NOTES

AMINOGLYCOSIDE ANTIBIOTICS. XIII SYNTHESIS AND ACTIVITY OF 4′-DEOXY-6′-N-METHYLAMIKACIN AND RELATED COMPOUNDS

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Although amikacin is resistant to most of the aminoglycoside-modifying enzymes[1], it is still susceptible to several enzymes, specifically AAC(6′)[2,3]* and ANT(4′)[4,5]. Aminoglycoside antibiotics have been modified in various ways to overcome inactivation by resistant strains of bacteria which produce one or more aminoglycoside-modifying enzymes. 6′-N-Methylation is known to be effective in blocking enzymatic acetylation of the 6′-amino group[7,8]. Recent studies[9~11] have shown that 4′-deoxykanamycins inhibit some strains of resistant organisms producing AAC(6′)-I and APH(3′)-II as well as ANT(4′).

Modification of amikacin at both the 4′- and 6′-positions has been attempted in order to make the antibiotic invulnerable to all of the known enzymatic mechanisms that cause inactivation of aminoglycosides. This paper describes the preparation and activity of 4′-deoxy-6′-N-methylamikacin and several related compounds.

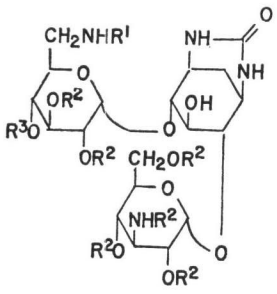
Synthesis

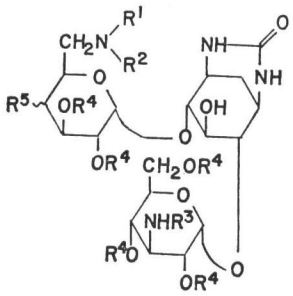
1,3-Ureidokanamycin A[12] (**1**), used as the starting material because of its advantage in selective 1-N-acylation in the final stage following removal of the ureido carbonyl, was benzyloxycarbonylated with N-benzyloxycarbonyloxy-5-norbornene-2,3-dicarboximide (Cbz-ONB)[13] giving a good yield (78%) of 6′-N-Cbz-1,3-ureidokanamycin A (**2**), m.p. 179~181°C, IR (KBr): 1700, 1645, 1520, 1270, 1145, 1050, 760, 695 cm^{-1}. NMR (D_2O): δ in ppm, 4.95 (2H, m, anomeric protons), 5.07 (2H, s), 7.35 (5H, s). Anal**. $C_{27}H_{40}N_4O_{14}\cdot H_2CO_3\cdot\frac{1}{2}H_2O$. The 4′-hydroxy derivative (**4**) was prepared in essentially the same manner as that employed in the preparation of 4′-deoxykanamycin A[9]. Acetylation of **2** with acetic anhydride in pyridine gave the hepta-N,O-acetyl derivative (**3**) in 96% yield, m.p. 150~151°C, IR (KBr): 1745, 1670, 1375, 1230, 1040 cm^{-1}. Anal. $C_{41}H_{54}N_4O_{21}\cdot\frac{1}{2}H_2O$. Removal of the Cbz group of **3** by catalytic hydrogenation with Pd-C in 80% ethanol resulted in 4′-O→6′-N acetyl migration leading to the key intermediate (**4**) in 95% yield, which showed a spot at Rf 0.23 on TLC ($CHCl_3$-EtOH, 7: 2) negative to ninhydrin and positive to anthrone, m.p. >300°C, IR (KBr): 3420, 1745, 1665, 1380, 1240, 1055 cm^{-1}. Anal. $C_{33}H_{48}N_4O_{19}\cdot\frac{1}{2}H_2O$. Treatment of **4** with mesyl chloride in pyridine gave the 4′-O-mesylate (**5**) in 73% yield, m.p. 215~216°C, IR (KBr): 3460, 1755, 1655, 1380, 1240, 1185, 1040, 970 cm^{-1}. NMR (DMSO-d_6): δ in ppm, 3.10 (3H, s, Ms). Anal. $C_{34}H_{50}N_4O_{21}S$. Reaction of **5** with potassium thiolacetate (AcSK) in DMF at 80°C for 3 days in the presence of 18-Crown-6 afforded the thiolacetate (**6**) in 65% yield, m.p. 168~170°C, IR (KBr): 3400, 1740, 1660, 1375, 1235, 1040 cm^{-1}. Anal. $C_{33}H_{50}N_4O_{19}S\cdot\frac{1}{2}H_2O$. The reaction without 18-Crown-6 gave only a poor yield of **6**. Desulfurization of **6** with Raney Nickel by refluxing in ethanol gave a 92% yield of the 4′-deoxygenated derivative (**7**), m.p. 162~163°C. IR (KBr): 3400, 1740, 1655, 1375, 1230, 1050, 760 cm^{-1}. Anal. $C_{33}H_{48}N_4O_{18}\cdot H_2O$. Hydrolysis of **7** with barium hydroxide by heating under reflux in a mixture of dioxane and water provided 4′-deoxy-1,3-ureidokanamycin A (**8**) in 80% yield, m.p. 189~189.5°C (recrystallized from ethanol). NMR (D_2O): δ in ppm, 1.2~2.1 (4H, m, $2\times CH_2$), 4.92 (1H, d, J=3.0 Hz), 5.05 (1H, d, J=3.0 Hz). Anal. $C_{19}H_{34}N_4O_{11}\cdot\frac{1}{2}C_2H_5OH\cdot 3H_2O$. Selective 6′-N-benzyloxycarbonylation of **8** afforded the 6′-N-Cbz derivative (**9**) in 81% yield, m.p. 168~170°C. IR (KBr): 1700, 1650, 1520, 1135, 760, 695 cm^{-1}. NMR (D_2O): δ in ppm, 1.5 (2H, m),

