NOTES

AMINOGLYCOSIDE ANTIBIOTICS. II.*
CONFIGURATIONAL AND POSITIONAL ISOMERS OF BB-K8

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BB-K8 (**1**) is a new semisynthetic derivative of kanamycin acylated with L(−)-γ-amino-α-hydroxybutyric acid (L-HABA) at the C-1 amino group of the 2-deoxystreptamine moiety. The chemistry[1] and antimicrobial activity[1,2] of BB-K8 have been reported. The present paper describes the two configurational isomers of BB-K8, which bear the DL- and D-HABA residue at the C-1 amino group of kanamycin A, as well as the three positional isomers of BB-K8, which are acylated with L-HABA at the C-3, C-6′ and C-3″ amino groups of kanamycin A.

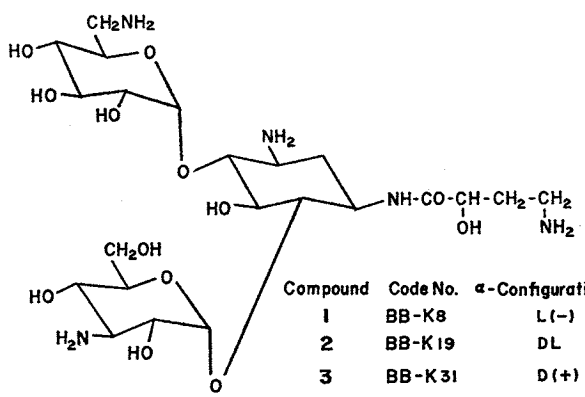
Configurational Isomers of BB-K8

DL-HABA was prepared by known methods either from γ-aminobutyric acid[3,4] or from γ-butyrolactone[5,6]. The optical resolution of DL-α-hydroxy-γ-phthalimidobutyric acid, an intermediate in the latter method, by *d*-amphetamine was reported by SAITO *et al.*,[6] whereas we have successfully carried out the resolution with dehydroabiethylamine (DAA)[7] in ethanol to separate the less-soluble DAA salt of the L-isomer ($C_{32}H_{42}N_2O_5 \cdot H_2O$**, mp 94～95°C, $[\alpha]_D^{24}$ +10.8° (*c* 2.5, MeOH), yield 86%) from the more-soluble DAA salt of the D-isomer ($C_{32}H_{42}N_2O_5 \cdot H_2O$, mp 119～120°C, $[\alpha]_D^{27}$ +26.8 (*c* 2.5, MeOH), yield 62%). Each of the diastereomeric salts was treated with aqueous NaOH to generate the optically active γ-phthalimido acids, which were hydrolyzed with HCl to L-HABA ($C_4H_9NO_3$, mp 218～219°C, $[\alpha]_D^{25}$ −30° (*c* 2.5, H_2O), yield 67%) and D-HABA ($C_4H_9NO_3$, mp 222～223°C, $[\alpha]_D^{25}$ +28° (*c* 2.5, H_2O), yield 57%).

The selective N-acylation at the C-1 amino group of kanamycin A with DL-HABA and D-HABA was carried out by essentially the same method as reported previously[1] to give BB-K19 (**2**, $C_{22}H_{48}N_5O_{13} \cdot 2H_2CO_3$, mp 180～181°C (dec.), $[\alpha]_D^{21}$ +94.5° (*c* 2.0, H_2O), TLC (S-110***) Rf 0.17) and BB-K31 (**3**, $C_{22}H_{48}N_5O_{13} \cdot 2H_2CO_3 \cdot 2H_2O$, mp 179～180°C (dec.), $[\alpha]_D^{28}$ +106° (*c* 1.25, H_2O), TLC (S-110) Rf 0.16), respectively (Fig. 1).

The minimal inhibitory concentration (MIC) of BB-K8 (**1**) and its configurational isomers, **2** and **3**, were determined by a two-fold agar dilution method with the results shown in Table 1. Compounds **2** and **3** showed antibacterial spectra

Fig. 1.



