AMINOGLYCOSIDE ANTIBIOTICS. XII
EFFECT OF N-ALKYLATION IN KANAMYCIN ANTIBIOTICS*

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(Received for publication April 17, 1978)

Four N-ethyl derivatives of kanamycin A and five N-ethyl derivatives of kanamycin B were synthesized. The antimicrobial activity of N-ethyl kanamycin derivatives was determined against aminoglycoside-sensitive and resistant organisms. The structure-activity relationship of these compounds is discussed with reference to the activity of N-acyl kanamycin derivatives.

Frequent use of aminoglycoside antibiotics in the treatment of serious Gram-negative infections has been accompanied by the emergence of multiple drug-resistant strains of bacteria carrying R-factors. This has stimulated the study of resistance mechanisms and mechanisms for circumventing the drug inactivation caused by resistant bacteria. Thus, aminoglycoside antibiotics have been chemically modified in various ways to make them resistant to enzymatic inactivation. One approach is the elimination of the hydroxy groups which are targets for enzymatic attacks, yielding, for example, 3′,4′-dideoxykanamycin B[2]. Acylation of the 1-amino group with L-4-amino-2-hydroxybutyric acid (L-AHBA) or its congeners, as exemplified by amikacin[3], produces a pronounced effect against inactivation by many aminoglycoside-modifying enzymes. It has been reported recently that 1-N-ethylation of sisomicin was effective in making the antibiotic active against sisomicin-resistant organisms. This semisynthetic sisomicin derivative is called netilmicin[4].

We have studied the N-alkylation of kanamycins A and B to explore the effect on enzymatic inactivation. This paper describes the preparation and activity of N-ethyl derivatives of kanamycin and discusses structure-activity relationships for both sensitive and resistant organisms.

Preparation of N-Ethyl Derivatives of Kanamycins A and B

Kanamycin A has four amino groups at the 1-, 3-, 6′- and 3″-positions, and kanamycin B has an additional amino group at the 2′-position. Introduction of an ethyl group on each of the amino groups was performed *via* a key intermediate in which all amino groups other than the target amino group were blocked, providing four N-ethyl derivatives of kanamycin A and five N-ethyl derivatives of kanamycin B.

The synthesis and properties of the N-ethyl derivatives of kanamycins A and B are summarized in Tables 1 and 2. 1-N-Ethylkanamycin A was prepared from amikacin, the 1-N-AHBA derivative of kanamycin A. Amikacin was benzylated with benzaldehyde and sodium borohydride giving tetra-N-benzyl-amikacin, which was subjected to hydrazinolysis to yield a key intermediate bearing a free amino group at the 1-position. Ethylation of the intermediate with acetaldehyde and sodium boro-

* Part of this paper was presented at the 2nd Tokyo Symposium on Microbial Drug-resistance, October 26~28, 1977.

Table 1. Preparation of N-ethyl derivatives of kanamycins A and B

Compound	Structure	Overall yield	Starting material	Key intermediate
1	[KM-A]-1-N-Et	38%	[KM-A]-1-N-AHBA	3,3″,6′-tri-N-benzyl
2	[KM-A]-3-N-Et	40%	[KM-A]-3-N-AHBA	1,3″,6′-tri-N-benzyl
3	[KM-A]-6′-N-Et	30%	[KM-A]-6′-N-Cbz	1,3,3″-tri-N-acetyl
4	[KM-A]-3″-N-Et	24%	[KM-A]-1,3-urea	6′-N-Cbz-1,3-ureido
5	[KM-B]-1-N-Et	19%	[KM-B]-1-AHBA	2′,3,3″,6′-tetra-N-tosyl
6	[KM-B]-3-N-Et	9%	[KM-B]-6′-N-Cbz	
7	[KM-B]-2′-N-Et	2%	[KM-B]-1,3-urea	3″,6′-di-N-Cbz-1,3-ureido
8	[KM-B]-6′-N-Et	41%	[KM-B]-6′-N-Cbz	1,2′,3,3″-tetra-N-Cbe***
9	[KM-B]-3″-N-Et	1%	[KM-B]-1,3-urea	2′,6′-di-N-Cbz-1,3-ureido

