AMINOGLYCOSIDE ANTIBIOTICS. IX

STRUCTURE-ACTIVITY RELATIONSHIP IN 1-N-ACYL DERIVATIVES OF KANAMYCIN A (AMIKACIN ANALOGS)

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Twenty-three 1-N-acyl derivatives of kanamycin A were prepared and their *in vitro* activities against kanamycin-sensitive and resistant organisms were compared with amikacin.

The selective N-acylation of kanamycin at the C-1 amino group with L(−)-γ-amino-α-hydroxybutyric acid (L-AHBA) gave amikacin (BB-K 8)[1] with a broad antibacterial spectrum including kanamycin-resistant microorganisms. Similar N-acylation of kanamycin with DL- or D-AHBA gave compounds[2] with nearly the same antibacterial pattern as that of amikacin but with one-half to one-fourth the activity of amikacin. On the other hand, N-acylation with L-AHBA at either the 3-NH_2, 3″-NH_2 or 6′-NH_2 group gave compounds with little activity[2]. This paper reports the activities of 1-N-acyl derivatives of kanamycin A with various carboxylic acid residues. Details of their chemical syntheses will be reported elsewhere.

Materials and Methods

Amikacin analogs

Twenty-three 1-N-acylated derivatives of kanamycin A (**1** through **23**), shown in Fig. 1 through Fig. 6, have been prepared by the procedure similar to that reported previously[1], which comprises the reaction of 6′-N-benzyloxycarbonylkanamycin A with the N-hydroxysuccinimide ester of an appropriate side chain acid followed by reductive removal of the protecting group(s). The structures of the products prepared in the present study have been confirmed by the amide carbonyl band (*ca.* 1640 cm^{-1}, IR) and also by alkaline hydrolysis to regenerate kanamycin A and the side chain acid. Microanalyses of the products reported here agreed with the expected formula as the carbonate and/or the hydrate.

In vitro activity

The minimal inhibitory concentrations (MIC) of **1** through **23** were determined by a two-fold agar dilution method against a variety of microorganisms including 26 gram-negative bacteria (11 *Escherichia coli*, 2 *Klebsiella pneumoniae*, 1 *Serratia marcescens*, 6, *Pseudomonas aeruginosa* and 6 *Proteus* species), 3 staphylococci and 3 mycobacteria, among which several strains are known to produce one or more aminoglycoside inactivating enzymes. Nutrient agar (Eiken) plates and STEERS' multiple inoculator apparatus were used in the experiments.

Relative activity

The *in vitro* activity of the 23 compounds was compared with that of amikacin and the relative activity (RA_i, relative to amikacin) of each compound for each of the 32 test organisms was calculated.

$$RA_i = \frac{\text{MIC value of amikacin against strain "i"}}{\text{MIC value of test compound against strain "i"}} .$$

Table 1. *In vitro* activity of 1-N-acyl derivatives of kanamycin A relative to amikacin against 32 test organisms

Compound	Number of test organisms with indicated RA_i									Mean relative activity $RA_m \times 1000$
	≦1/128	1/64	1/32	1/16	1/8	1/4	1/2	1	2	
1					1	11	17	3		400
2						2	23	6	1	570
3							10	20	2	840
4	1	2	2	8	13	6				90
5	5	13	12	1	1					20
6	5	9	13	3	2					25
7	3	1	12	15		1				40
8	5	2	16	7	1	1				30
9				1	11	17	3			200
10		3	10	7	9	3				60
11			1	1	9	11	8	2		240
12	2	8	13	6	1		2			35
13	1	2	9	13	6		1			55
14		2	9	15	6					55
15	24	6	1	1						8
16		1	2	10	14	5				95
17		2	8	13	9					60
18	11	14	7							10
19	4	16	6	6						20
20		3	8	17	4					50
21	28	2	2							6
22	28	2	2							6
23	27	3	2							7
Amikacin								32		1,000

The geometric mean of the individual relative activities (RA_i) was then obtained for each compound and referred to as "mean relative activity" (RA_m) in this paper.

$$RA_m = 2^{(\Sigma \cdot \log_2 RA_i)/N}$$

where N is the number of test organisms employed.

Results

The relative *in vitro* activities of the 23 amikacin analogs against 32 test organisms are summarized and shown in Table 1. The mean relative activity (RA_m) of each compound is also shown in the last column of the Table. The MIC data for the 23 compounds obtained with several aminoglycoside-resistant organisms whose inactivating enzymes have been identified are shown in Table 2 comparatively with those of kanamycin and amikacin.