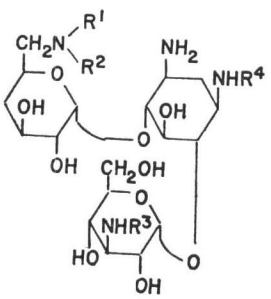
* Abbreviation for aminoglycoside-modifying enzymes, see ref. 6.

** Microanalyses for C, H, N and S (when present) are coincident with the calculated value of indicated formula within ±0.5%.

Fig. 1. 4′-Deoxy-6′-N-methylamikacin and related compounds.







1 R^1, R^2 & R^3=H
2 R^1=Cbz R^2 & R^3=H
3 R^1=Cbz R^2 & R^3=Ac
4 R^1 & R^2=Ac R^3=H
5 R^1 & R^2=Ac R^3=Ms
Cbz: Benzyloxycarbonyl
Ms: Methanesulfonyl
Ac: Acetyl
Me: Methyl
AcS: Acetylthio
Bn: Benzyl
AHBA: L-4-Amino-2-hydroxybutyryl

6 R^2=H R^1, R^3 & R^4=Ac R^5=AcS
7 R^2 & R^5=H R^1, R^3 & R^4=Ac
8 R^1, R^2, R^3, R^4 & R^5=H
9 R^1=Cbz R^2, R^3, R^4 & R^5=H
10 R^1=Cbz R^3=Ac R^2, R^4 & R^5=H
11 R^3=Ac R^1, R^2, R^4 & R^5=H
12 R^1=Bn R^3=Ac R^2, R^4 & R^5=H
13 R^1=Bn R^2=Me R^3=Ac R^4 & R^5=H
17 R^1=Bn R^2=Me R^3, R^4 & R^5=H
18 R^1 & R^3=Bn R^2=Me R^4 & R^5=H