Compound	Code No.	α-Configuration
1	BB-K8	L(−)
2	BB-K19	DL
3	BB-K31	D(+)

* Part I of this series: BB-K8, a new semisynthetic aminoglycoside antibiotic.[1]
** Microanalysis agreed with the indicated when presented in this paper.
*** See foot note of Table 2.

Table 1. Antibacterial spectra of HABA derivatives of kanamycin A

Test organism	Code #	MIC (mcg/ml)						
		Kanamycin	BB-K8 (1)	BB-K19 (2)	BB-K31 (3)	BB-K6 (6)	BB-K29 (7)	BB-K11 (8)
Staphylococcus aureus Smith	Sa-2	0.4	0.2	0.8	1.6	12.5	25	25
,, ,, 209P (R-4)	Sa-4	1.6	0.8	1.6	3.1	50	100	50
,, ,, A20239	Sa-10	100	0.8	1.6	6.3	>100	100	100
Escherichia coli NIHJ	Ec-1	0.8	0.4	1.6	1.6	25	50	25
,, ,, Juhl	Ec-3	1.6	0.8	1.6	3.1	50	50	50
,, ,, A20363 (ML-1630)	Ec-5	>100	0.8	1.6	3.1	>100	50	25
,, ,, A20365	Ec-7	100	0.2	0.4	0.4	>100	6.3	6.3
,, ,, K12	Ec-8	0.8	0.4	0.8	0.8	25	25	25
,, ,, NR79/W677	Ec-9	6.3	0.8	1.6	12.5	100	25	50
,, ,, JR35/C600	Ec-10	>100	0.2	0.8	0.8	>100	12.5	25
,, ,, W677	Ec-52	0.2	0.4	0.8	0.8	50	6.3	25
,, ,, JR66/W677	Ec-53	>100	0.4	1.6	6.3	>100	50	>100
Klebsiella pneumoniae D-11	Kp-1	0.2	0.1	0.2	0.4	12.5	6.3	12.5
,, ,, Type 22, #3038	Kp-8	>100	0.8	3.1	6.3	>100	50	>100
Pseudomonas aeruginosa D-15	Pa-1	12.5	0.8	3.1	3.1	>100	>100	>100
,, ,, A9930	Pa-3	12.5	0.2	0.4	1.6	100	25	50
,, ,, H9	Pa-4	>100	6.3	12.5	12.5	>100	>100	>100
,, ,, A20718 (strain 130)	Pa-16	50	3.1	—	—	>100	>100	>100
Proteus vulgaris A9436	Pv-1	0.4	0.2	0.2	0.4	12.5	12.5	12.5
Proteus mirabilis A9554	Pm-1	0.8	0.8	1.6	1.6	25	50	50
Proteus morganii A9553	Pg-1	0.8	0.4	0.8	0.8	25	25	50
,, ,, A20031	Pg-2	0.8	0.8	1.6	3.1	25	50	50
Mycobacterium 607	M6-1	0.4	0.4	0.8	3.1	25	25	25
Mycobacterium phlei	Mp-1	0.4	0.2	0.4	1.6	25	6.3	12.5
Mycobacterium ranae	Mr-1	0.4	0.4	0.8	3.1	25	12.5	25

similar to that of **1** but with less intrinsic activity than **1**. Compound **2** is approximately half as active as **1**, while **3** with the D-configuration side chain is about one-fourth as active as **1**. Some of the kanamycin-resistant organisms (*e.g.* Ec-9, Ec-53, Kp-8 and Sa-10 in Table 1) showed relatively greater resistance to compound **3**, the MICs being 8~16 times higher than those of **1**.

