PROPERTIES OF GLYCOSIDES OF NEAMINE, KANAMYCIN A AND GENTAMICIN C_1

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Bioassays of glycosides of neamine, kanamycin A and gentamicin C_1 showed that against most susceptible bacteria the potency was between 6 and 40 % that of the parent compound. These alkali-labile and acid-stable glycosides appeared to be N-glycosides.

Although many derivatives have been prepared of a number of the aminoglycoside antibiotics[1] few reports are available on the preparation of glycosides of this group[2]. We have prepared by chemical procedures glucosides, maltosides, galactosides, lactosides, mannosides, rhamnosides, and ribosides of the aminoglycoside antibiotics, neamine, kanamycin A, and gentamicin C_1. While the derivatives do not have higher biopotency *in vitro* than the parent compound, most have significant activity against the test organisms.

Materials and Methods

Antibiotics: Neamine was prepared by acid hydrolysis of neomycin B[3]. Kanamycin A was the gift of Bristol Laboratories. Schering Corporation furnished gentamicin C_1.

Carbohydrates: Glucose was obtained from Matheson Coleman and Bell Company, maltose from Fisher Scientific Company, and galactose from Pfanstiehl Laboratories, Inc. Sigma Chemical Company was the source of the rhamnose, ribose, and mannose. All sugars were used without further purification. Maltose-U-^{14}C was purchased from Amersham/Searle.

Bioassays: Antibiotic potency was determined using 2-fold dilution tests in MUELLER-HINTON Broth. All tests were incubated for 24 hours (or overnight) at 37°C. The test organisms included *Staphylococcus aureus* FDA 209P, *Escherichia coli* W, *Enterobacter cloacae* (Bristol), *Proteus morganii* (Bristol), *Proteus mirabilis* (Schering), *Serratia marcesens* (Bristol), *Serratia* species (Schering), *Diplococcus pneumoniae* (Bristol), and *Pseudomonas aeruginosa* (ATCC 10145).

Physical measurements: Optical rotations were measured in a Bendix series 100 Automatic Polarimeter equipped with a 1-cm cell, and using solutions dissolved in distilled water. Microanalyses were performed by the Spang Microanalytical Laboratories, Ann Arbor, Michigan.

Preparation of glycosides: Aminoglycoside antibiotic and sugar were dissolved in McILVAINE's buffer or phosphate buffer so that the molar ratio of aminoglycoside antibiotic to sugar was 0.3. After dissolving the sugars and antibiotic the pH was adjusted to between 7.6 and 8.0 by addition of NaOH or HCl. The solutions were incubated at 30°C or 37°C (depending upon the antibiotic) for 48~72 hours. In most experiments the concentration of sugar was 4 g/40 ml of buffer.

Analyses of incubation mixtures: Samples from the incubation mixtures were analyzed by paper ionophoresis at pH 1.9 (formic acid - acetic acid solution) with a current of 50 volts/cm for 20~40 minutes (see ref. 4) The location of the aminoglycoside components was determined by spraying with ninhydrin reagents and by bioautography using *S. aureus* FDA 209P. Those samples where radioactive maltose-U-^{14}C was a component (to form kanamycin-

maltoside-^{14}C) were treated with DAVIES' method[5] which included adsorption on phosphocellulose paper followed by counting in a Packard Liquid Scintillation Counter using BRAY's solution[6] as scintillation liquid.

Isolation of aminoglycoside antibiotic glycosides: The pH of the reaction mixture was adjusted to pH 8.0 with NaOH and the sample diluted to about 30 mg/ml aminoglycoside. The diluted solution was passed over a 1.1×50 cm column of CG-50 (NH_4^+ cycle) resin. The column was washed with 50 ml of distilled water and the aminoglycoside antibiotics eluted using one liter of $0 \sim 0.5$ N gradient of NH_4OH (10 ml fractions were usually collected). The fraction collector and the column were located in a room maintained at $4 \sim 6$°C. The glycoside of the antibiotic was often found in fractions 25 to 40 while the untransformed antibiotic was eluted later. The fractions containing the derivative were pooled and lyophilized to give white powders. All preparations appeared to be contaminated with ammonium carbonate (frequently a problem with aminoglycoside antibiotics).

Results and Discussion

Incubation of neamine, kanamycin A, and gentamicin C_1 with a number of aldohexoses and aldodisaccharides at pH $7 \sim 9$ resulted in formation of new derivatives as shown by paper ionophoresis. The optimum pH for the reaction, the optimum temperature, and the length

Table 1. Physical and chemical analyses of glycosides of aminoglycoside antibiotics

A. Chemical analyses

Glycosides	Analyses
1. Kanamycin-glucoside	calculated for monoglucoside: C, 44.6; H, 7.12; N. 8.67% found: C, 40.58; H, 7.08; N, 8.05%. (no ash) C/N ratio: calc., 6.00; found, 5.88
2. Kanamycin-maltoside	calculated for monomaltoside: C, 44.55; H, 6.93; N, 6.93% found: C, 40.93; H, 6.97; N, 7.00% (no ash) C/N ratio: calc., 7.50; found, 7.18
3. Kanamycin-galactoside	calculated for monogalactoside: C, 44.6; H, 7.12; N, 8.67% found: C, 38.44; H, 7.06; N, 7.50% (trace ash present) C/N ratio: calc., 6.00; found, 6.01
4. Kanamycin-lactoside	calculated for monolactoside: C, 44.55; H, 6.93; N, 6.93% found: C, 42.5; H, 7.18; N, 6.25% (trace ash present) C/N ratio: calc., 7.50; found, 7.9
5. Kanamycin-rhamnoside	calculated for monorhamnoside: C, 42.6; H, 7.12; N. 8.67% found: C, 36.85; H, 6.76; N, 8.55% C/N ratio: calc., 6.00; found, 5.03.