14 R^1=Bn R^2=Me R^3 & R^4=H
15 R^1=Bn R^2=Me R^3=H R^4=CbzAHBA
16 R^1=H R^2=Me R^3=H R^4=AHBA
19 R^1 & R^3=Bn R^2=Me R^4=H
20 R^1 & R^3=Bn R^2=Me R^4=CbzAHBA
21 R^1=Bn R^2, R^3 & R^4=H
22 R^1, R^2, R^3 & R^4=H
23 R^1, R^2 & R^3=H R^4=AHBA
24 R^1, R^3 & R^4=H R^2=Me

2.2 (2H, m), 4.9 (1H, d, J=3.0 Hz), 5.0 (1H, d, J=3.5 Hz), 5.06 (2H, s), 7.34 (5H, s). Anal. $C_{27}H_{40}N_4O_{13}\cdot 3H_2O$. Treatment of **9** with acetic anhydride in MeOH gave a quantitative yield of the 3″-N-acetyl derivative (**10**), m.p. 178~180 °C. IR (KBr): 1700, 1640, 1530, 1270, 1030, 760, 700 cm^{-1}. Anal. $C_{29}H_{42}N_4O_{14}\cdot 2H_2O$. Hydrogenolysis of **10** with 10% Pd-C gave a quantitative yield of the 6′-free amino derivative (**11**), m.p. 207~209°C (recrystallized from ethanol). IR(KBr): 1640, 1040 cm^{-1}. NMR (D_2O): δ in ppm, 2.06 (3H, s, Ac), 5.01 (1H, d, J=3.0 Hz), 5.08 (1H, s, J=3.0 Hz). Anal. $C_{21}H_{36}N_4O_{12}\cdot C_2H_5OH\cdot \frac{1}{2}H_2O$. Treatment of **11** with benzaldehyde at 60°C for an hour in MeOH followed by reduction with sodium borohydride afforded a quantitative yield of the 6′-N-benzyl derivative **12**, m.p. 178~179°C (recrystallized from isopropanol). IR (KBr): 1640, 1030, 750, 695 cm^{-1}. NMR (D_2O): δ in ppm, 2.02 (3H, s, Ac), 4.95 (1H, d, J=3.0 Hz), 5.02 (1H, d, J=3.5 Hz), 7.31 (5H, s). Anal. $C_{28}H_{42}N_4O_{12}\cdot \frac{1}{2}C_3H_7OH\cdot H_2O$. Methylation of **12** with aq. formaldehyde and sodium cyanoborohydride in methanol at room temperature gave the 6′-N-benzyl-6′-N-methyl derivative **13** in quantitative yield, m.p. 171~173°C. IR (KBr): 1640, 1500, 1450, 1375, 1130, 1030, 740, 695 cm^{-1}. NMR (D_2O): δ in ppm, 2.05 (3H, s, Ac), 2.25 (3H, s, N-CH_3), 5.0 (4H, m, 1′-H, 1″-H & $\underline{CH_2}$Ar). Anal. $C_{29}H_{44}N_4O_{12}\cdot \frac{3}{2}H_2O$. Hydrazinolysis of **13** with 100% hydrazine hydrate in a sealed tube at 140°C for 2 days gave 6′-N-benzyl-4′-deoxy-6′-N-methylkanamycin A (**14**) in 74% yield, m.p. 221~222°C (dec.). IR(KBr): 1590, 1450, 1135, 1030, 740, 700 cm^{-1}. NMR (D_2O): δ in ppm, 2.28 (3H, s, N-CH_3), 5.00 (1H, d, J=3.0 Hz), 5.17 (1H, d, J=3.0 Hz), 7.33 (5H, s, Ar). Anal. $C_{26}H_{44}N_4O_{10}\cdot H_2O$. Acylation of **14** with an equimolar N-(L-4-Cbz-amino-2-hydroxybutyryloxy)-5-norbornene-2,3-dicarboximide (L-Cbz-AHBA-ONB) in aqueous THF, followed by separation on a CG-50 (NH_4^+) column gave some recovered **14** (53%) and the crude acylation product **15**, which was subjected to hydrogenolysis with 10% Pd-C in 80% aqueous acetic acid overnight at room temperature. Subsequent CG-50 (NH_4^+) column chromatography afforded a 13% yield (from **14**) of 4′-deoxy-6′-N-methylamikacin (**16**), m.p. 170~171°C. IR (KBr): 1640, 1575, 1480, 1325, 1135, 1030 cm^{-1}.

