KANAMYCIN 6′-ACETATE AND RIBOSTAMYCIN 6′-ACETATE, ENZYMATICALLY INACTIVATED PRODUCTS BY *PSEUDOMONAS AERUGINOSA*

Sir:

As reported in a previous paper,[1] 3′,4′-dideoxykanamycin B is converted to its 6′-acetate by an enzymatic reaction with ATP, coenzyme A, acetate and an enzyme solution prepared from *Pseudomonas aeruginosa* GN 315. In this communication, we report the inactivation of kanamycin and ribostamycin (SF-733)[2] by the same enzyme solution of *P. aeruginosa* GN 315, producing kanamycin 6′-acetate and ribostamycin 6′-acetate.

The enzyme solution (S-100) was prepared by the procedure described in a previous paper.[1] The inactivation was carried out at 37°C for 3 hours in the following reaction mixture: 100 mg (0.2 mmoles) of kanamycin or 91 mg (0.2 mmoles) of ribostamycin in 25 ml of water, 4,842 mg (8.0 mmoles) of disodium ATP trihydrate in 100 ml of 0.8 % sodium bicarbonate, 25 mg (0.03 mmoles) of coenzyme A in 50 ml of water, 25 ml of the enzyme solution (20 mg/ml protein), 25 ml of 100 mM magnesium acetate in 60 mM 2-mercaptoethanol and 25 ml of 1 M potassium phosphate buffer (pH 7.8). After 3 hours incubation, kanamycin and ribostamycin were completely inactivated as shown by the disc plate method using *Bacillus subtilis* PCI 219.

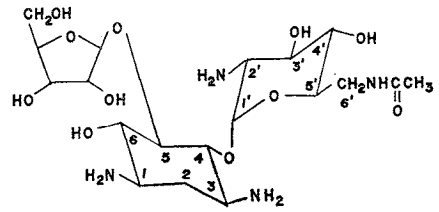
The inactivated kanamycin was isolated as follows: The reaction mixture was heated in a boiling water bath for 10 minutes and filtered. The filtrate was diluted to 1,000 ml with water and passed through a column of Amberlite CG-50 (50 ml of NH_4^+ form). After washing the column with 250 ml of water, the inactivated kanamycin, which could be detected by ninhydrin and RYDON-SMITH[3] reactions on high-voltage paper electrophoresis, was eluted with 0.5 % ammonia. The eluate (30 ml) was concentrated to 3 ml and the concentrated solution was subjected to column chromatography on Amberlite CG-50 (20 ml of NH_4^+ form). After washing the column with 100 ml of water, the inactivated kanamycin was eluted with 0.1 % ammonia. Thus, 72 mg of purified inactivated kanamycin was obtained as a white powder.

The inactivated kanamycin melts at 162~167°C with decomposition. Anal. calcd. for $C_{20}H_{38}N_4O_{12}\cdot H_2O$: C 44.11, H 7.40, N 10.28. Found: C 43.84, H 7.41, N 10.08. It shows no inhibition against *B. subtilis* PCI 219 at 100 μg/ml. It gives positive ninhydrin and RYDON-SMITH reactions. On high-voltage paper electrophoresis under 3,000 volts for 20 minutes, using formic acid – acetic acid – water (25 : 75 : 900, in volume), the inactivated kanamycin moves a distance of 12.6 cm to the cathode, while kanamycin moves 15.7 cm. It shows no UV maximum except end absorption. The IR spectrum shows amide bands I and II (1650 and 1570 cm^{-1}).

It was confirmed to be identical with kanamycin 6′-acetate[4] which was obtained by an enzymatic inactivation using an enzyme prepared from *E. coli* K-12 R-5, in all respects including pmr spectrum. The pmr spectrum of the inactivated kanamycin in D_2O, using tetramethylsilane as an external reference ($\delta=0$), showed one N-acetyl signal at δ 2.47. The spin decoupling experiments indicated that three amino groups at C-1, C-3 and C-3″ were not acetylated. The methylene protons of C-6′ shifted to lower field (*ca.* δ 3.7), compared to kanamycin (*ca.* δ 3.4). The results indicate that the amino group in the 6-amino-6-deoxyglucose moiety is acetylated.

Inactivated ribostamycin was isolated by resin chromatography as described above, yielding 60 mg of purified inactivated ribostamycin. The inactivated ribostamycin is a white powder melting at 103~108°C with decomposition. Anal. calcd. for $C_{19}H_{36}N_4O_{11}\cdot H_2O$: C 44.35, H 7.44, N 10.88. Found: C 44.07, H 7.50, N 11.12. It shows no

Fig. 1. The structure of the inactivated ribostamycin



inhibition against *B. subtilis* PCI 219 at 100 μg/ml. It gives positive nynhydrin and RYDON-SMITH reactions. On high-voltage paper electrophoresis under 3,000 volts for 20 minutes, the inactivated ribostamycin moves a distance of 12.5 cm to the cathode, while ribostamycin moves 16.2 cm. It shows only end absorption in the UV spectrum and amide bands I and II (1650 and 1570 cm^{-1}) in its IR spectrum. The pmr spectrum of the inactivated ribostamycin in D_2O showed one N-acetyl signal at δ 2.49. The double resonance experiments indicated that the signal of methylene protons on C-6′ (*ca.* δ 3.5 in the spectrum of ribostamycin) shifted to lower filed (*ca.* δ 3.8 in the inactivated ribostamycin). From these results, the inactivated ribostamycin is ribostamycin 6′-acetate.

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