B. Specific optical rotations

Compounds	$[\alpha]_D^{25}$	Compounds	$[\alpha]_D^{25}$
Neamine	64°	Kanamycin A-riboside	96°
Neamine-glucoside	55.5	Kanamycin A-maltoside	98
Neamine-galactoside	58	Kanamycin A-lactoside	72.5
Neamine-maltoside	75	Gentamicin C_1	108
Neamine-lactoside	42.3	Gentamicin C_1-glucoside	87.5
Kanamycin A	132	Gentamicin C_1-galactoside	109.5
Kanamycin A-glucoside	88.6	Gentamicin C_1-mannoside	87
Kanamycin A-galactoside	100	Gentamicin C_1-rhamnoside	104
Kanamycin A-rhamnoside	97.8	Gentamicin C_1-maltoside	96.5
Kanamycin A-mannoside	87.9	Gentamicin C_1-lactoside	75

Table 2. Antibacterial activity of glycosides of aminoglycoside antibiotics

A. Glycosides of neamine

Microorganism	Minimal inhibitory concentrations, mcg/ml				
	Neamine	Neamine-glucoside	Neamine-maltoside	Neamine-galactoside	Neamine-lactoside
Diplococcus pneumoniae	>125	>125	>63	>125	>125
Staphylococcus aureus	4	32	32	8	32
Escherichia coli	32	125	>63	125	>125
Enterobacter cloacae	8	63	63	32	125
Proteus morganii	16	125	>63	63	125
Serratia marcesens	8	63	63	32	125

B. Glycosides of kanamycin A

Microorganism	Minimal inhibitory concentrations, mcg/ml			
	Kanamycin A	Kanamycin A-glucoside	Kanamycin A-galactoside	Kanamycin A-maltoside
D. pneumoniae	63	125	63	>125
S. aureus	1	16	2	16
E. coli	2	16	8	125
E. cloacae	1	16	4	125
P. morganii	2	32	8	63
Pseudomonas aeruginosa	4	63	63	>125
S. marcesens	2	32	32	125

Microorganism	Minimal inhibitory concentrations, mcg/ml			
	Kanamycin A-lactoside	Kanamycin A-rhamnoside	Kanamycin A-mannoside	Kanamycin A-riboside
D. pneumoniae	>125	125	>125	63
S. aureus	16	2	8	8
E. coli	63	4	32	16
E. cloacae	32	2	16	8
P. morganii	32	8	16	8
Ps. aeruginosa	125	16	63	32
S. marcesens	>125	4	63	8

C. Glycosides of Gentamicin C_1

Microorganism	Minimal inhibitory concentrations, mcg/ml			
	Gentamicin C_1	Gentamicin C_1-glucoside	Gentamicin C_1-galactoside	Gentamicin C_1-lactoside
S. aureus	0.03	0.3	0.3	0.75
E. coli	0.075	3.0	0.75	3.0
P. mirabilis	3.0	15.5	7.5	>25
Ps. aeruginosa	0.075	3.0	0.75	7.5
Serratia sp.	0.75	3.0	3.0	17.5

of the incubation period varies with the sugar and antibiotic involved. Best conversions were of the order of 40 % of the added aminoglycoside antibiotic.

Initial studies suggested that the glycosides of the amino glycoside antibiotics were unstable in alkaline solution, and relatively stable in acid solution. Samples of kanamycin A-maltoside-U-^{14}C were dissolved in buffers and incubated at various temperatures. The half-lives in hours at 4°C for pH 3 to pH 11 was >1,000 hours; at 26°C for pH 3, 300 hours for pH 7

220 hours, and for pH 11, 90 hours. At 40°C the half-lives for pH 3 were 56 hours, for pH 7, 36 hours, and for pH 11, 16 hours. Analysis of the mixtures after incubation at pH 7 and pH 11 showed antibiotics with mobility in paper ionophoresis equivalent to kanamycin A, and in paper chromatography (using 2-butanone-*iso* - propanol - 6.5 N NH_4OH, 8:2:3, (ref. 2) the same as kanamycin A.

Hydrolysis of kanamycin A maltoside with 6 N HCl for 15 minutes at 120°C followed by paper chromatography using *n*-butanol - pyridine - water (6:4:3) showed the same decomposition products formed when kanamycin A was treated in this manner (chromatogram sprayed with ninhydrin). Hydrolysis with 1 N HCl for 1 hour at 100°C followed by mass spectral analysis of the mixture showed the presence of hydroxymethylfurfural.

The microanalyses of the lyophilized powders obtained from the CG-50 columns gave C/N ratios (see Table 1 A) that might be expected for addition of one hexose unit or one disaccharide unit for kanamycin-glucoside, kanamycin-maltoside, kanamycin-galactoside, and kanamycin-lactoside. The specific optical rotations of the aminoglycoside antibiotics and their derivatives are shown in Table 1 B. The rotations are in the range of what might be expected if one mole of sugar was added to the aminoglycoside antibiotic.

In Table 2, the antibacterial activities of the glycosides are compared with the activities of the parent compound. In every instance the activity of the derivative was considerably lower than that of the parent compound. In animal tests, kanamycin A-glucoside and gentamicin C_1-glucoside had about the same ratio of PD_{50} to that of the parent compound as noted in the *in vitro* potency tests, *e.g.* 10 %.

The identification of the compounds as N-glycosides of the aminoglycoside antibiotics rests on the observations of alkali lability, interpretation of CMR spectra, and some studies on the N-acetylkanamycin where glycosylation did not occur.

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