SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF 1-N [(S)-ω-AMINO-2-HYDROXYALKYL] KANAMYCIN A DERIVATIVES

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Four 1-N-aminohydroxy-alkyl derivatives of kanamycin A were prepared and their *in vitro* activities against aminoglycoside-sensitive and aminoglycoside-resistant organisms were compared with amikacin. 1-N-[(S)-4-Amino-2-hydroxybutyl] kanamycin A (Fig. 1, compound **2**, code no. UK-18,892) was equipotent to amikacin in all these tests and in mouse protection studies.

Amikacin, 1-N-[(S)-4-amino-2-hydroxybutyryl] kanamycin A, has been reported[1] to be highly active against both kanamycin-sensitive and kanamycin-resistant bacteria. In contrast, several other 1-N-acyl derivatives of kanamycin A have been shown to possess low antibacterial activity.[2] We undertook a program of 1-N-alkyl modifications of kanamycin A to investigate structure-activity relationships in this area, and this paper describes the synthesis and antibacterial properties of some of the compounds produced.

Materials and Methods

Kanamycin A-1-N-Alkylated Derivatives

1-N-(S)-ω-Amino-2-hydroxyalkyl derivatives of kanamycin A were prepared by diborane reduction of the corresponding 1-N-acyl derivatives, using the trifluoroacetate salts to improve solubility. The 1-N-acyl derivative (150 mg) was converted to its trifluoroacetate salt by dissolving in anhydrous trifluoroacetic acid followed by evaporation under reduced pressure. The resulting salt was dissolved in dry tetrahydrofuran (5 ml) and treated portionwise with a 1 M solution of diborane in tetrahydrofuran (20 ml), under a nitrogen atmosphere. After 6 hours at 50°C the reaction mixture was cooled and excess diborane was destroyed by the addition of a small amount of water. After evaporation under reduced pressure, the residue was taken up in water (10 ml), adjusted to pH 12 with 0.1 N sodium hydroxide solution and then to pH 5 using 2 N hydrochloric acid. The resulting solution was chromatographed on a column of Amberlite CG-50 ion-exchange resin (50 ml), in the ammonium ion form, eluting with dilute ammonia solution (linear increase, 0.1 to 1 M). Fractions containing the product were combined and evaporated under reduced pressure to yield the product as an amorphous solid (Table 1). The requisite 1-N-acyl compounds were synthesized by the method of NAITO *et al.*[2]

The structures of the products prepared in the present study have been confirmed by ^{13}C-n.m.r. and/or mass spectral data. Microanalyses of the products reported are in agreement with the expected formulae calculated as the carbonate salts.

Micro-organisms

The bacteria designated 'aminoglycoside-sensitive' were pathogenic strains well adapted to growth under laboratory conditions. The aminoglycoside-resistant organisms were bacteria with proven inactivating mechanisms and were obtained from Prof. J. DAVIES, University of Wisconsin.

M.I.C. Determinations

Minimum inhibitory concentrations (M.I.C.) of the compounds were determined by a standard dilution method in Diagnostic Sensitivity Test Agar (DSTA-Oxoid). Serial dilutions (in 2-fold steps) of the compounds were prepared in the agar plates over the required concentration range and, after the

surface moisture was removed by drying at 28°C, the organisms were inoculated on the surface of the agar using a multi-point inoculator (Denley Instruments Ltd.). The standard inoculum consisted to a 1 in 100 dilution of an overnight broth culture (Brain-Heart Infusion) and subsequent incubation was for18 hours at 37°C.

Experimental Infections in Mice

Acute systemic infections in mice were produced by intraperitoneal inoculation of standardized bacterial cultures suspended in 5% hog gastric mucin. The challenge dose was generally 1 to 10 lethal doses, *i. e.* 1 to 10 times the number of organisms needed to kill 100% of the mice within 72 hours, depending on the organism. The dosage regimen for all experimental infections was 0.5 and 4 hours post-infection and all compounds were administered subcutaneously. After 72 hours, a 50% protective dose value (PD_{50}), expressed in mg/kg body-weight, was calculated by a probit method. The PD_{50} values expressed were calculated from the dose of compound given in each injection.

