THE STRUCTURE DETERMINATION OF AN ENZYMATIC INACTIVATION PRODUCT OF 3′, 4′-DIDEOXYKANAMYCIN B

Sir:

As described in the preceding communication[1], 3′, 4′-dideoxykanamycin B (DKB) was inactivated in the presence of ATP by an enzyme preparation obtained from *Escherichia coli* JR66/W677 carrying R factor. In this communication, the structure of the inactivated antibiotic is shown to be 3′, 4′-dideoxykanamycin B-2″-adenylate by nuclear magnetic resonance study.

We reported that the inactivated DKB was a mono-adenylyl DKB from the data of UV and IR, color reactions, and elementary analysis ($C_{18}H_{37}N_5O_8 \cdot C_{10}H_{12}N_5O_6P \cdot 3H_2O$). The proton magnetic resonance (pmr) spectrum of inactivated DKB in D_2O solution (20 mg/0.3 ml, pH 8.0, Fig. 1) using tetramethylsilane as an external reference ($\delta=0$) showed signals for two protons at δ 8.63 and δ 8.85 ppm attributable to the adenine-ring protons, six protons at δ 6.53, 5.28, 4.98, 4.83 and *ca.* 4.6 ppm (2-H). These six protons were assigned to the ribose-ring protons by successive double resonance experiments and the comparison with disodium 5′-adenylate in D_2O solution (15 mg/0.3 ml, Table 1). Therefore, these observations confirm the presence of one mole of 5′-adenylic acid in the molecule. Irradiation at δ 5.45 ppm (J=3.6, 1″-H) caused the complex signal at δ 4.3 ppm (2″-H) to collapse to a triplet type signal. Irradiation at δ 3.49 ppm (3″-H) caused the same region (2″-H) to collapse to a broad doublet type signal. Irradiation at δ 5.45 and 3.49 ppm (triple resonance) caused the same position (2″-H) to collapse to a doublet (J=8.0). Irradiation at δ 4.32 ppm (2″-H) caused the signals at δ 5.45 ppm (1″-H) and δ 3.50 ppm (3″-H) to collapse to a singlet and doublet (J=10.0), respectively. The chemical shift of the 3″-H to high-field was attributed to the amino group. These results indicate that the (1″-H), (2″-H) and (3″-H) signals described above are attributable to the 3-aminoglucose moiety (Table 2). In the case of DKB in D_2O solution (base, 17 mg/0.3 ml), the (1″-H), (2″-H) and (3″-H) signals of the 3-aminoglucose moiety are observed at δ 5.52, 3.98 and 3.46 ppm, respectively. The 2″-H signal of inactivated DKB is shifted 0.34 ppm to lower-field when com-

Fig. 1. PMR spectrum of 3′,4′-dideoxykanamycin B-2″-adenylate.

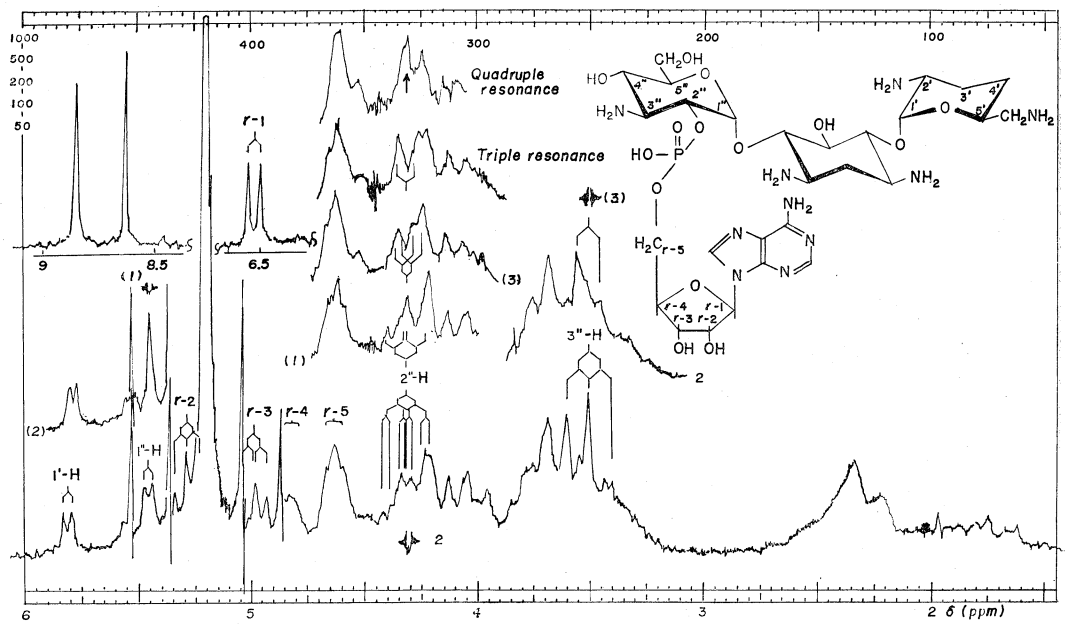

Table 1. Chemical shifts and coupling constants for the ribose moiety in 5′-AMP and DKB-2″-adenylate

	Chemical shift (δ)*					Coupling constant (Hz)					
	1′-H	2′-H	3′-H	4′-H	5′-H	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',P}$	$J_{5',P}$
5′-AMP (disodium)	6.51	5.20	4.95	4.79	4.45	5.5	5.0	3.5	3.5	0.5**	4.5
DKB-2″-adenylate	6.53	5.28	4.98	4.83	*ca.* 4.6	5.5	5.0	3.5	—	—	—

* δ (ppm) from tetramethylsilane as external reference
** determined from ^{31}P decoupling spectrum

Table 2. Chemical shifts and coupling constants for the 3-aminoglucose moiety in DKB and DKB-2″-adenylate

	Chemical shifts (δ)			Coupling constant (Hz)			
	1″-H	2″-H	3″-H	$J_{1'',2''}$	$J_{2'',3''}$	$J_{3'',4''}$	$J_{2'',P}$
DKB (base)	5.52	3.98	3.46	3.8	10.5	10.0	—
DKB-2″-adenylate	5.45	4.32	3.50	3.6	10.0	—	8.0

pared with DKB. The lower-field shift indicates that the 2″-H signal is affected by the phosphoric ester function. Similar shifts were observed in the spectra of phosphorylated kanamycin, paromamine and dihydrostreptomycin[2]. Irradiation of the 1″-H, 3″-H and ^{31}P (40.489023 MHz) signals caused the complicated signal of 2″-H to reduce to a singlet as shown in Fig. 1. These quadruple resonance results conclusively show that adenylic acid is attached at the C-2 position of the 3-aminoglucose moiety of DKB.

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2) Naganawa, H.; S. Kondo, K. Maeda & H. Umezawa: Structure determinations of enzymatically phosphorylated products of aminoglycosidic antibiotics by proton magnetic resonance. J. Antibiotics 24: 823～829, 1971