Positional Isomers of BB-K8

Since there are four acylable amino groups in kanamycin A, two in the 2-deoxystreptamine (DOS) and one each in the 6-amino-6-deoxy-D-glucose (6-AG) and 3-amino-3-deoxy-D-glucose (3-AG) moieties, three other positional isomers of BB-K8 are possible. For convenience, the four amino groups in the kanamycin molecule are designated as N^1, N^2, N^3 and N^4 in the order of reactivity to an acylating agent. It has been reported[1] that BB-K8 is the N^2-acylation product of kanamycin with L-HABA. The selective acylation at the other amino group, N^1, N^3 or N^4, with L-HABA was achieved on either the intact kanamycin A or the suitably N-protected kanamycin derivatives. Amino groups were protected by the carbobenzoxy (Cbz) group with use of N-(benzyloxycarbonyloxy)succinimide (**4**) and the acylation was carried out also by the activated ester method using the N-hydroxysuccinimide ester of N-Cbz-L-HABA (**5**). The N-protecting groups were finally removed by catalytic hydrogenolysis over palladium-on-charcoal. The synthetic

Fig. 2. Preparation of L-HABA derivatives of kanamycin

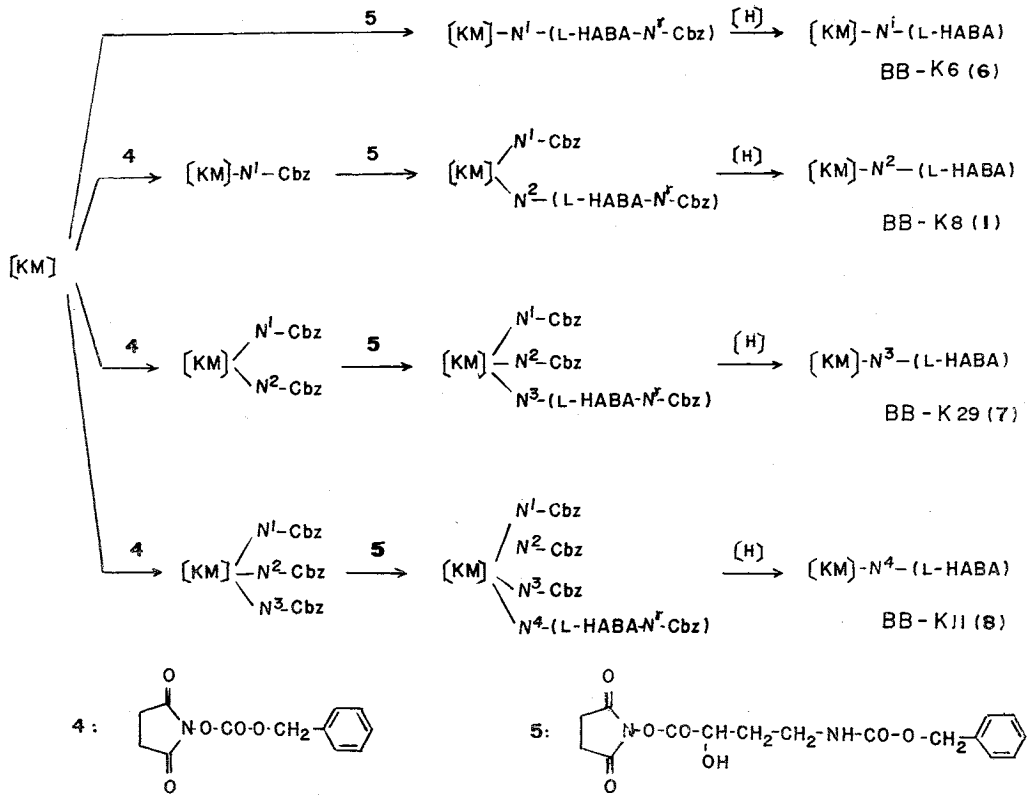


Table 2. Properties of L-HABA derivatives of kanamycin A

Compound	Code No.	Site of acylation	mp (°C)	$[\alpha]_D$ in H_2O	TLC		Activity*** (u/mg)
					S-110*	S-116**	
1	BB-K8	C-1 (N^2)	203~204°(dec.)	+99° (*c* 1.0, 23°C)	0.17	0.45	1,000
6	BB-K6	C-6′(N^1)	184~187°(dec.)	+109° (*c* 1.0, 26°C)	0.29	0.60	33
7	BB-K29	C-3(N^3)	181~183°(dec.)	+83.5° (*c* 1.0, 22°C)	0.24	0.55	15
8	BB-K11	C-3″(N^4)	202°(dec.)	+92° (*c* 0.75, 29°C)	0.15	0.38	10
Kanamycin A					0.43	0.78	

* silica gel plate, $CHCl_3$-MeOH-28% NH_4OH-H_2O (1:4:2:1)
** silica gel plate (developed ×2), upper layer of $CHCl_3$-MeOH-17% NH_4OH (2:1:1)
*** agar diffusion assay (*B. subtilis* PCI 219 plate, relative to BB-K8)

routes are schematically shown in Fig. 2.

