The Novel Enzymatic 3''-*N*-Acetylation of Arbekacin by an Aminoglycoside 3-*N*-Acetyltransferase of *Streptomyces* Origin and the Resulting Activity†

KUNIMOTO HOTTA*, ATSUKO SUNADA, JUN ISHIKAWA and SATOSHI MIZUNO

National Institute of Infectious Diseases,
1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

YOKO IKEDA and SHINICHI KONDO

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

(Received for publication May 19, 1998)

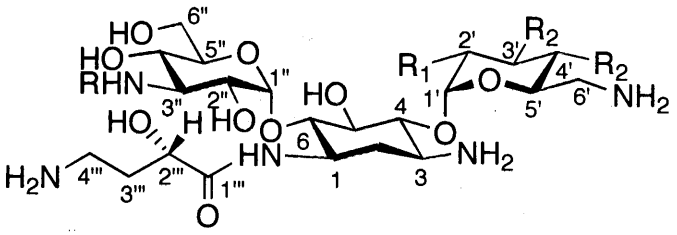
Kanamycin group antibiotics were subjected to enzymatic acetylation by a cell free extract containing an aminoglycoside 3-*N*-acetyltransferase, AAC(3)-X, derived from *Streptomyces griseus* SS-1198PR. Characterization of the incubated reaction mixtures by TLC and antibiotic assay revealed that a product retaining activity was specifically formed from arbekacin, an anti-MRSA semisynthetic aminoglycoside. The structural determination demonstrated that acetylation occurred at the 3''-amino group in arbekacin and amikacin, and at the 3-amino group in dibekacin as in the case of kanamycin. These results should reflected the effect of the (*S*)-4-amino-2-hydroxybutyryl side chain which is present in arbekacin and amikacin, but absent in dibekacin and kanamycin. The 3''-*N*-acetylation is the first finding in the enzymatic modifications of aminoglycoside antibiotics. 3''-*N*-Acetylarbekacin showed antibiotic activity as high as that of 2'-*N*-acetylarbekacin reported previously, whereas 3''-*N*-acetylamikacin showed no substantial activity. Thus, our results illuminated a novel aspect of arbekacin distinct from the other aminoglycosides.

Enzymatic mechanisms of resistance to aminoglycoside antibiotics were first reported in 1967 by UMEZAWA and his colleagues.[1,2] They demonstrated all the three types of aminoglycoside-modifying enzymes in clinically-isolated resistant bacteria carrying R plasmids; *i.e.*, an aminoglycoside acetyltransferase, AAC(6')[3] as well as a phosphotransferase, APH(3'),[4] that inactivated kanamycin (KM), and an adenylyltransferase, AAD(3''),[5] that inactivated streptomycin (SM). On the basis of these findings, they predicted structures refractory to these enzymes and eventually synthesized dibekacin (DKB) as the first rationally designed semisynthetic aminoglycoside.[6] Subsequently, arbekacin (ABK)[7] having the 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] side chain were synthesized as in the case of amikacin (AMK).[8] Among a lot of aminoglycoside derivatives synthesized for active agents against resistant bacteria, only ABK showed an excellent activity against all of methicillin-resistant *Staphylococcus aureus* (MRSA) strains containing any sort of aminoglycoside-modifying enzyme available in the 1980s. Therefore, ABK was approved as a chemotherapeutic agent for the treatment of infections caused by MRSA, since 1990 in Japan,[9~11] and has been used extensively until today. Only a small number of MRSA strains with a moderate level (12.5~25 μg/ml) of ABK-resistance due to a bifunctional enzyme APH(2'')/AAC(6') have emerged[12,13] so that the incidence of ABK-registant MRSA strains has been very low. However, one cannot rule out the possibility of emergence in the future of novel ABK-resistant strains, since ABK contains several amino and hydroxyl groups that may be modified by enzymes such as AAC(3), AAC(2') and AAC(6').

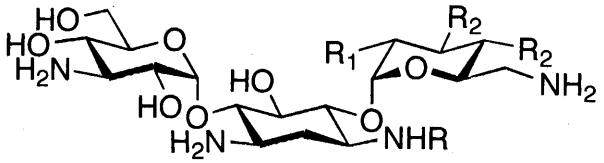
To check these possibility, we attempted to use AACs

† A part of this work was presented at ASM General Meeting (96th) that was held on May 21, 1996 in New Orleans, La. and at the 43rd Annual Meeting of the Eastern Branch of the Japan Society of Chemotherapy held on November 15, 1996 in Tokyo.