NMR (D_2O+DCl): δ in ppm, 1.1~2.5 (6H, m, 3×CH_2), 2.72 (3H, s, N-CH_3), 5.12 (1H, d, J=3.0 Hz, 1″-H), 5.50 (1H, d, J=3.5 Hz, 1′-H). Anal. $C_{23}H_{45}N_5O_{12}\cdot 2H_2CO_3\cdot\frac{1}{2}H_2O$.

Compound **16** was also prepared by the following alternative route. Hydrolysis of **13** with aqueous barium hydroxide by refluxing for 20 hours gave a quantitative yield of 6′-N-benzyl-4′-deoxy-6′-N-methyl-1,3-ureidokanamycin A (**17**), m.p. 154~156°C. IR (KBr): 1640, 1125, 1025, 745, 695 cm^{-1}. Anal. $C_{27}H_{42}N_4O_{11}\cdot\frac{3}{2}H_2O$. Benzylation of **17** with benzaldehyde and sodium borohydride gave the 3″,6′-di-N-benzyl derivative (**18**) in 97% yield, m.p. 156~157°C. IR (KBr): 1640, 1455, 745, 700 cm^{-1}. Anal. $C_{34}H_{48}N_4O_{11}\cdot\frac{1}{2}H_2O$. Hydrazinolysis of **18** in the usual manner gave 3″,6′-di-N-benzyl-4′-deoxy-6′-N-methylkanamycin A (**19**) in 93% yield, m.p. 136~137°C. IR (KBr): 1445, 1130, 1075, 1025, 725, 685 cm^{-1}. Anal. $C_{33}H_{50}N_4O_{10}\cdot H_2O$. Acylation of **19** with Cbz-AHBA-ONB followed by silica gel chromatography gave recovered **19** (42%) and the crude acylated product **20**, which was hydrogenated with 10% Pd-C in aq. acetic acid overnight to give 4′-deoxy-6′-N-methylamikacin (**16**) in 18% yield from **19**, identical with the product obtained by the foregoing method.

Two new bioactive derivatives, 4′-deoxyamikacin (**23**) and 4′-deoxy-6′-N-methylkanamycin A (**24**), were also prepared from intermediates, **12** and **14**, respectively, used in the preparation of **16**.

Hydrazinolysis of **12** gave 6′-N-benzyl-4′-deoxykanamycin A (**21**) in 83% yield, m.p. 239~240°C (dec.). IR (KBr): 1585, 1450, 1135, 1070, 1040, 745, 700 cm^{-1}. Anal. $C_{25}H_{42}N_4O_{10}\cdot\frac{1}{2}H_2CO_3\cdot\frac{1}{2}H_2O$. Treatment of **21** with an equimolar amount of Cbz-AHBA-ONB in aqueous THF followed by catalytic reduction with 10% Pd-C overnight and subsequent chromatography on CG-50 (NH_4^+ & Cu^{++}-NH_4^+) gave the non-acylated product, 4′-deoxykanamycin A[9] (**22**) in 21% yield and the acylated product, 4′-deoxyamikacin (**23**) in 5% yield. TLC (S-110): Rf 0.21. Anal. $C_{22}H_{43}N_5O_{12}\cdot H_2CO_3\cdot H_2O$. Compound **23** was also prepared from 4′-deoxykanamycin A (**22**) by 6′-N-benzyloxycarbonylation followed by acylation with Cbz-AHBA and hydrogenolysis in 7% yield.[14]

4′-Deoxy-6′-N-methylkanamycin A (**24**) was prepared from the intermediate **14** by catalytic hydrogenation with Pd-C in 50% aqueous acetic acid in a quantitative yield, m.p. 260~265°C (dec.). IR (KBr): 1135, 1070, 1035 cm^{-1}. Anal. $C_{19}H_{38}N_4O_{10}\cdot H_2CO_3\cdot\frac{1}{2}H_2O$.