In the following discussion of structure-activity relationship, the twenty-three derivatives are classified into six groups according to the type of side chain acid.

ω-Amino-α-hydroxyalkanoic Acid Derivatives, Homologs of Amikacin (Fig. 1)

1-N-acyl derivatives of kanamycin A with L-α-hydroxy-ω-aminoalkanoic acid (homologs of

Table 2. MIC (mcg/ml) of 1-N-acyl derivatives of kanamycin A against aminoglycoside antibiotics-resistant strains

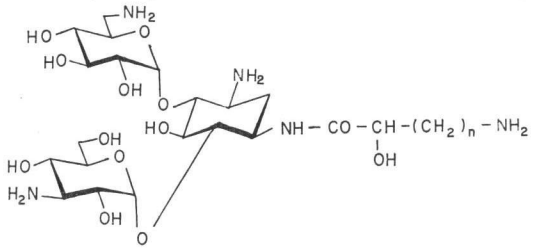
Code#	Ec-8	Ec-5	Ec-9	Ec-10	Ec-53	KP-8	Pa-4
Organism	*E. coli* K-12	*E. coli* K-12 ML-1630	*E. coli* NR 79/W677	*E. coli* JR 35/C 600	*E. coli* JR 66/W 677	*Kl. pneumoniae* Type 22 #3038	*Ps. aeruginosa* H_9
Inactivating enzyme(s)	—	NPT_1 [3]	KAT[4]	NPT_1 [5]	NPT_2 +GAS[6-8]	NPT_2 +GAS[8,9]	NPT[10,11]
Kanamycin	0.8	>100	6.3	100	>100	>100	>100
Amikacin	0.4	0.8	0.8	0.2	0.4	0.8	6.3
1	1.6	3.2	3.2	0.4	0.8	1.6	12.5
2	0.8	1.6	1.6	0.4	0.8	1.6	6.3
3	0.4	0.8	1.6	0.4	0.8	0.8	12.5
4	3.1	3.1	50	3.1	50	50	100
5	12.5	50	100	12.5	>100	>100	>100
6	12.5	50	100	12.5	100	100	100
7	12.5	12.5	100	3.1	50	100	100
8	12.5	25	100	6.3	100	>100	100
9	1.6	3.1	3.1	0.8	3.1	6.3	25
10	3.1	6.3	50	3.1	12.5	25	>100
11	1.6	1.6	1.6	0.4	3.1	3.1	100
12	12.5	25	50	12.5	50	50	12.5
13	3.1	12.5	50	6.3	50	50	100
14	3.1	12.5	25	3.1	12.5	25	50
15	50	50	100	100	>100	>100	>100
16	3.1	12.5	25	1.6	25	12.5	50
17	6.3	6.3	25	6.3	25	50	>100
18	25	>100	100	>100	>100	>100	>100
19	25	50	>100	12.5	100	100	>100
20	6.3	12.5	25	6.3	12.5	12.5	>100
21	100	>100	>100	50	>100	>100	>100
22	100	>100	>100	100	>100	>100	>100
23	50	100	100	25	>100	>100	>100

* NPT_1: Neomycin-kanamycin 3′-O-phosphotransferase I
NPT_2: Neomycin-kanamycin-butirosin 3′-O-phosphotransferase II
KAT: Kanamycin 6′-N-acetyltransferase
GAS: Gentamicin 2″-O-adenylate synthetase

amikacin) exhibited antibacterial spectra similar to that of amikacin with activity against kanamycin-sensitive and resistant organisms though the intrinsic activity was highest with amikacin. The second most active derivative was the next higher homolog of amikacin with the L(−)-δ-amino-α-hydroxyvaleric acid (L-AHVA) residue (**3**), which showed almost the same activity as amikacin against kanamycin-sensitive organisms but was slightly less (1∼1/2) active than amikacin against kanamycin-resistant strains of *E. coli* and *P. aeruginosa*. The shorter chain homolog with L-β-amino-α-hydroxypropionic acid (L-AHPA) residue (**2**) was in general approximately one-half as active as amikacin.

