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STRUCTURE DETERMINATION OF AMIKACIN DERIVATIVES MODIFIED BY ENZYMES FROM RESISTANT S. AUREUS STRAINS

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Amikacin is known to be refractory to a variety of aminoglycoside-modifying enzymes produced by gram-negative and gram-positive bacteria[1]. It has been reported recently[2 ~ 6] that certain staphylococcal strains can inactivate kanamycin, tobramycin and amikacin. We wish to report the structure determination of amikacin derivatives modified by two resistant strains of *Staphylococcus aureus*, A21978 and A21980[7].

Amikacin was incubated with an enzyme preparation of *S. aureus* A21978* in the presence of adenosine triphosphate (ATP), affording two modified amikacin derivatives designated MAK-A and MAK-B which showed no antibiotic activity. The crude products were purified by repeating chromatographies on Amberlite CG-50 (NH_4^+ form) and silica gel columns and finally by preparative TLC.

MAK-A : mp 231 ~ 235°C (dec.) ; Rf 0.49 (S-110)** ; $\lambda_{max}^{H_2O}$ 260 nm (ε 12,400); PMR (D_2O, ppm from DHO): -0.31 (1H, d, J = 4 Hz, 1"-H), -0.53 (1H, d, J = 4 Hz, 1'-H), -1.29 (1H, d, J = 5.5 Hz, ribose 1-H), -3.34 (1H, s, adenine 8-H), -3.59 (1H, s, adenine 2-H). Anal. Calc'd for $C_{32}H_{55}N_{10}O_{19}P \cdot 2.5H_2O$: C, 40.04; H, 6.30; N, 14.59. Found: C, 39.81; H, 5.98; N, 14.22. MAK-B : mp 269 ~ 273°C (dec.); Rf 0.28 (S-110); no UV absorption; PMR (D_2O, ppm from DHO): -0.33 (1H, d, J = 4 Hz, 1"-H), -0.70 (1H, d, J = 4 Hz, 1'-H). Anal. Calc'd. for $C_{22}H_{44}N_5O_{16}P \cdot 2.5H_2O$: C, 37.18; H, 6.95; N, 9.86. Found: C, 37.01, H, 6.86; N, 9.87. MAK-A and B gave positive ninhydrin and Hanes[8] tests, and liberated amikacin on treatment with alkaline phosphatase of calf intestine. MAK-A was hydrolyzed to amikacin by phosphodiesterase of snake venom, while MAK-B was not. These data suggest that MAK-A is an O-adenylylated amikacin and MAK-B is an O-phosphorylated amikacin. Mild acid hydrolysis of both compounds (4N HCl, 85°C, 4 hours) gave ninhydrin positive spots on TLC corresponding

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* Another strain, *S. aureus* A21980, gave the identical inactivation products MAK-A and MAK-B. The detailed inactivation procedures used by Dr. Julian Davies, who kindly provided the crude preparations of inactivated amikacins, are given in ref. 7.

** Silica gel TLC : $CHCl_3 - CH_3OH - 28\%\ NH_4OH - H_2O$ (4 : 1 : 2 : 1)

Table 1 ^{13}C Chemical shifts of modified amikacins, MAK-A and MAK-B (in D_2O, Varian FT-80)