Activity

The minimum inhibitory concentrations (MIC) of 4′-deoxy-6′-N-methylamikacin (**16**), 4′-deoxyamikacin (**23**) and 4′-deoxy-6′-N-methylkanamycin A (**24**) were determined against both aminoglycoside-sensitive and -resistant organisms by the twofold agar dilution method in MUELLER-HINTON agar and the activity was compared with amikacin and kanamycin A. The MIC values presented in Table 1 are representative of those obtained from a larger group of test organisms.

Against six strains of kanamycin-sensitive organisms which did not produce any aminoglycoside-modifying enzymes, **23** had about the same activity as amikacin, while **16** was 1/2~1/4 as active as amikacin. A similar MIC ratio was observed for organisms producing APH(3′)-I, APH(3′)-II, AAC(2′), AAC(3′)-I, AAC(3′)-II, AAC(6′)-I, AAC(6′)-III and ANT(2″), all of which were sensitive to amikacin. Against these organisms, **16** was 1/2~1/4 less active than amikacin and **23** showed activity equal to or slightly less than that of amikacin. This indicates that the three antibiotics have comparable resistance to these enzymes and that the MIC variations obtained with them are merely a reflection of the differences in their intrinsic activity observed in tests against non-enzyme-producing organisms.

As expected, improvement of the MIC ratio of **16** to amikacin was observed for both ANT(4′) and AAC(6′)-producing strains. This was also the case for the ratio of **23** to amikacin with ANT(4′) producers. Three strains producing ANT(4′) were 2~4 times more susceptible to **16** and 4~8 times more susceptible to **23** than amikacin. Taking into account the relative intrinsic activity of these compounds, **16** is as resistant as **23** to the action of ANT(4′). Against *Pseudomonas aeruginosa* GN 315, an AAC(6′)-IV producer resistant to amikacin, **16** was 2 times more active than amikacin, while **23** did not show an improved MIC value.

4′-Deoxy-6′-N-methylkanamycin (**24**) was 1/2~1/4 as active as the parent antibiotic kanamycin A against six sensitive organisms, with a spectrum broadened to include strains producing amino-

Table 1. Antibacterial activity of 4′-deoxy-6′-N-methylamikacin (**16**), 4′-deoxyamikacin (**23**) and 4′-deoxy-6′-N-methykanamycin A (**24**).

Organism	Aminoglycoside-modifying enzyme	MIC (mcg/ml)				
		16	**23**	Amikacin	**24**	Kanamycin A
S. aureus Smith	—	1.6	0.4	0.4	1.6	0.4
B. brevis ATCC 8185	—	1.6	0.4	0.4	3.1	0.8
E. coli K12	—	0.8	0.4	0.4	3.1	0.8
K. pneumoniae D11	—	1.6	0.4	0.4	1.6	0.8
Pr. vulgaris A9436	—	1.6	0.4	0.2	0.8	0.4
E. cloacae A9655	—	1.6	0.8	0.4	12.5	1.6
E. coli ML 1630	APH(3′)-I	6.3	1.6	1.6	>100	>100
E. coli A20107	APH(3′)-II	3.1	1.6	0.8	25	>100
Ent. cloacae A21006	〃	1.6	0.8	0.8	50	>100
Pr. mirabilis A21222	AAC(2′)	1.6	0.8	0.8	3.1	0.8
E. coli A20895	AAC(3)-I	1.6	0.8	0.4	6.3	1.6
Ps. aeruginosa A20741	AAC(3)-II	25	6.3	6.3	>100	>100
E. coli NR79/W677	AAC(6′)-I	3.1	0.8	0.8	6.3	>100
Ps. aeruginosa GN4925	AAC(6′)-III	6.3	1.6	0.8	>100	100
Ps. aeruginosa GN315	AAC(6′)-IV	12.5	25	25	25	>100
E. coli A20732	ANT(2″)	1.6	0.8	0.4	12.5	50
S. epidermidis A22033	ANT(4′)	1.6	0.8	6.3	25	50
B. brevis IFO 12334	〃	3.1	3.1	6.3	25	>100
S. aureus A22054	〃	3.1	1.6	6.3	12.5	>100