Fig. 1. Structures.



Arbecacin R=H R_1=NH_2 R_2=H
Amikacin R=H R_1=OH R_2=OH
3″-*N*-Acetylarbekacin R=$COCH_3$ R_1=NH_2 R_2=H
3″-*N*-Acetylamikacin R=$COCH_3$ R_1=OH R_2=OH



Dibekacin R=H R_1=NH_2 R_2=H
Kanamycin R=H R_1=OH R_2=OH
3-*N*-Acetyldibekacin R=$COCH_3$ R_1=NH_2 R_2=H
3-*N*-Acetylkanamycin R=$COCH_3$ R_1=OH R_2=OH

available from actinomycetes such as aminoglycoside-producers. First, we exposed ABK to AAC(2′) derived from a kasugamycin-producing strain, *Streptomyces kasugaensis* MB273. Consequently it turned out that ABK was readily converted to the 2′-*N*-acetyl derivative retaining antibiotic activity, indicating that AAC(2′)-dependent aminoglycoside resistant bacteria could not be resistant to ABK.[14] While, an inactivated product, 3-*N*-acetyl KM was isolated by the enzymatic reaction of KM with an AAC(3) obtained from *S. griseus* SS-1198PR, a KM-resistant mutant derived from a wild type SM-producing strain (*S. griseus* SS-1198). Although this enzyme was first designated AAC(3)-V,[15] it was renamed AAC(3)-X as a new aminoglycoside-modifying enzyme.[16]

In the present study, ABK, AMK and DKB were exposed to AAC(3)-X of *S. griseus* origin. Interestingly, ABK and AMK that have the same 1-*N*-acyl side chain turned out to be modified by acetylation at the 3″-amino group that has never been reported in any aminoglycoside, whereas DKB lacking the side chain was converted to 3-*N*-acetyl DKB by the intrinsic action of AAC(3)-X (Fig. 1). In this paper, the formation, isolation, structural elucidation and antimicrobial activity of enzymatic reaction products of ABK, AMK and DKB by AAC(3)-X are presented.

Results

In a preliminary study on acetylation with a cell free extract of *S. lividans* TK21/pANT3-1 containing the AAC(3)-X gene (*aac(3)-Xa*) cloned from a KM-resistant mutant of SM-producing *S. griseus* SS-1198, ABK was completely converted to a single modified product monitored by TLC, similar to KM and DKB. The antibiotic assay of the reaction mixtures demonstrated that no apparent inactivation occurred with ABK suggesting that the acetylation product of ABK retained antibiotic activity as comparable as that of ABK. By contrast, the reaction mixtures of DKB and KM lost the activity.[15] While, acetylation of AMK proceeded so slowly that it was not completed even by 96-hour incubation.

The acetylated product in the enzymatic reaction mixture of ABK and a cell free extract of *S. lividans* TK21/pANT3-1 was isolated by column chromatography on Amberlite CG-50 (NH_4^+) resin in a good yield. FAB-MS of the product provided *m/z* 595 $(M+H)^+$ indicating monoacetylated ABK. As shown in Tables 1 and 2, the ^1H and ^{13}C NMR spectra of the acetylated product were compared with those of ABK in an acidic D_2O. The ^1H NMR spectrum of the product showed an acetyl CH_3 signal at δ 2.07 and the 3″-H signal at δ 4.10 shifted to lower field than that of ABK (δ 3.45). The ^{13}C

Table 1. ^{1}H NMR spectral data.