Further elongation of the side chain (n=4, **4**) considerably lowered the activity to 1/4∼1/8 against most of the kanamycin-sensitive organisms and to a greater extent (1/16∼1/128) against

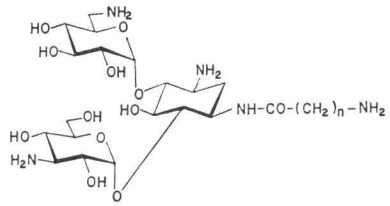
Fig. 1. ω-Amino-α-hydroxyalkanoic acid derivatives, homologs of amikacin



	Compound				
	1	**2**	Amikacin	**3**	**4**
n	1	1	2	3	4
Config. of α-OH	DL	L	L	L	L
mp (°C, dec)	195~200	200~205	203~204	214~215	200~203
R_f (S-110)*	0.33	0.33	0.16	0.18	0.23
Mean relative activity $RA_m \times 1{,}000$	400	570	1,000	840	90

* TLC: silica gel plate F_{254}, $CHCl_3$-CH_3OH-28% NH_4OH-H_2O (1:4:2:1)

Fig. 2. ω-Aminoalkanoic acid derivatives



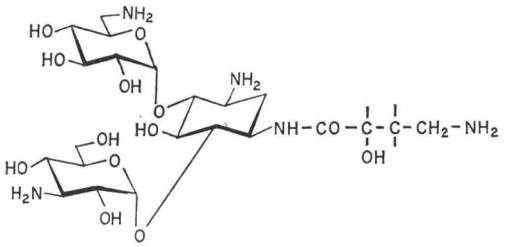
	Compound			
	5	**6**	**7**	8
n	1	2	3	4
mp (°C, dec)	200~203	188~191	175~180	185~190
R_f (S-110)	0.38	0.32	0.22	0.22
$RA_m \times 1{,}000$	20	25	40	30

the kanamycin-resistant organisms (*e.g.* Ec-9, Ec-53, Kp-8 and *Pseudomonas* species in Table 2).

ω-Aminoalkanoic Acid Derivatives (Fig. 2)

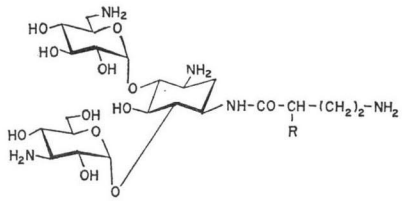
1-N-ω-Aminoalkanoylkanamycins having no hydroxy group in the α-position showed much reduced activity compared to the corresponding α-hydroxy derivatives. As in the α-OH-ω-NH_2 analogs, the butyryl side chain provided the best compound (**7**) in this series, even though it was only 1/16~1/32 as active as amikacin against most of the test organisms and still less active against the resistant strains of Ec-9, Ec-53 and Kp-8. This was also the case in all the other compounds of this series, **5**, **6** and **8**, in which a greater decrease of the relative activity to amikacin was seen with the three kanamycin-resistant strains than with the other test organisms.

Fig. 3. Substituted-AHBA derivatives



	Compound		
	9	**10**	**11**
Side chain	CO—CH(OH)—CH(OH)—CH_2—NH_2	CO—CH(OH)—C(CH_3)(CH_3)—CH_2—NH_2	CO—C(CH_3)(OH)—CH_2—CH_2—NH_2
α-Config.	DL-*erythro*	DL	DL
mp (°C, dec)	210~216	190~195	195~198
R_f (S-110)	0.17	0.35	0.27
RA_m ×1,000	200	60	240

Fig. 4. Modification of the α-OH group

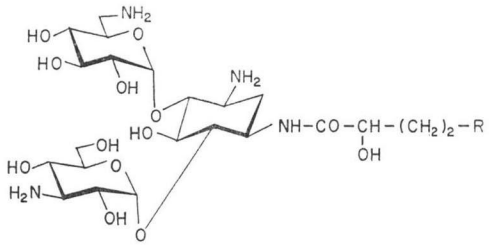


	Compound			
	7	**12**	**13**	**14**
R	H	NH_2	α-H β-OH	OAc
Config. of α-R	—	L	β-DL	DL
mp (°C, dec)	175~180	>185	174~180	165~167
R_f (S-110)	0.22	0.18	0.31	0.60
RA_m ×1,000	40	35	45	55

Substituted AHBA Derivatives (Fig. 3)

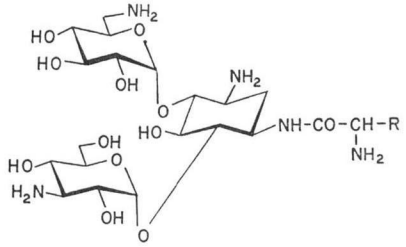
Compounds with an additional hydroxy group at the β-position (**9**) or a methyl group at the α-position (**11**) retained antibacterial activity against both kanamycin-sensitive and kanamycin-resistant strains, although they were in general 4~16 times less active than amikacin. Addition of two methyl groups in the β-position (**10**) resulted in more marked decrease of the activity. However, these compounds were acylated with the racemic amino acids and a substantial increase of the activity might be expected if the side chain acid with L-configuration were used.