Table 2. NMR and physical data of N-ethyl derivatives of kanamycins A and B

Compound	Site of ethylation	Mp. °C(dec)	Rf[2]	$CH_3(\delta)$, J(Hz)	1′-H(δ), J(Hz)	1″-H(δ), J(Hz)
1	1-N	169~174	0.47	1.30(t) 7.0	5.48(d) 3.0	5.08(d) 3.0
2	3-N	167~170	0.55	1.30(t) 7.0	5.40(d) 3.0	5.12(d) 3.0
3	6′-N	161~165	0.38	1.30(t) 7.0	5.45(d) 3.0	5.11(d) 3.0
4	3″-N	167~169	0.47	1.34(t) 7.0	5.49(d) 3.0	5.10(d) 3.0
5	1-N	160~165	0.43	1.34(t) 7.1	5.90(d) 3.5	5.11(d) 3.5
6	3-N	154~157	0.52	1.37(t) 7.0	6.00(d) 3.8	5.13(d) 3.4
7	2′-N	157~163	0.52	1.13(t)[1] 7.0	5.23(d)[1] 4.0	5.03(d)[1] 3.0
8	6′-N	173~176	0.39	1.30(t)[1] 7.0	5.90(d) 4.0	5.07(d) 3.2
9	3″-N	150~155	0.42	1.35(t) 6.9	5.93(d) 3.8	5.10(d) 3.3

[1] measured in the form of free base; the rest were measured at pD 2.

[2] TLC: Merck silica gel 60 F_{254}, 10% $AcONH_4$ - acetone - c.NH_4OH (9 : 5 : 1)

hydride followed by catalytic hydrogenation with palladium on charcoal yielded the desired 1-N-ethylkanamycin A (**1**).

Similarly benzylation of 3-N-AHBA-kanamycin A[5] followed by hydrazinolysis afforded the 3-amino intermediate. N-Acetylation of 6′-N-Cbz*-kanamycin A[3] followed by catalytic reduction gave 6′-amino-tri-N-acetylkanamycin A for 6′-N-ethylation. Selective benzyloxycarbonylation of 1,3-ureidokanamycin A[6] gave an intermediate for 3″-N-ethylation. These intermediates were ethylated by a procedure similar to that used for 1-N-ethylation and following deprotection afforded 3-N-ethylkanamycin A (**2**), 6′-N-ethylkanamycin A (**3**), and 3″-N-ethylkanamycin A (**4**).

The N-ethyl derivatives of kanamycin B shown in Table 1 were prepared in a similar manner. The 1-N-AHBA derivative of kanamycin B[7] was tosylated to give the penta-N-tosylate, which was subjected to hydrazinolysis to give the 1-amino-tetra-N-blocked intermediate. 1,3-Ureido-kanamycin B[6] was selectively carbobenzoxylated to give 6′-N-Cbz-1,3-ureido-kanamycin B, which was *t*-butoxycarbonylated giving a mixture of the 2′-N-Boc** and 3″-N-Boc derivatives. Carbobenzoxylation of the mixture, treatment with trifluoroacetic acid and subsequent separation by chromatography afforded 2′- and 3″-amino-tetra-N-blocked intermediates. Ethoxycarbonylation of 6′-N-Cbz-kanamycin B followed by hydrogenolysis gave 6′-amino-tetra-N-Cbz-kanamycin B. Ethylation of the foregoing key intermediates followed by deblocking afforded 1-N-ethylkanamycin B (**5**), 2′-N-ethylkanamycin B (**7**), 6′-N-

* Cbz : benzyloxycarbonyl ** Boc : *t*-butoxycarbonyl *** Cbe : Ethoxycarbonyl

ethylkanamycin B (**8**), and 3″-N-ethylkanamycin B (**9**). 3-N-Ethylkanamycin B (**6**) was prepared by ethylation of 6′-N-Cbz-kanamycin B followed by chromatographic separation over CG-50 (NH_4^+) and subsequent deblocking by hydrogenolysis.

Antibacterial Activity

The intrinsic antibiotic activity of the N-ethylkanamycin derivatives was assessed by determining the minimum inhibitory concentrations (MIC) against 20 strains of kanamycin-sensitive organisms, which include *Escherichia coli* (5 strains), *Klebsiella pneumoniae* (3), *Proteus mirabilis* (2), *Shigella* species (2), *Enterobacter* species (2), *Staphylococcus aureus* (4) and *Bacillus* species (2). The activity for each of the derivatives relative to kanamycin A or B was calculated from the geometric mean MIC of the compound and that of kanamycin for each of the bacterial genera. The mean relative activity against a total of 20 sensitive organisms (RAm-S) is shown in the last line of Table 3, which represents the intrinsic activity of each derivative relative to kanamycin A or B whose activity is taken as 100. The results show that the N-ethylation of kanamycin A generally reduces the intrinsic activity of the parent antibiotic. 3″-N-Ethylkanamycin A (**4**) is the most active among the four derivatives retaining 66% of the overall activity of kanamycin A. The 1-N-ethyl derivative (**1**) is the second most active with 29% of kanamycin A activity, followed by 6′-N-ethylkanamycin A (**3**) which is 19% as active as kanamycin A. The 3-N-ethyl derivative (**2**) is the least active and has only 1% of the activity of kanamycin A.