Proton	Chemical shift (δ ppm)					
	ABK	3"AcABK	AMK	3"AcAMK	DKB	3AcDKB
1-H	4.15 ddd	4.13 m	4.14 m	4.11 m	3.68 ddd	3.57 ddd
2-Hax	1.88 ddd	1.88 ddd	1.84 ddd	1.79 ddd	2.00 ddd	1.79 ddd
2-Heq	2.27 ddd	2.28 ddd	2.27 ddd	2.25 ddd	2.58 ddd	2.27 ddd
3-H	3.54 ddd	3.55 m	3.63 ddd	3.64 m	3.62 m	**4.09** ddd
4-H	4.00 dd	4.00 dd	3.92 m	3.88 dd	4.07 dd	3.76 dd
5-H	3.84 dd	3.88 dd	3.93 m	3.96 dd	3.92 dd	3.86 dd
6-H	3.92 dd	3.92 dd	3.89 m	3.84 dd	3.86dd	3.74 dd
1'-H	5.80 d	5.83 d	5.55 d	5.52 d	5.83 d	5.70 d
2'-H	3.59 ddd	3.6 m	3.71 dd	3.68 d	3.61 m	3.48 ddd
3'-H	2.07 m	2.07 m	3.81 dd	3.78 m	2.05 m	1.84 m
						2.00 m
4'-H	1.65 dddd	1.65 dddd	3.42 dd	3.39 dd	1.64 ddd	1.63 ddd
	1.96 m	1.97 m			1.95 m	1.87 m
5'-H	4.21 dddd	4.22 dddd	4.06 ddd	4.05 m	4.22 dddd	4.01 m
6'-H	3.15 dd	3.15 dd	3.22 dd	3.18 dd	3.13 dd	3.14 dd
	3.29 dd	3.30 dd	3.47 dd	3.45 dd	3.29 dd	3.27 dd
1"-H	5.20 d	5.18 d	5.20 d	5.15 d	5.16 d	5.11 d
2"-H	3.81 dd	3.59 dd	3.80 dd	3.55 dd	4.00 dd	3.96 dd
3"-H	3.45 dd	**4.10** dd	3.43 dd	**4.07** m	3.54 dd	3.52 dd
4"-H	3.71 dd	3.50 dd	3.73 dd	3.47 dd	3.75 dd	3.73 dd
5"-H	4.05 ddd	4.05 m	4.12 m	4.09 m	3.96 m	3.94 m
6"-H	3.81 dd	3.80 dd	3.83 m	3.80 m	3.82 dd	3.80 dd
	3.86 dd	3.85 dd			3.90 dd	3.86 dd
2'''-H	4.30 dd	4.29 dd	4.31 dd	4.26 dd		
3'''-H	2.0 m	1.99 m	2.00 dddd	1.98 m		
	2.20 dddd	2.17 dddd	2.20 m	2.16 m		
4'''-H	3.20 br t	3.19 br t	3.20 br t	3.17 br t		
Ac-CH_3		2.07 s		2.07 s		2.03 s

Bold letters show lower-field shifts due to the *N*-acetylation.

NMR spectrum showed an acetyl CO (δ 176.0) and CH_3 (δ 23.1) signals, and the β-carbons (C-2" and C-4") of the acetamide group shifted to lower field (δ 70.4 and 68.3, respectively). Thus, the structure of the product was elucidated to be 3"-*N*-acetyl ABK. The enzymatic reaction of AMK by 48-hour incubation gave 3"-*N*-acetyl AMK in 35% yield. However, the product of DKB was the 3-*N*-acetyl derivative similar to that of KM.[15]

As shown in Table 3, by the enzymatic reaction with the enzyme AAC(3)-X, ABK and AMK that have the same 1-*N*-acyl side chain, (*S*)-4-amino-2-hydroxybutyric acid, were converted into 3"-*N*-acetyl ABK and 3"-*N*-acetyl AMK, respectively, instead of 3-*N*-acetyl products. As expected, the modification product of DKB was confirmed to be 3-*N*-acetyl DKB. On the other hand, two known enzymes, AAC(3)-III produced by *Pseudomonas aeruginosa* PST1[17] and AAC(3)-IV produced by *Escherichia coli* JR225,[18] were not able to acetylate ABK and AMK, but readily converted KM and DKB to the 3-*N*-acetyl derivatives.

Furthermore, a new product, 3"-*N*-acetyl ABK showed a substantial antibiotic activity (55% activity of ABK against *Bacillus subtilis* PCI219) as same as 2'-*N*-acetyl ABK (42% activity of ABK).[15] Minimum inhibitory concentrations of 3"-*N*-acetyl ABK are shown in Table 4.

Discussion

It was surprise to us to learn that AAC(3)-X was capable of converting both ABK and AMK to novel

Table 2. ^{13}C NMR spectral data.