Fig. 5. Modification of the γ-NH_2 group



	Compound					
	15	**16**	**17**	**18**	**19**	**20**
R	H	$NHCH_3$	$NHCOCH_2NH_2$	$NHCOCH_3$	$CONH_2$	COOH
α-Config.	DL	DL	L	L	L	L
mp (°C, dec)	190~193	202~203	174~175	175~178	249~250	175~181
R_f (S-110)	0.54	0.11	0.45	0.57	0.37	0.70
RA_m ×1,000	8	95	60	10	20	50

Fig. 6. α-Aminobutyric acid derivatives



	Compound			
	12	**21**	**22**	**23**
Side chain	CO—CH—CH_2—CH_2 NH_2 NH_2	CO—CH—CH—CH_3 NH_2 OH	CO—CH—CH_2—CH_2 NH_2 OH	CO—CH—CH_2—CH_3 NH_2
α-Config.	L	L	DL	DL
mp (°C, dec)	>185	201	185~186	170~171
R_f (S-110)	0.18	0.47	0.46	0.61
RA_m ×1,000	35	6	6	7

Modification of the α-OH Group (Fig. 4)

The elimination (7), modification (**12** and **14**) and shift to the β-position (**13**) of the α-hydroxy group in the amikacin side chain reduced the activity against most of the test organisms to 1/8~1/64 except for Ec-9, Ec-53 and Kp-8, against which activity was even less (1/32~1/128).

Modification of γ-NH_2 Group (Fig. 5)

N-Methylation (**16**) and N-glycylation (**17**) of the γ-amino group gave compounds which retained antibacterial activity against kanamycin-sensitive and resistant organisms though they were about 8~16 time less active than amikacin. However, the activity nearly disappeared

when the terminal basic group was removed (**15**, **18** and **19**), with the exception of **20** which possessed an acid group at the γ-position. This particular compound still retained considerable activity (1/8~1/64 of amikacin) though with relatively decreased activity against *Pseudomonas* species.

α-Aminobutyric Acid Derivatives (Fig. 6)

The α, γ-diaminobutyric acid derivative, **12**, showed moderate activity (1/16~1/64 of amikacin) while three other α-aminobutyric acid derivatives, **21**, **22** and **23**, were virtually inactive. Activity was nearly lost with the α-amino-γ-hydroxybutyric acid derivative (**22**) in which the OH and NH_2 groups in the side chain are located in the reverse position to those of amikacin.

Discussions

A series of 1-N-acyl analogs of butirosins have been prepared by HASKELL *et al.*[12] and TSUKIURA *et al*[13]. Various 1-N-acyl derivatives of neamine have also been made by SAEKI *et al.*[14] and the structure-activity relationships were discussed. KONDO *et al.*[15] reported the syntheses and activities of 1-N-acyl derivatives of kanamycin A with D-, L- and DL-isoseryl side chains, the latter two derivatives being equivalent to compounds **2** and **1**, respectively, in this paper.

Twenty-three amikacin analogs with various types of acyl residue have been prepared and their activities compared with amikacin. The presence of a terminal basic group (ω-NH_2 or ω-$NHCH_3$) in the side chain seems to be most important for antibacterial activity in this series of compounds. Removal of the terminal basic function (**15**, **18**~**20**) substantially lowered the intrinsic activity of the compounds to 1/20~1/100 of amikacin's activity. The α-hydroxy group also plays an important role in the activity, and removal or any modification of the α-hydroxy group of amikacin (**5**~**8**, **12**~**14**) resulted in a considerable decrease of activity (1/20~1/50). Lack of both the ω-NH_2 (or basic) and the α-OH groups (**21**, **22**, **23**) made the derivatives virtually inactive or less than 1/140 the activity of amikacin. However, the derivatives having both functions in the proper position (**1**~**4**, **9**~**11**, **16**, **17**) retained the substantial activity of amikacin against the kanamycin-sensitive and resistant strains, with amikacin still being the best followed by its higher and lower homologs (compounds **3** and **2**).

Another observation in the present study was that the structural modification of the side chain acid in amikacin affected the compounds' activity against certain groups of kanamycin-resistant organisms, such as Ec-9 (kanamycin 6′-acetyl-transferase), Ec-53 and Kp-8 (neomycin-kanamycin phosphotransferase II and gentamicin 2″-O-adenylate synthetase), to a greater extent than against one class of kanamycin-resistant organisms (Ec-5 and Ec-10: neomycin-kanamycin phosphotransferase I) and kanamycin-sensitive organisms.

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