Table 5 shows the activity against 20 strains of kanamycin-resistant organisms, which are classified into six groups according to the type of aminoglycoside-modifying enzymes* produced

* abbreviation for aminoglycoside-modifying enzymes, see ref. 1.

Table 3. Relative activity of N-ethyl derivatives of kanamycins A and B, and N-AHBA derivatives of kanamycin A

Organisms	No. of strains tested	N-Ethylkanamycin A (Kanamycin A=100)				N-Ethylkanamycin B (Kanamycin B=100)				N-AHBA-kanamycin A (Kanamycin A=100)			
		1 (1-N)[1]	**2** (3-N)	**3** (6′-N)	**4** (3″-N)	**5** (1-N)	**6** (3-N)	**7** (2′-N)	**8** (6′-N)	**9** (3″-N)	**10** (1-N)	**11** (3-N)	**12** (6′-N)	**13** (3″-N)
Escherichia coli	5	33	1	19	66	57	16	50	50	115	200	1	1	3
Klebsiella pneumoniae	3	25	1	16	79	40	10	25	50	79	159	3	2	3
Proteus mirabilis	2	18	1	25	71	71	18	50	50	100	200	3	2	3
Shigella sp.	2	25	1	18	50	25	9	35	25	71	141	4	2	3
Enterobacter sp.	2	35	2	18	100	71	18	71	25	141	141	4	1	4
Staphylococcus aureus	4	25	1	18	50	35	9	42	30	59	119	2	1	3
Bacillus sp.	2	50	1	25	71	35	12	35	18	71	283	3	2	3
RAm-S[2]		29	1	19	66	45	12	42	35	87	168	3	1	3

[1] Site of N-alkylation or N-acylation [2] Mean relative activity against kanamycin-sensitive organisms

Table 4. Kanamycin-resistant organisms producing aminoglycoside-modifying enzymes

Kanamycin-resistant organisms	Aminoglycoside-modifying enzyme	Kanamycin-resistant organisms	Aminoglycoside-modifying enzyme
Escherichia coli A20363	APH(3′)-I	*Staphylococcus aureus* A22054	ANT(4′)
〃 A20365	〃	*Staphylococcus epidermidis* A22033	〃
〃 A20665	〃	*Bacillus brevis* IFO 12334	〃
Enterobacter cloacae A20364	〃		
Serratia marcescens A20333	〃	*Pseudomonas aeruginosa* A20741	AAC(3)-II
Klebsiella pneumoniae A20328	〃	〃 A20896	〃
Escherichia coli A20107	APH(3′)-II	*Proteus mirabilis* A21222	AAC(2′)*
〃 A20520	〃	*Proteus rettgeri* A20921	〃
Enterobacter cloacae A21006	〃	*Providencia stuartii* A20894	〃
〃 A21008	〃	〃 A21051	〃
Escherichia coli A20732	ANT(2″)	*Escherichia coli* A21218	AAC(6′)
Enterobacter cloacae A20953	〃	*Enterobacter cloacae* HMC-26	〃
		Serratia marcescens A21226	〃

* tested only for kanamycin B derivatives

Table 5. Resistance index* of N-ethyl derivatives of kanamycins A and B, and N-AHBA derivatives of kanamycin A

Aminoglycoside modifying enzymes	No. of strain	N-Ethylkanamycin A (Kanamycin A=1)				N-Ethylkanamycin B (Kanamycin B=1)					N-AHBA-Kanamycin A (Kanamycin A=1)			
		1 (1-N)	**2** (3-N)	**3** (6′-N)	**4** (3″-N)	**5** (1-N)	**6** (3-N)	**7** (2′-N)	**8** (6′-N)	**9** (3″-N)	**10** (1-N)	**11** (3-N)	**12** (6′-N)	**13** (3″-N)
APH(3′)-I	6	31	89	4	1	6	4	1	2	1	342	119	25	42
APH(3′)-II	4	55	100	7	2	100	5	1	2	1	107	79	35	20
ANT(2″)	2	39	35	25	1	71	4	1	16	1	435	66	35	17
ANT(4′)	3	3	100	8	1	1	7	1	2	1	38	33	79	17
AAC(6′)	3	1	158	25	1	1	26	1	29	1	87	8	40	6
AAC(2′)	4	—	—	—	—	2	33	6	2	1	—	—	—	—
AAC(3)-II	2	20	70	6	2	3	8	1	3	1	70	33	25	8