Carbon	Chemical shift (δ ppm)					
	ABK	3"AcABK	AMK	3"AcAMK	DKB	3AcDKB
C-1	49.5	49.5	49.6	49.6	50.4	51.0
C-2	31.1	31.1	30.9	30.8	28.6	**30.7**
C-3	49.6	49.7	48.6	48.3	49.5	48.1
C-4	78.5	78.5	80.0	79.8	77.8	**78.6**
C-5	75.6	75.7	73.2	72.7	75.1	76.0
C-6	80.8	80.6	80.9	80.6	84.6	84.9
C-1'	95.9	95.9	96.3	95.5	96.0	95.4
C-2'	49.5	49.5	71.6	71.5	49.3	49.6
C-3'	21.3	21.3	73.1	73.1	21.3	21.6
C-4'	26.1	26.2	71.5	71.5	26.2	26.1
C-5'	66.7	66.7	69.5	69.4	66.8	65.7
C-6'	43.3	43.4	41.1	41.0	43.4	43.2
C-1"	98.8	99.2	98.7	99.1	101.4	101.3
C-2"	68.7	**70.4**	68.8	**70.4**	68.8	68.9
C-3"	55.9	54.7	56.1	54.7	55.7	55.7
C-4"	66.3	**68.3**	66.3	**68.2**	66.2	66.1
C-5"	72.9	73.4	72.7	73.1	73.8	73.6
C-6"	60.5	61.2	60.5	61.0	60.7	60.6
C-1'"	176.3	176.3	176.3	176.3		
C-2'"	70.4	70.4	70.4	70.3		
C-3'"	31.6	31.6	31.6	31.5		
C-4'"	37.7	37.8	37.7	37.7		
Ac-CO		176.0		176.0		174.5
Ac-CH_3		23.1		23.0		23.0

Bold letters show β-carbon shifts due to the *N*-acetylation.

Table 3. Enzymatic *N*-acetylation of aminoglycoside antibiotics.

Aminoglycoside-modifying enzyme	Producing strain	ABK	AMK	DKB	KM
		Acetylation (MIC μg/ml)	Acetylation (MIC μg/ml)	Acetylation (MIC μg/ml)	Acetylation (MIC μg/ml)
AAC(3)-III	*P. aeruginosa* PST1	No (12.5)	No (12.5)	3-*N*-acetyl (>100)	3-*N*-acetyl (>100)
AAC(3)-IV	*E. coli* JR225	No (0.39)	No (1.56)	3-*N*-acetyl (50)	3-*N*-acetyl (1.56)
AAC(3)-X	*S. lividans* TK21/pANT3-1	3"-*N*-acetyl (<2.5)	Slow 3"-*N*-acetyl (<2.5)	3-*N*-acetyl (>400)	3-*N*-acetyl (>400)

MICs against enzyme-producing strains are shown in parentheses.

monoacetylated derivatives, 3"-*N*-acetyl ABK and 3"-*N*-acetyl AMK, respectively, while the enzyme produced 3-*N*-acetyl derivatives of both KM and DKB as we expected. The 3"-*N*-acetylation should reflect a steric hindrance effect of the acyl side chain common to both ABK and AMK. The 3"-*N*-acetylation will take place on the opposite side of the 3-amino group possibly due to the effect of the side chain. The long arm of the panthoteine residue in acetyl CoA molecule may also be critical to the 3"-*N*-acetylation. It should also be noted

Table 4. Minimum inhibitory concentrations of 3″AcABK and ABK.