All mice used were female, strain CD-1 with an average weight of 20 g and were obtained from the Charles River Company.

Table 1. Synthetic yield and electrophoretic behaviour of 1-N-alkyl derivatives*

Compound	% yield	Electrophoretic mobility relative to amikacin**
1	27	0.6
2	50	0.6
3	35	0.7
4	63	0.85

* These compounds were all amorphous solids which melted within the range 145°~155°C.

** Thin-layer electrophoresis on glass plates coated with silica (0.25 mm thick). Using a 1:1.5 mixture of 2 M formic acid and 2 M acetic acid and a potential of 900 volts for 45 minutes. Detection was by means of hypochlorite/starch iodide and under these conditions amikacin (**6**) had a relative mobility of 1.0.

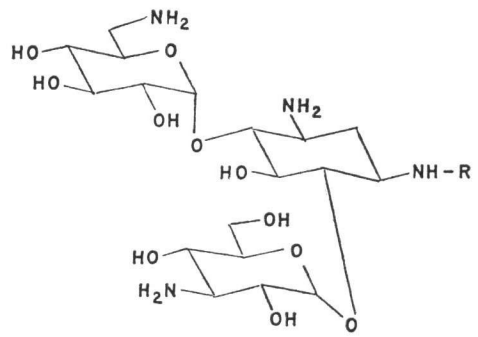
Results

Four 1-N-aminohydroxyalkyl derivatives of kanamycin A (**1**~**4**, Fig. 1) were prepared by reduction of the corresponding 1-N-acyl derivatives (**5**~**8**, respectively). The *in vitro* activities of these compounds against 25 aminoglycoside-sensitive strains of bacteria are shown in Table 2 in comparison with amikacin (**6**).

From the data shown in Table 2 it is apparent that these (S)-ω-amino-2-hydroxyalkyl derivatives possess an antibacterial spectrum similar to that of amikacin with good activity against *Staphylococcus aureus* and a wide range of gram-negative bacteria. The most potent of the four alkyl compounds is compound **2**, (UK-18,892), the (S)-4-amino-2-hydroxybutyl derivative, with the corresponding hexyl analog (**4**) having the lowest activity.

Good activity was also shown by these (S)-ω-amino-2-hydroxyalkyl derivatives against several aminoglycoside-resistant organisms possessing known inactivating enzymes (Table 3). It

Fig. 1.



	R	n
1	$\cdot CH_2CH(OH)\cdot(CH_2)_nNH_2$ (S)	1
2	〃	2
3	〃	3
4	〃	4
5	$\cdot CO\cdot CH(OH)\cdot(CH_2)_nNH_2$ (S)	1
6	〃	2
7	〃	3
8	〃	4

appears from these data that these compounds, like amikacin, are resistant to several forms of enzymatic inactivation and this has been confirmed in a study[3] in which UK–18,892 was evaluated against over 200 recently isolated aminoglycoside-resistant bacterial strains. In excess of 96% of these strains were inhibited by a concentration of <6.2 mcg/ml of UK–18,892. Resistance to enzymatic inactivation has also been demonstrated for UK–18,892 using isolated enzyme preparations[4]. The efficacy of UK–18,892 has been examined in mouse-protection tests against lethal bacterial infections, and the results of these studies are shown in Table 4. Amikacin was evaluated in parallel and the results reflect in broad terms the similar *in vitro* antibacterial potencies of UK–18,892 and amikacin, indicating that both compounds have similar pharmacokinetic properties and stability in the mouse.

Discussion

A series of 1-N-acyl derivatives of kanamycin A were reported by NAITO *et al.*[2] who showed that optimum activity was associated with the 4-amino-2-hydroxybutyryl derivative (amikacin). In order to investigate the effect of replacing the amide group by an amino function, we have synthesized four 1-N-(S)-ω-amino-2-hydroxyalkyl derivatives of kanamycin A.

