

The kanamycin B derivative (1.18 g) prepared by the procedure described in example 4 was dissolved in pyridine (15 ml) and the solution, after addition of methanesulfonyl chloride (CH₃SO₂Cl, 0.66 g), was allowed to stand at room temperature for 1.5 hours. The solution was concentrated to one-third of the original volume and the solution was poured into water. Resulting precipitates were filtered and washed with water to give a solid, 1.32 g, mp 198° C., $[\alpha]_D^{21} + 107^\circ$ (c 1.5, dimethylformamide); NMR spectrum (in pyridine-d₅): ν 6.60 and 6.45 (each 3-proton singlet assignable to methyl protons).

Example 6.

The synthesis of 2''-O-benzoyl-3',4'-dideoxy-3',4'-didehydro-penta-N-ethoxycarbonyl-4'',6''-O-isopropylidenekanamycin B.

To a solution of the kanamycin B derivative (1.0 g), prepared by the procedure described in example 5, in dimethylformamide (20 ml), sodium iodide (11 g) and zinc dust (5 g) were added and the mixture was heated at 95° C. for 1 hour under vigorous stirring. The mixture was poured into a hot mixture of chloroform (100 ml) and water (100 ml) under stirring and the chloroform layer isolated was washed with water, dried over sodium sulfate and evaporated to give a solid (940 mg). The solid was treated with hot ethyl acetate and the solvent-insoluble solid (310 mg) was recrystallized from a mixture of methanol-chloroform-ethyl acetate, mp 282—284° C., $[\alpha]_D^{22} + 36^\circ$ (c 0.4, dimethylformamide); NMR spectrum (in pyridine-d₅): ν 4.06 (2-proton a little broadened singlet, H—3' and 4').

Example 7.

The synthesis of 2''-O-benzoyl-3',4'-dideoxy-penta-N-ethoxycarbonyl-4'',6''-O-isopropylidenekanamycin B.

A solution of the kanamycin B derivative (1.15 g), prepared by the procedure described in example 6, in a mixture of *p*-dioxane (45 ml), methanol (25 ml) and water (25 ml) was hydrogenated with hydrogen under 2 atm. pressure in the presence of platinum oxide (0.3 g), which was preactivated before use, at room temperature for 5 hours. The resulting solution was filtered and evaporated to give a colorless powder, 1.10 g, mp 254–256° C., $[\alpha]_D^{22} + 87^\circ$ (c 0.2, dimethylformamide); NMR spectrum (in pyridine- d_5): ν 7.8–8.5 (4-proton broad signal, H–3',4').

Example 8.

The synthesis of 2''-O-benzoyl-3',4'-dideoxy-penta-N-ethoxycarbonylkanamycin B.

The kanamycin B derivative (980 mg) prepared by the procedure described in example 7 was dissolved in a mixture of acetic acid (8 ml) and water (5 ml) and the solution was heated at 100° C. for 5 minutes. Resulting precipitates were filtered and dried, 750 mg, mp 247–248° C., $[\alpha]_D^{23} + 89^\circ$ (c=2.7, dimethylformamide). Found: C, 52.41; H, 6.54; N, 7.48%. Calcd. for $C_{40}H_{61}N_5O_{10}$: C, 52.45; H, 6.71; N, 7.65%.

Example 9.

The synthesis of 3',4'-dideoxykanamycin B. The kanamycin B derivative (150 mg), prepared by the procedure described in example 8, and barium hydroxide octahydrate (2.3 g) were added to a mixture of water (5 ml) and *p*-dioxane (4 ml), and the resulting mixture was heated in a boiling water bath for 8 hours. Carbon dioxide was introduced and the resulting precipitates were removed by centrifuge. The supernatant layer isolated was evaporated. The residue obtained was dissolved in water and the solution, after filtration, was chromatographed on a column of Amberlite (Registered Trade Mark) IRC-50 (H^+ form) with 0.1–0.4N ammonium hydroxide. The fraction containing 3',4'-dideoxykanamycin B was concentrated. Addition of acetone gave crystals, 47 mg, $[\alpha]_D^{20} + 130^\circ$ (c 0.65, water). Found: C, 47.71; H, 8.38; N, 15.31%. Calcd. for $C_{18}H_{27}N_5O_8$: C, 47.88; H, 8.26; N, 15.51%.