Test organism	Aminoglycoside-modifying enzyme	MIC (μg/ml) 3″AcABK	ABK
Staphylococcus aureus FDA209P		0.78	≤0.20
S. aureus Smith		1.56	≤0.20
S. aureus Ap01	AAD(4',4")	25	1.56
S. aureus MS15009 GMr		>100	>100
S. aureus MS9610		3.13	0.39
S. aureus MS16526	APH(2")/AAC(6'), AAD(4',4")	50	12.5
S. epidermidis 109	AAD(4',4")	6.25	≤0.20
Micrococcus flavus FDA16		>100	1.56
M. luteus PCI1001		>100	0.78
Bacillus anthracis		0.78	≤0.20
B. subtilis PCI219		0.39	≤0.20
B. subtilis NRRL B-558		0.78	≤0.20
B. cereus ATCC10702		3.13	0.78
Corynebacterium bovis 1810		>100	0.39
Mycobacterium smegmatis ATCC607		6.25	0.20
Escherichia coli NIHJ		3.13	≤0.20
E. coli K-12		3.13	0.39
E. coli K-12 R5	AAC(6')-1	>100	12.5
E. coli K-12 R388		3.13	<0.20
E. coli K-12 J5 R11-2	APH(3')-I	3.13	0.39
E. coli K-12 ML1629	APH(3')-I	12.5	0.78
E. coli K-12 ML1630		12.5	0.78
E. coli K-12 ML1410		12.5	0.78
E. coli K-12 ML1410 R81	APH(3')-I	12.5	0.78
E. coli K-12 LA290 R55	AAD(2")	6.25	0.78
E. coli K-12 LA290 R56		>100	>100
E. coli K-12 LA290 R64		3.13	≤0.20
E. coli K-12 C600 R135	AAC(3)-I	3.13	0.39
E. coli W677		3.13	≤0.20
E. coli JR66/W677	APH(3')-II, AAD(2")	25	1.56
E. coli JR225	AAC(3)-IV	12.5	0.39
Klebsiella pneumoniae PCI602		6.25	0.78
K. pneumoniae 22#3038	APH(3')-II, AAD(2")	12.5	1.56
Shigella dysenteriae JS11910		12.5	1.56
S. flexneri 4b JS11811		3.13	1.56
S. sonnei JS11746		12.5	0.78
Salmonella typhi T-63		6.25	0.39
S. enteritidis 1891		50	1.56
Proteus vulgaris OX19		12.5	0.78
Providencia rettgeri GN311		1.56	0.78
P. rettgeri GN466		1.56	0.39
Providencia sp. Pv16	AAC(2')	25	1.56
Providencia sp. 2991	AAC(2')	100	3.13
Serratia marcescens		100	6.25
Serratia sp. SOU		>100	12.5
Serratia sp. 4		>100	>100
Pseudomonas aeruginosa A3		1.56	0.78
P. aeruginosa No.12		25	3.13
P. aeruginosa H9	APH(3')-II	25	3.13
P. aeruginosa H11		50	12.5
P. aeruginosa TI-13	APH(3')-I	12.5	1.56
P. aeruginosa GN315	AAC(6')-4	>100	6.25
P. aeruginosa 99	AAC(3)-I	25	6.25
P. aeruginosa B-13	APH(3')-I, -II	50	6.25
P. aeruginosa 21-75	APH(3')-III	>100	25
P. aeruginosa PST1	AAC(3)-III	25	3.13
P. aeruginosa ROS134/PU21		>100	100
P. aeruginosa K-Ps102		50	6.25
Stenotrophomonas maltophilia GN907		>100	100

that AACs derived from *P. aeruginosa* and *E. coli* failed to do the 3″-*N*-acetylation, although these enzymes produced 3-*N*-acetyl derivatives from KM and DKB. This means that AAC(3)-X has a unique catalytic property.

The other point to note is that 3″-*N*-acetyl ABK showed a substantial antibiotic activity whereas no significant activity was observed with 3″-*N*-acetyl AMK in spite of their structural similarity. The differences can be seen at the 2′-, 3′- and 4′-positions where ABK has amino, deoxy and deoxy, respectively, instead of all hydroxyl groups in AMK. It seems possible that the presence of extra amino group in ABK in comparison with AMK plays a critical role for the antibiotic activity. However, this explanation cannot be acceptable for the substantial activity of 2′-*N*-acetyl ABK when compared with the activity of 2′-*N*-acetyl DKB since there is no difference in the numbers of amino group between them.[14] Therefore, the reason for the antibiotic activity of the monoacetyl derivatives of ABK remains unsolved.

However, there is no doubt that the antibiotic activity of the monoacetyl derivatives is the advantage for ABK to keep itself refractory to the emergence of resistant bacteria depending on AAC(3) and AAC(2′). The activity of acetylated derivatives may allow us to call ABK the third generation aminoglycoside antibiotic.