Table 2. Geometric mean MIC against kanamycin-sensitive strains of 4′-deoxy-6′-N-methylamikacin (**16**), 4′-deoxyamikacin (**23**), 6′-N-methylamikacin (**25**) and amikacin.

Organism	No. of strains	Geometric mean MIC (mcg/ml)			
		16	**23**	**25**	Amikacin
Escherichia coli	5	1.8	0.89	1.4	0.59
Klebsiella pneumoniae	3	2.5	0.62	1.6	0.62
Proteus mirabilis	2	3.1	0.8	1.6	0.8
indole(+) *Proteus* sp.	5	2.7	0.8	1.4	0.44
Providencia sp.	4	1.6	0.46	1.3	0.23
Enterobacter cloacae	2	2.2	0.8	1.6	0.55
Shigella sp.	2	2.2	1.1	2.2	0.55
Staphylococcus aureus	2	2.2	0.55	1.1	0.55
Bacillus sp.	2	0.8	0.27	0.8	0.2
Total	27	2.0	0.67	1.4	0.45
RAm-S (%)[a]		22	68	33	100

[a] Relative inhibitory activity against kanamycin A sensitive strains.

glycoside-modifying enzymes except for APH(3′)-I, AAC(3)-II and AAC(6′)-III producers. It has been shown[9,11] that 4′-deoxykanamycins inhibit APH(3′)-II-poducing organisms. This is also the case for **24** although to a lesser extent than for 4′-deoxykanamycin A. It can be seen that **24** was resistant to AAC(6′)-IV, but not to AAC(6′)-III. Also **24** showed moderate activity against *E. coli* A20732, an ANT(2″) producing organism, which was resistant to both kanamycin

Table 3. Geometric mean MIC against organisms producing aminoglycoside-modifying enzymes and resistance index (R-index) of 4′-deoxy-6′-N-methylamikacin (**16**), 4′-deoxyamikacin (**23**), 6′-N-methylamikacin (**25**) and amikacin.

Aminoglycoside-modifying enzyme produced	No. of strains	**16**		**23**		**25**		Amikacin	
		mean MIC (mcg/ml)	R-index	mean MIC (mcg/ml)	R-index	mean MIC (mcg/ml)	R-index	mean MIC (mcg/ml)	R-index
APH(3′)-I[b,c]	4	6.3	1.6	1.6	2.0	3.1	2.2	2.2	1
APH(3′)-II[a]	4	2.6	1.4	1.3	0.9	3.1	1.2	0.8	1
ANT(2″)[a]	2	3.1	0.8	0.8	1.0	1.6	1.0	0.55	1
ANT(4′)[a,b,d]	5	9.5	21	3.1	21	132	1.0	44	1
AAC(2′)[a]	3	3.1	1.5	1.0	1.5	2.0	1.5	1.0	1
AAC(3)-I[b,e]	2	1.1	1.1	0.8	0.5	1.1	0.8	0.27	1
AAC(3)-II[a]	2	35	0.8	6.3	1.5	18	1.1	6.3	1
AAC(3)-III[f]	1	3.1	1.1	0.8	1.5	1.6	1.5	0.8	1
AAC(6′)-I[b]	1	3.1	1.1	0.8	1.5	3.1	0.8	0.8	1
AAC(6′)-III[b]	1	6.3	0.6	1.6	0.7	3.1	0.8	0.8	1
AAC(6′)-IV[a,g]	2	8.9	13	25	1.5	6.3	12	25	1

[a] see, ref 16. [b] see, Table 1. [c] *E. cloacae* A20364, *E. aerogenes* A20896, *K. pneumoniae* A20328. [d] *S. aureus* A22059, *S. aureus* A21978. [e] *P. stuartii* A20734. [f] *K. pneumoniae* A22552. [g] *P. aeruginosa* A20897.

and 4′-deoxykanamycin.