Example 10.

The synthesis of penta-N-salicylidenekanamycin B.

To a suspension of kanamycin B base (400 mg) in aqueous methanol (1:8, 20 ml), salicylaldehyde (520 mg) was added and the resulting solution was poured into water. Precipitates were filtered and dried yielding 750 mg.

Example 11.

The synthesis of 3',4';4'',6''-di-O-cyclohexylidene-penta-N-salicylidenekanamycin B (in general formula I', $R=C_6H_4(OH)$, $X=Y=(CH_2)_5C$, $Z=H$).

Penta-N-salicylidenekanamycin B (700 mg) prepared by the procedure described in example 10 was dissolved in dry dimethylformamide (12 ml) and the solution, after addition of cyclohexanone dimethylketal (500 mg) and anhydrous *p*-toluenesulfonic acid (30 mg), was heated at 50° C. for 1 hour, and then a small amount of the solvent was removed by evaporation in vacuo. The resulting solution was poured into water. The resultant precipitates were filtered and dried to yield 710 mg.

Example 12.

The synthesis of 2''-O-benzoyl-3',4';4'',6''-di-O-cyclohexylidene-penta-N-salicylidenekanamycin B (in general formula I', $R^1=C_6H_4(OH)$, $X^1=Y^1=(CH_2)_5C$, $Z=COC_6H_5$).

The kanamycin B derivative (590 mg) prepared by the procedure described in example 11 was dissolved in pyridine (10 ml) and to the solution, benzoyl chloride (120 mg) was added. After allowing to stand at room temperature for 1 hour, the solution was poured into water. The resultant precipitates were filtered and dried to yield 612 mg.

Example 13.

The synthesis of 2''-O-benzoyl-4'',6''-O-cyclohexylidene-penta-N-ethoxycarbonylkanamycin B.

A mixture of the kanamycin B derivative (600 mg), prepared by the procedure described in example 12, in aqueous acetic acid (1:3, 20 ml) was heated at 95° C. for 30 minutes. The reaction mixture was evaporated and the residue was treated with

ether. The ether insoluble part was dissolved in aqueous acetone (1:1, 15 ml) and to the solution, after addition of anhydrous sodium carbonate (400 mg), ethoxycarbonyl chloride (270 mg) was added and stirring was continued for 5 hours at room temperature. The resulting precipitates were filtered, washed with water and dried to give a solid (515 mg), $[\alpha]_D^{20} +98^\circ$ (c 1.5, dimethyl-formamide). The substance could be transformed to 3',4'-dideoxykanamycin B by a series of similar procedures described in examples 5—9.

WHAT WE CLAIM IS:—

1. The compound 3',4'-dideoxykanamycin B.
2. A process for the synthesis of 3',4'-dideoxykanamycin B starting from the kanamycin B which comprises protecting the amino and all or a part of the hydroxyl groups other than 3' and 4'-hydroxyl groups of kanamycin B by a conventional method, sulfonylating the 3' and 4'-hydroxyl groups to afford a derivative having disulfonic ester groups, removing the 3',4'-disulfonic esters by known methods to give a 3',4'-unsaturated compound, reducing the 3',4'-unsaturated compound and removing the residual protecting groups.
3. A process as claimed in Claim 2 and substantially as described in any one of the specific examples hereinbefore set forth.
4. 3',4'-dideoxykanamycin B whenever prepared by the method claimed in either of Claims 2 and 3.

For the Applicants,

F. J. CLEVELAND & COMPANY,
Chartered Patent Agents,

Lincoln's Inn Chambers, 40/43, Chancery Lane, London, WC2A 1JQ.

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For the Applicants,
F. J. CLEVELAND & COMPANY,
Chartered Patent Agents,
Lincoln's Inn Chambers, 40/43, Chancery Lane, London, WC2A 1JQ.

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