Experimental

General

The optical rotations were taken on a Perkin-Elmer 241 polarimeter. FAB-MS spectra were measured on a Jeol JMS-SX102 mass spectrometer. ^1H and ^{13}C NMR spectra in D_2O at pD 1.5~2.0 were taken on a Jeol JNM-EX400 spectrometer. ^1H NMR spectra were recorded at 400 MHz using TMS ($\delta=0$) as an internal standard, and ^{13}C NMR spectra at 100 MHz using dioxane ($\delta=67.4$) as an internal standard. Chemical shifts were assigned by ^1H-^1H COSY, DEPT, HMQC, HMBC and NOE experiments. High-voltage paper electrophoresis[19] (HVPE) was carried out on a CAMAG HVE system at 3,300 V for 15 minutes, using HCOOH-CH_3COOH-H_2O (25:75:900, pH 1.8) as an electrolyte solution, and stained with ninhydrin and Rydon-Smith reagents. The relative mobility (Rm) to alanine was calculated. TLC was performed on a silica gel plate (E. Merck, Art. 5715) developed with $CHCl_3$-MeOH-17% aqueous ammonia (1:4:3).

All antibiotics, kanamycin (KM), amikacin (AMK), dibekacin (DKB) and arbekacin (ABK) as each sulfate were obtained from Meiji Seika Kaisha, Ltd. Other chemicals were commercially available.

Antibiotic Activity

Antimicrobial activities against *Bacillus subtilis* PCI219 were determined by an ordinary cup or paper disc method in Mycin Assay Agar (Difco). Minimum inhibitory concentrations (MICs) were determined by the serial two-fold agar dilution method in Bacto Mueller-Hinton Medium (Difco) after incubation at 37°C for 17 hours.

AAC(3) Producing Strains

S. lividans TK21/pANT3-1 transformed with a KM-resistant plasmid, pANT3-1 containing a KM resistance (AAC(3)-X) gene derived from *S. griseus* SS-1198PR[15] was used as the AAC(3)-X producer. The AAC(3)-X gene was discovered through the protoplast regeneration of the wild type strain, *S. griseus* SS-1198 and cloned into pIJ702 to produce pANT3-1. *S. lividans* TK21/pANT3-1 is highly resistant to KM and DKB (>400 μg/ml), and sensitive to ABK and AMK (<2.5 μg/ml) (Table 3). AAC(3)-III producing strain, *P. aeruginosa* PST1[17] and AAC(3)-IV producing *E. coli* JR225[18] were kindly provided by Professor J. DAVIES.

Cell Free Extracts

A cell free extract (S-30) of *S. lividans* TK21/pANT3-1 was prepared by the method described in a previous paper.[15] Cells of *P. aeruginosa* PST1 and *E. coli* JR225 were disrupted sonically and then centrifuged at 100,000 × *g* for 2 hours at 4°C to obtain cell free extracts (S-100).

Isolation of 3″-*N*-Acetylarbekacin (3″AcABK)

The enzymatic reaction was carried out in a mixture (20 ml) consisting of ABK (20.4 mg, 36.9 μmol) in H_2O 15 ml, 50 mM acetyl CoA (Na salt, Sigma) 2 ml, the cell free extract of *S. lividans* TK21/pANT3-1 (69.6 mg protein/ml) 1 ml and 1 M phosphate buffer (pH 7.0) 2 ml at 37°C for 20 hours. The mixture was passed through a column of Amberlite CG-50 (NH_4^+, 20 ml). After being washed with H_2O (40 ml) followed by 0.4% aqueous ammonia (100 ml), the column was eluted with 1% aqueous ammonia (100 ml). Approximately 2-ml fractions of the eluate were collected, and both ninhydrin-positive and Rydon-Smith-positive products in the fractions were detected by HVPE and TLC. Fractions 18~38 were concentrated to give pure 3″AcABK (16.0 mg, 73%) as the colorless powder, $[\alpha]_D^{25}$ +79° (*c* 0.59,

H_2O); FAB-MS (positive) m/z 595 $(M+H)^+$; HVPE Rm 1.71 (ABK: 2.01); TLC Rf 0.26 (ABK: 0.20). The ^1H and ^{13}C NMR spectra are shown in Tables 1 and 2. The antibacterial activity against *B. subtilis* PCI219 by an ordinary cup method showed 55% activity of ABK. The MICs are shown in Table 3.