Carbon		Chemical shift (ppm downfield from TMS) Amikacin base	Amikacin +DCl	MAK-A base	MAK-A +DCl	MAK-B base	MAK-B +DCl	ATP. 4Na
(DOS)	C-1	50.4	49.5	50.4	49.6	50.4	49.6	
	C-2	35.1	30.9	32.4	30.9	31.9	30.9	
	C-3	49.4	48.7	49.3	48.7	49.1	48.4	
	C-4	87.6	79.8	87.0	80.0	85.4	79.9	
	C-5	75.4	73.2	75.4	73.4	75.1	72.8	
	C-6	81.2	81.2	81.4	81.0	81.1	81.0	
(6-AG)	C-1'	99.2	96.2	99.3	96.3	98.9	95.4	
	C-2'	72.7	71.7	72.3	71.6	72.0[a]	71.2[j**]	
	C-3'	73.7	73.1	72.9[a]	72.2[e**]	76.3[i*]	78.2[k*]	
	C-4'	71.8	71.7	75.9[b*]	75.5[b*]	72.0[a]	71.1[l**]	
	C-5'	73.7	69.5	72.3[a]	68.6[f**]	71.4	69.3	
	C-6'	42.4	41.2	41.5	40.9	41.5	41.1	
(3-AG)	C-1"	100.3	98.7	99.6	98.8	99.1	98.7	
	C-2"	72.5	68.8	71.1	68.8	70.0	68.8	
	C-3"	54.9	56.2	55.0	56.1	55.3	56.2	
	C-4"	70.1	66.4	69.9	66.3	68.9	66.3	
	C-5"	72.8	72.7	72.9	72.8	72.8	72.8	
	C-6"	61.2	60.7	61.1	60.5	60.9	60.6	
(L-AHBA)	C=O	177.2	176.1	176.3	176.3	176.2	176.3	
	C-α	70.7	70.5	70.6	70.4	70.4	70.4	
	C-β	36.5	31.6	35.0	31.6	34.4	31.6	
	C-γ	38.1	37.9	37.8	37.8	37.7	37.8	
(Ribose)	C-1'			88.1	89.1			88.1
	C-2'			74.9	75.3			75.3
	C-3'			70.6	71.0			70.9
	C-4'			84.4[c**]	84.8[g**]			84.4[m**]
	C-5'			66.1[d*]	66.0[h*]			66.2[n*]
(Adenine)	C-2			153.7	o			153.3
	C-4			149.7	o			149.1
	C-5			119.3	o			118.8
	C-6			156.2	o			155.6
	C-8			140.7	o			140.4

a) unresolved doublet.

b~n) doublet with the following J_{C-P} : (b) 5.8 Hz, (c) 8.0 Hz, (d) 5.2 Hz, (e) 2.8 Hz, (f) 3.4 Hz, (g) 8.8 Hz, (h) 5.0 Hz, (i) 4.0 Hz, (j) 2.4 Hz, (k) 4.6 Hz, (l) 4.8 Hz, (m) ca. 8.0 Hz, (n) ca. 4.0 Hz.

o) distinct resonances not observed.

*) ^2J (^{13}C - ^{31}P).

**) ^3J (^{13}C - ^{31}P).

to 3-amino-3-deoxyglucose (3-AG), 2-deoxystreptamine (DOS) and L-4-amino-2-hydroxybutyric acid (L-AHBA), but only a faint spot for 6-amino-6-deoxyglucose (6-AG).

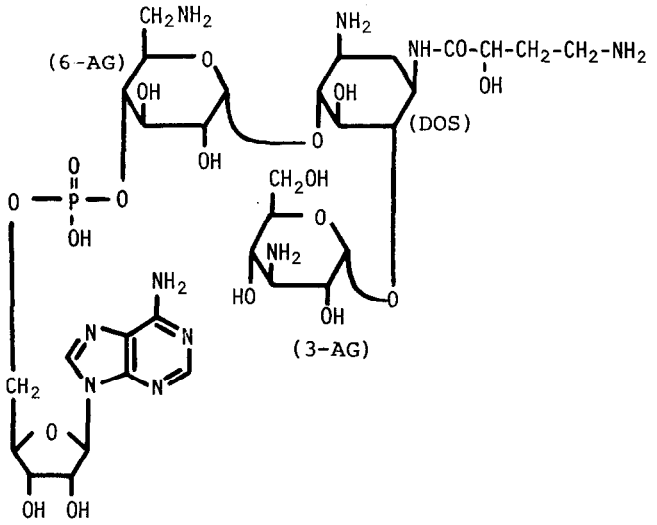
The ^{13}C NMR spectra of MAK-A and B were measured under basic and acidic conditions. The assignment of the chemical shifts of these compounds (Table 1) was made on the basis of comparisons with those of amikacin[9] and adenosine triphosphate (ATP), taking into consideration the β-carbon shift on protonation of amino groups and the multiplicity of resonance by ^{13}C - ^{31}P coupling[10].