* Resistance Index (R-Index)=RAm-R/RAm-S

(Table 4). In addition, four strains of AAC(2′)-producing organisms were included in testing the kanamycin B derivatives. The geometric mean MIC* of a given compound was obtained for each group of resistant organisms and compared with that of kanamycin A or B to calculate the mean relative activity against resistant organisms (RAm-R). The ratio of RAm-R/RAm-S is defined as "resistant index" (R-index) and the values are shown in Table 5 for each of the kanamycin derivatives for each group of resistant organisms. It can be seen from the table that 3″-N-ethylation (compound **4**) has essentially no effect with regards to resistance to aminoglycoside-modifying enzymes. 1-N-Ethylation (compound **1**) produced a marked effect on APH(3′)-I and II, ANT(2″) and AAC(3)-II (R-index: 31, 55, 39 and 20) with little effect on AAC(6′)and ANT(4′). Although the intrinsic activity of the 3-N-ethyl derivative (**2**) is poor, compound **2** shows a broad spectrum of resistance against various types of modifying enzymes, including APH(3′)-I and II, ANT(4′), AAC(6′), AAC(3)-II, and ANT(2″). The 6′-N-ethyl derivative (**3**) shows a good R-index for ANT(2″) and AAC (6′).

In the kanamycin B series, the order of intrinsic activity parallels that of the kanamycin A derivatives (Table 3): 3″-N (87%) > 1-N(45%) > 2′-N(42%) > 6′-N(35%) > 3-N(12%). The activity of the 3″-N-ethyl derivative (**9**) against *E. coli*, *Enterobacter* and *P. mirabilis* is equal to or greater than that of kanamycin B. The 3-N-ethyl derivative (**6**), showing 12% of the activity of kanamycin B, is the least active of the five, although relatively more active than the corresponding 3-N-ethylkanamycin A (**2**). As shown in Table 5, 1-N-ethylkanamycin B (**5**) shows a good R-index (100 and 71) against APH(3′)-II and ANT (2″), and a small R-index (6) against APH(3′)-I. 3-N-Ethylkanamycin B (**6**) is effective against AAC(6′) and AAC(2′). The 2′-N-ethyl derivative (**7**) is slightly active against AAC(2′). The 6′-N-ethyl derivative (**8**) is moderately active against ANT(2″) and AAC(6′). The 3″-N-ethyl derivative (**9**) is ineffective against any of the enzymes.

In order to compare the effect of N-alkyl and N-AHBA-substitution, the activity of N-AHBA derivatives (**10**～**13**)[5] is shown in Tables 3 and 5. The 1-N-AHBA derivative of kanamycin A (**10**, amikacin) shows better intrinsic activity when compared to that of kanamycin A against kanamycin-sensitive organisms (Table 3), while the other N-AHBA derivatives show only 1～3% the activity of kanamycin A. This contrasts with the finding that significant activity was retained in the N-ethyl derivatives of kanamycin A (**1**, **3** and **4**) except for the 3-N-ethyl derivative (**2**). As shown in Table 5, amikacin (**10**) showed a high R-index (38～435) against all of the inactivating enzymes tested, while the 1-N-ethyl derivative (**1**) gave a high value against only APH(3′)-I and II, and ANT(2″). Despite their lack of intrinsic activity, the other three N-AHBA positional isomers (**11**～**13**) show considerable resistance to APH(3′)-I and II, ANT(2″), ANT(4′), and AAC(3)-II. A similar resistance to these enzymes is observed for the 1-N- and 3-N-ethylkanamycin A derivatives (**1** and **2**).

Generally, the N-ethyl derivatives of kanamycin B show higher activity against sensitive strains compared to the corresponding kanamycin A derivatives, which reflects greater intrinsic activity of kanamycin B *vs.* kanamycin A. The effect of N-ethylation on various types of modifying enzymes is generally greater in kanamycin A than kanamycin B in both scope and intensity, as evidenced in the 1-N-AHBA-substitution[8]. Ethylation of the 1-amino group produced a marked effect on APH(3′)-I and II, and ANT(2″) in kanamycin A, and APH(3′)-II and ANT(2″) in kanamycin B. The effect of 3-N-ethylation on various R-indices was much more significant in kanamycin A than kanamycin B. The 6′-N-ethyl-kanamycins A and B showed a relatively high resistance to AAC(6′) and ANT(2″).

* Cut off point for MIC determination : 1,600 mcg/ml

Ethylation at the 3″-amino group of kanamycins A and B was of least effect on the intrinsic activity as well as the spectrum of the parent antibiotics. Although 1- or 3-N-ethylation of kanamycins A and B resulted in increased resistance to the action of various aminoglycoside-modifying enzymes, the magnitude of the effect was not sufficiently large to offset the decrease in intrinsic activity.

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