Isolation of 3″-*N*-Acetylamikacin (3″AcAMK)

An enzyme reaction mixture (10 ml, 37°C, 48 hours) consisting of AMK (10 mg, 17.1 μmol) in H_2O 7.0 ml, 50 mM acetyl CoA 1.0 ml, the cell free extract of *S. lividans* TK21/pANT3-1 (69.6 mg protein/ml) 1.0 ml and 1 M phosphate buffer (pH 7.0) 1.0 ml was passed through a column of Amberlite CG-50 (NH_4^+, 20 ml). After being washed with H_2O (40 ml) and 0.4% aqueous ammonia (100 ml), the column was eluted with 1.5% aqueous ammonia. Approximately 2-ml fractions of the eluate were collected, and both ninhydrin-positive and Rydon-Smith-positive products in the fractions were detected by HVPE. Fractions 10~14 showing Rm 1.33 were concentrated to obtain pure 3″AcAMK (3.8 mg, 35%) as the colorless powder, $[\alpha]_D^{25}$ +94° (*c* 0.19, H_2O); FAB-MS (positive) m/z 628 $(M+H)^+$; HVPE Rm 1.33 (AMK: 1.56); TLC Rf 0.16 (AMK: 0.13). The ^1H and ^{13}C NMR spectra are shown in Tables 1 and 2. The antibacterial activity against *B. subtilis* PCI219 showed 3% activity of AMK.

Isolation of 3-*N*-Acetyldibekacin (3AcDKB)

An enzyme reaction mixture (10 ml, 37°C, 2 hours) consisting of DKB (10 mg, 22.1 μmol) in H_2O 6.84 ml, 50 mM acetyl CoA 1.0 ml, the cell free extract of *S. lividans* TK21/pANT3-1 (69.6 mg protein/ml) 0.16 ml and 1 M phosphate buffer (pH 7.0) 1.0 ml was passed through a column of Amberlite IRC-50 (NH_4^+, 20 ml). After being washed with H_2O (40 ml), the column was eluted with 0.3% aqueous ammonia. Approximately 3.5-ml fractions of the eluate were collected, and ninhydrin-positive products in the fractions were detected by TLC. Fractions 30~50 were concentrated to 3 ml and the product in the concentrate was rechromatographed on a column of Amberlite CG-50 (NH_4^+, 10 ml). After being washed with H_2O (20 ml) and 0.2% aqueous ammonia (40 ml), the column was eluted with 50 ml of 1.0% aqueous ammonia and each 1-ml fraction was collected. From fractions 8~17, pure 3AcDKB (8.2 mg, 75%) was obtained as the colorless powder. $[\alpha]_D^{25}$ +100° (*c* 0.37, H_2O); FAB-MS (positive) m/z 494 $(M+H)^+$; HVPE Rm 1.67 (DKB: 1.79); TLC Rf 0.55 (DKB: 0.47). The ^1H and ^{13}C NMR spectra are shown in Tables 1 and 2. The antibacterial activity against *B. subtilis* PCI219 showed only 0.2% activity of DKB.