4′-Deoxy-6′-N-methylamikacin (**16**) was compared in detail with 4′-deoxyamikacin (**23**), 6′-N-methylamikacin (**25**)[15] and amikacin against both aminoglycoside-sensitive and -resistant organisms. The intrinsic activity of the four antibiotics was assessed by MIC determinations against 27 strains of kanamycin-sensitive organisms, including *Escherichia coli* (5 strains), *Klebsiella* species (3), *Proteus mirabilis* (2), indole-positive *Proteus* species (5), *Providencia* species (4), *Enterobacter cloacae* (2), *Shigella* species (2), *Staphylococcus aureus* (2), and *Bacillus* species (2). Table 2 shows the results in terms of geometric mean MIC against each bacterial species. The last line in Table 2 shows the mean activity relative to amikacin against sensitive organisms (RAm-S)[16], which is calculated as fllows:

[(Geometric mean MIC of amikacin)/(Geometric mean MIC of test compound)] × 100. The calculated value represents the intrinsic activity of each compound relative to amikacin whose activity is taken as 100.

Amikacin is the most active of the four antibiotics against kanamycin-sensitive strains. 4′-Deoxygenation and 6′-N-methylation reduced the activity of amikacin by about 1/3 and 2/3, respectively (RAm-S; 86% and 33%). 4′-Deoxy-6′-N-methylamikacin (**16**) possessed approximately 22% of the activity of amikacin. This figure suggests that the two substitutions affected the activity of **16** additively [2/3 × 1/3 = 2/9 = 22%].

The activity of the four antibiotics against 27 strains of aminoglycoside-resistant organisms that produce aminoglycoside-modifying enzymes is shown in Table 3 in terms of geometric mean MIC obtained for each group of resistant organisms producing a common enzyme. Table 3 also gives a "resistance index (R-index)"[16] which is the ratio of RAm-R/RAm-S, where RAm-R (the mean activity relative to amikacin against resistant organisms) was calculated in a similar manner to that previously described for RAm-S. Thus, the R-index represents the antibiotics resistance relative to amikacin against each class of modifying enzymes.

As can be seen in the table, 4′-deoxy-6′-N-methylamikacin (**16**) is the most refractory of the 4 compounds to the action of inactivating enzymes although its intrinsic activity is the lowest. 4′-Deoxyamikacin (**23**) is 21 times as effective as amikacin against ANT(4′) and 6′-N-methylamikacin (**25**) is 12 times more effective against AAC(6′)-IV than amikacin, while 4′-deoxy-6′-N-methylamikacin (**16**) showed both effects, being 21 and 13 times more resistant than amikacin to the inactivation by ANT(4′) and

AAC(6′)-IV, respectively.

Against the resistant organisms producing APH(3′)-I, APH(3′)-II, ANT(2″), AAC(2′), AAC (3)-I, AAC(3)-II, AAC(6′)-I and AAC(6′)-III, all of which are inhibited by amikcain, **16**, **23** and **25** showed R-index values very close to those of amikacin (1.6～2.2 for APH(3′)-I and 0.5～1.5 for others). This suggests that 4′-deoxygenation and 6′-N-methylation do not affect amikacin's resistance to these inactivating enzymes indicating that the 4′-OH and 6′-NH_2 groups of amikacin play little if any part in amikacin's defense against the above-mentioned enzymes.

References

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