The presence of the adenosine moiety in MAK-A was indicated by the ^{13}C spectrum which showed five outstanding signals due to the adenine nucleus in the range of 119.3 ~ 156.2 ppm and five peaks for the ribose moiety at 66.1 ~ 88.1 ppm (three singlets and two doublets coupled with phosphorus). The carbon shifts due to the DOS, 3-AG and L-AHBA moieties of MAK-A and B, which appeared as single lines, are in good agreement with those of amikacin. This fact, coupled with the results of acid hydrolysis, indicates that modifications occurred on the 6-AG moiety of the compounds. In addition, the readily discernible C-1' and C-6' signals of the 6-AG moieties of MAK-A and B resonated as singlets at 99.3 and 98.9 ppm and at 41.5 and 41.5 ppm, respectively, indicating that the adenylyl or phosphoryl group should be on the C-3' or C-4' of the 6-AG moiety in the modified compounds.

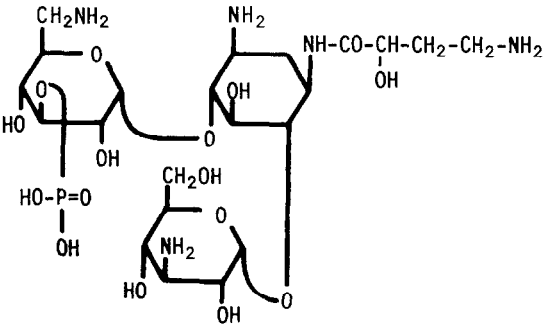
In the ^{13}C-NMR spectrum of MAK-A measured under a protonated condition, three well-resolved doublets appeared at 68.6, 72.2 and 75.5 ppm (coupling with phosphorus). The doublet at the most upfield region (68.6 ppm) was assigned to the C-5' of 6-AG moiety (β-shift on protonation). The splitting of C-5' signal ($^3J_{C-P}$ = 3.4 Hz) and the sharp singlet of C-2' at 71.6 ppm indicated that the site of adenylylation in MAK-A is not on the C-3' but on the C-4' hydroxyl group. Accordingly, two doublets at 75.5 ppm ($^2J_{C-P}$ = 5.8 Hz) and 72.2 ppm ($^3J_{C-P}$ = 2.8 Hz) were reasonably assigned to the C-4' and C-3' signals, which showed a downfield shift by 3.8 ppm and a upfield shift by 0.9 ppm, respectively, compared with that of amikacin.

In the spectrum of MAK-B taken under acidic condition, the C-5' signal appeared as a singlet at 69.3 ppm, which was nearly the same as that of amikacin (69.5 ppm). Therefore, the C-4' hydroxyl group should not be phosphorylated and the site of phosphorylation in MAK-B is on the C-3' hydroxyl group. The doublet at 78.2 ppm ($^2J_{C-P}$ = 4.6 Hz) was attributed to the C-3' signal which was deshielded by 5.1 ppm from that of amikacin. Two doublets at 71.2 ppm ($^3J_{C-P}$ = 2.4 Hz) and 71.1 ppm ($^3J_{C-P}$ = 4.8 Hz) were ascribed to the C-2' and C-4', which were shifted upfield by 0.5 ppm and 0.6 ppm, respectively, from that of amikacin.

The structures of enzymatically modified amikacin derivatives, MAK-A and MAK-B, are thus determined to be 4'-O-adenylylamikacin and 3'-O-phosphorylamikacin, respectively, as shown on the next page.



MAK-A (4'-O-adenylylamikacin)



MAK-B (3'-O-phosphorylamikacin)

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