All four alkyl compounds were active against a wide range of bacteria, including aminoglycoside-resistant strains. UK–18, 892, 1-N-[(S)-4-

Table 2. Activity *in vitro* against aminoglycoside-sensitive bacteria (MIC in mcg/ml)

Organism	Amikacin*	1	2	3	4
E. coli E104	3.1	6.2	3.1	3.1	6.2
E110	3.1	6.2	3.1	1.6	6.2
E116	1.6	6.2	1.6	1.6	6.2
E172	1.6	6.2	1.6	1.6	6.2
E 10	1.6	6.2	3.1	3.1	12.5
E 36	3.1	6.2	3.1	3.1	12.5
E 51	3.1	6.2	3.1	3.1	12.5
E173	1.6	3.1	0.8	1.6	3.1
P. mirabilis					
P133	3.1	6.2	3.1	3.1	12.5
P8	1.6	3.1	1.6	1.6	6.2
P. vulgaris					
P136	1.6	3.1	3.1	1.6	6.2
P124	0.8	3.1	0.8	1.6	3.1
Ps. aeruginosa					
Ps56	0.8	6.2	0.8	1.6	6.2
Ps52	0.8	6.2	0.8	1.6	6.2
Ps48	1.6	6.2	0.8	1.6	6.2
Ps169	3.1	6.2	1.6	1.6	6.2
Klebsiella					
K37	1.6	3.1	1.6	1.6	3.1
K38	0.4	1.6	0.8	1.6	1.6
K. aerogenes					
K33	1.6	3.1	1.6	1.6	6.2
K39	1.6	6.2	1.6	1.6	6.2
K40	1.6	3.1	1.6	1.6	6.2
S. aureus					
S223	0.8	1.6	0.4	0.8	1.6
S222	0.4	1.6	0.4	0.8	6.2
S202	3.1	6.2	3.1	3.1	12.5
S208	0.8	1.6	0.8	0.8	3.1

* The amikacin used in this study was obtained commercially as Biklin (Grunenthal).

Table 3. Activity *in vitro* against aminoglycoside-resistant bacteria (MIC in mcg/ml)

Organism	Resistance* mechanism	Amikacin	Kanamycin A	1	2	3	4
Ps. aeruginosa (130)	AAC (3)	1.6	50	6.2	1.6	1.6	6.2
Ps. aeruginosa (209)	AAC (3)/ AP (3′)**	1.6	50	6.2	1.6	3.1	6.2
E. coli (JR35/25)	AP (3′)-I	3.1	>100	12.5	3.1	6.2	12.5
E. coli (JR66/W677)	AAD (2″)/ AP (3′)-II	3.1	>100	12.5	3.1	6.2	6.2

* AAC (3); 3-acetyltransferase. AP (3′); 3′-phosphotransferase. AAD (2″); 2″-adenylsynthetase.
** Classification as AP (3′)-I or -II not proven

amino-2-hydroxybutyl] kanamycin A (**2**), was the most potent of these four derivatives with activity similar to that of amikacin which was included as a comparative agent. The propyl and pentyl analogs (**1** and **3** respectively) had slightly lower activity whilst the hexyl analog **4** was 25~50% as potent as UK-18,892. In contrast, in the acyl series[2], the corresponding propionyl analog **5** was 57% as active, the pentanoyl analog **7** was 84% as active and the hexanoyl analog **8** was only 9% as active as the butyryl analog, amikacin. UK-18,892 was also evaluated in comparison with amikacin in a series of mouse-protection tests against lethal bacterial infections and both compounds afforded similar degrees of protection.

In view of the excellent antibacterial activity of UK-18,892, this compound is being progressed to more extensive pre-clinical evaluation.

Table 4. PD_{50} values (mg/kg body weight) of UK-18,892 and amikacin against acute bacterial infections in mice

Organism	PD_{50} (mg/kg)	
	UK-18,892	Amikacin
E. coli 266	2.6	2.0
E. coli 36	3.5	2.4
Staph. aureus 5	1.6	1.6
Staph. aureus 8	1.0	0.7
P. mirabilis 4	2.7	5.7
P. mirabilis 8	6.5	3.7
Klebsiella 8	1.6	1.9
Klebsiella 33	0.5	1.0
Pseudomonas 48	4.0	4.7
Pseudomonas 56	16.2	18.6

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