BB-K6 (**6**, $C_{22}H_{43}N_5O_{13}\cdot 2H_2CO_3$), which is the acylation product at the most reactive amino function (N^1), was prepared by reacting intact kanamycin A with an equimolar amount of **5**. The actual location for N^1 has been determined to be the C-6′ amino group in the 6-AG moiety by a method similar to that used by UMEZAWA *et al.*[13] Compound 6 was treated with $NaNO_2$ in aqueous acetic acid and then hydrolyzed in 4 N HCl. From the hydrolyzate, 6-AG was isolated and identified but neither 3-AG nor DOS was detected.

BB-K29 (7, $C_{22}H_{43}N_5N_{13}\cdot 2H_2CO_3$), which is the N^3-acylation product, was prepared from **5** and the N^1, N^2-di-Cbz-kanamycin ($C_{84}H_{43}N_4O_{15}\cdot 3H_2O$, mp 182~186°C, TLC (S-114*) Rf 0.38), which was obtained by reacting kanamycin with 2 moles of **4**. The site of acylation in **7** was established to be the C-3 amino group in

* Silica gel plate, MeOAc-*n*-PrOH-28% NH_4OH (45:105:60).

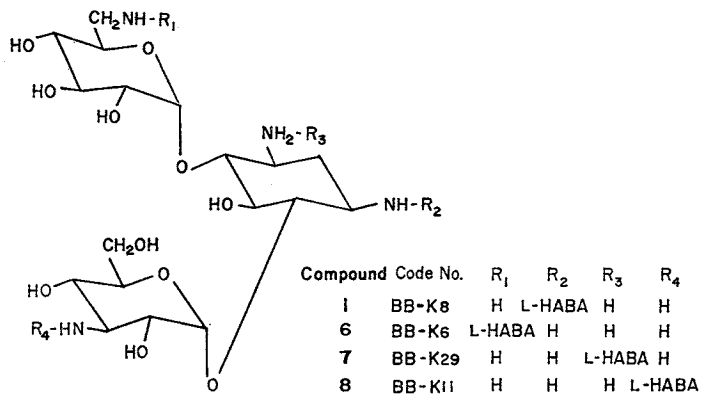
the DOS moiety by the following experiments: SCHIFF's base of **7** with *p*-methoxybenzaldehyde (tetra-N-*p*-methoxybenzylidene BB-K29, $C_{54}H_{67}N_5O_{17}$, mp 155~157°C) was reduced by $NaBH_4$ to the tetra-N-*p*-methoxybenzyl derivative ($C_{54}H_{75}N_5O_{17}\cdot H_2O$, mp 161~165°C) which was then hydrolyzed with 6 N HCl to yield 1-N-*p*-methoxybenzyl-2-deoxystreptamine ($C_{14}H_{22}N_2O_4\cdot H_2O$, mp 75~78°C, TLC (S-114) Rf 0.45, ORD (H_2O): $[\alpha]_{280}^{trough}$ −390°, $[\alpha]_{265}^{peak}$ −330°, $[\alpha]_{232}^{trough}$ −1130°. CD(H_2O): $[\theta]_{273}$ −508, $[\theta]_{223}$ −3810), which was determined to be the enantiomer of that obtained from BB-K8 by the same sequence of reactions[1].