References

1) UMEZAWA, H.: Biochemical mechanism of resistance to aminoglycosidic antibiotics. *In* Advances in Carbohydrate Chemistry and Biochemistry. Vol. 30. *Ed.*, R. S. TIPSON & D. HORTON, pp. 183~225, Academic Press, New York, San Francisco, London, 1974
2) UMEZAWA, H. & S. KONDO: Mechanisms of resistance to aminoglycoside antibiotics. *In* Handbook of Experimental Pharmacology. Vol. 62. Aminoglycoside Antibiotics. *Ed.*, H. UMEZAWA & I. R. HOOPER, pp. 267~292, Springer-Verlag, Berlin, Heidelberg, New York, 1982
3) UMEZAWA, H.; M. OKANISHI, R. UTAHARA, K. MAEDA & S. KONDO: Isolation and structure of kanamycin inactivated by a cell-free system of kanamycin-resistant *E. coli*. J. Antibiotics Ser. A, 20: 136~141, 1967
4) UMEZAWA, H.; M. OKANISHI, S. KONDO, K. HAMANA, R. UTAHARA, K. MAEDA & S. MITSUHASHI: Phosphorylative inactivation of aminoglycosidic antibiotics by *Escherichia coli* carrying R factor. Science 157: 1559~1561, 1967
5) UMEZAWA, H.; S. TAKASAWA, M. OKANISHI & R. UTAHARA: Adenylylstreptomycin, a product of streptomycin inactivated by *E. coli* carrying R factor. J. Antibiotics 21: 81~82, 1968
6) UMEZAWA, H.; S. UMEZAWA, T. TSUCHIYA & Y. OKAZAKI: 3′,4′-Dideoxykanamycin B active against kanamycin-resistant *Escherichia coli* and *Pseudomonas aeruginosa*. J. Antibiotics 24: 485~487, 1971
7) KONDO, S.; K. IINUMA, H. YAMAMOTO, K. MAEDA & H. UMEZAWA: Syntheses of 1-*N*-{(*S*)-4-amino-2-hydroxybutyryl}-kanamycin B and -3′,4′-dideoxykanamycin B active against kanamycin-resistant bacteria. J. Antibiotics 26: 412~415, 1973
8) KAWAGUCHI, H.; T. NAITO, S. NAKAGAWA & K. FUJISAWA: BB-K8, a new semisynthetic aminoglycoside antibiotics. J. Antibiotics 25: 695~708, 1972
9) UBUKATA, K.; N. YAMASHITA, A. GOTOH & M. KONNO: Purification and characterization of aminoglycoside-modifying enzymes from *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. 25: 754~759, 1984
10) KONDO, S.: Development of arbekacin and synthesis of new derivatives stable to enzymatic modifications by methicillin-resistant *Staphylococcus aureus* (in Japanese). Jpn. J. Antibiotics 47: 561~574, 1994
11) KOBAYASHI, Y.; H. UCHIDA & Y. KAWAKAMI: Arbekacin. Intl. J. Antimicrob. Agents 5: 227~230, 1995
12) KONDO, S.; A. TAMURA, S. GOMI, Y. IKEDA, T. TAKEUCHI & S. MITSUHASHI: Structures of enzymatically modified products of arbekacin by methicillin-resistant *Staphylococcus aureus*. J. Antibiotics 46: 310~315, 1993
13) KONDO, S.: Enzymatic modification of arbekacin in methicillin-resistant *Staphylococcus aureus* and potent activity of the 2″-amino derivatives. *In* Recent Advances in Chemotherapy. *Ed.*, J. EINHORN, C. E. NORD & S. R. NORRBY, pp. 210~211, American Society for Microbiology, Washington, D.C., 1994
14) HOTTA, K.; C.-B. ZHU, T. OGATA, A. SUNADA, J. ISHIKAWA, S. MIZUNO, Y. IKEDA & S. KONDO: Enzymatic

2′-*N*-acetylation of arbekacin and antibiotic activity of its product. J. Antibiotics 49: 458～464, 1996

15) HOTTA, K.; J. ISHIKAWA, M. ICHIHARA, H. NAGANAWA & S. MIZUNO: Mechanism of increased kanamycin-resistance generated by protoplast regeneration of *Streptomyces griseus*. I. Cloning of a gene segment directing a high level of an aminoglycoside 3-*N*-acetyltransferase activity. J. Antibiotics 41: 94～103, 1988

16) SHAW, K. J.; P. N. RATHER, R. S. HARE & G. H. MILLER: Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiological Reviews 57: 138～163, 1993

17) BIDDLECOME, S.; M. HAAS, J. DAVIES, G. H. MILLER, D. F. RANE & P. J. L. DANIELS: Enzymatic modification of aminoglycoside antibiotics: a new 3-*N*-acetylating enzyme from a *Psedomonas aeruginosa* isolate. Antimicrob. Agents Chemother. 9: 951～955, 1976

18) DAVIES, J. & S. O'CONNOR: Enzymatic modification of aminoglycoside antibiotics: 3-*N*-acetyltransferase with broad specificity that determines resistance to the novel aminoglycoside apramycin. Antimicrob. Agents Chemother. 14: 69～72, 1978

19) UMEZAWA, H. & S. KONDO: Electrophoresis of antibiotics. *In* Methods in Enzymology. Vol. 43. Antibiotics. *Ed.*, J. H. HASH, pp. 279～290, Academic Press, New York, 1975