BB-K11 (**8**, $C_{22}H_{43}N_5O_{13}\cdot 2H_2CO_3$), which is the acylation product at the least reactive amino function (N^4), was prepared from **5** and the N^1, N^2, N^3-tri-Cbz-kanamycin ($C_{42}H_{54}N_4O_{17}$, mp 258~263°C(dec.), TLC (S-114) Rf 0.43), the latter compound being obtained from the same reaction mixture from which the above-described N^1, N^2-di-Cbz-kanamycin was isolated. The site of acylation in **8** was determined to be the C-3″ amino group in the 3-AG moiety, since the deamination of **8** and subsequent acid hydrolysis gave 3-AG but neither 6-AG nor DOS. The structures of the above compounds are shown in Fig. 3.

Some of the physico-chemical properties of compounds **6**, **7** and **8** are shown in Table 2. Comparative periodate oxidation data on these compounds along with BB-K8 and kanamycin are shown in Table 3, and were consistent with the assigned structures.

In marked contrast to BB-K8, its positional isomers **6**, **7** and **8** are all very weakly active, having about 1~3% of the activity of BB-K8 when assayed on a *Bacillus subtilis* plate by the agar diffusion method (Table 2). The MICs of these compounds determined by serial agar dilution method are shown in Table 1. It may be worthwhile to note that, although compounds **7** and **8** are much less active than kanamycin against the sensitive organisms, they are considerably more active than kanamycin against the kanamycin-resistant organisms (*e.g.* Ec-5[8], Ec-7, Ec-10[9] in Table 1) which are known or presumed to inactivate kanamycin and neomycin by 3′-phosphorylation (neomycin phosphotransferase I[11]). Another interesting finding is

Fig. 3.



Compound	Code No.	R_1	R_2	R_3	R_4
1	BB-K8	H	L-HABA	H	H
6	BB-K6	L-HABA	H	H	H
7	BB-K29	H	H	L-HABA	H
8	BB-K11	H	H	H	L-HABA

Table 3. Periodate oxidation of L-HABA derivatives of kanamycin A

Compound	Code No.	Periodate consumed (mole)					
		1 hr	2 hr	5 hr	7 hr	24 hr	Theor.
1	BB-K8	2.3	2.9	3.2	3.5	4.2	4
6	BB-K6	4.1	4.1	4.1	4.2	4.4	4
7	BB-K29	3.1	3.7	4.0	4.2	4.7	4
8	BB-K11	1.1	1.6	1.9	2.1	2.5	2
Kanamycin A		3.1	3.7	4.0	4.2	4.7	4

that compound **7** is relatively more active than **8** against Ec-53 (*Escherichia coli* JR66/W677[10]) and Kp-8 (*Klebsiella pneumoniae* Type 22 #3038[10]) which are known to produce the neomycin phosphotransferase II[11]. The general antibacterial pattern of compound **6** is similar to that of kanamycin though the intrinsic activity of **6** is much lower than the latter.

Enzymatic inactivation experiments were carried out on compounds **7** and **8** with an enzymatic solution obtained from *E. coli* JR66/W677 according to the published method[12]. Compound **7** was recovered intact from the enzymatic reaction mixture. However, the enzymatic reaction of compound **8** yielded the inactivated product (**9**) which was isolated by ion-exchange chromatography (Amberlite CG-50, NH_4^+ form) and characterized. Compound **9** analyzed as $C_{22}H_{43}N_5O_{13}\cdot HPO_3\cdot H_2CO_3\cdot H_2O$, gave positive HANES reaction and showed no UV absorption maximum. Treatment of **9** with alkaline phosphatase regenerated compound **8**. Periodate oxidation experiment on **9** showed no consumption (0.11 mole) of the reagent, while **8** consumed 2.13 moles in a comparative test. Thus, compound **9** appeared to be 3′-phosphate of compound **8**.

It is very likely from the comparative antibacterial spectra presented here and also from the inactivation experiments that L-HABA acylation at the C-1 or C-3 amino group of kanamycin (compound **1** or **7**) has given rise to a resistance to both neomycin phosphotransferase I and II, and that acylation at C-3″ with L-HABA (compound **8**) might block the enzymatic action of neomycin phosphotransferase I but not of II. Acylation at the C-6′ amino group (compound **6**) seems to have no effect on the action of phosphorylative enzymes.

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