ISOLATION AND CHARACTERIZATION OF LIVIDOMYCIN A INACTIVATED BY *PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA COLI* CARRYING R FACTOR

Sir:

As reported in a previous paper[1], liviodomycin A[2] is inactivated by an enzyme obtained from *Pseudomonas aeruginosa* TI-13, resistant to this antibiotic. In this paper, we report the structure elucidation of the inactivated product and its identity with the product inactivated by an enzyme solution obtained from *Escherichia coli* K-12 ML 1410 R-81 carrying R factor resistance to the antibiotic.

Crude inactivated product (150 mg) prepared by the procedures described in a previous paper[1] was dissolved in 2 ml of 0.1 M ammonium formate and chromatographed over CM Sephadex C-25 (50 ml) equilibrated with 0.1 M ammonium formate. After washing the column with each 500 ml of 0.1 M and 0.4 M ammonium formate, the inactivated product, which could be detected by RYDON-SMITH[3] and HANES[4] reactions, was eluted with 0.8 M ammonium formate. The eluate (71 ml) was passed through a column of Amberlite CG-50 (50 ml of NH_4^+ form) and the inactivated product was eluted with 0.5% ammonia. Thus, 92 mg of purified inactivated lividomycin A was obtained as a white powder.

The inactivated lividomycin A does not melt up to 210°C. $[\alpha]_D^{20}$ +52.2° (*c* 1.48, H_2O). Anal. calcd. for $C_{29}H_{56}N_5O_{21}P \cdot 5H_2O$: C 37.38, H 7.14, N 7.52, P 3.32. Found: C 37.49, H 7.70, N 7.26, P 3.42. The bioactivity against *Bacillus subtilis* PCI219 is less than 1/1,000 of that of lividomycin A in the usual cylinder plate assay. It gives positive ninhydrin, RYDON-SMITH and HANES reactions. On high-voltage paper electrophoresis at 3,000 volts for 20 minutes, using formic acid - acetic acid - water (25 : 75 : 900 in volume), the inactivated lividomycin A moves to the cathode by 10.8 cm, while lividomycin A moves by 12.3 cm. It shows no UV maximum except end absorption. A band at 970 cm^{-1} (phosphoric ester) is observed in IR spectrum. The inactivated lividomycin A consumed 6.2 moles of periodate for 24 hours as does lividomycin A (5.9 moles). Methanolysis of the inactivated lividomycin A (refluxed in 0.4 N hydrogen chloride in methanol for 6 hours) gave 3'-deoxyparomamine, methyl lividotriosaminide phosphate and a small amount of methyl lividobiosaminide phosphate.

These results indicate that the inactivated lividomycin A is a monophosphate at either the 2- or 5-hydroxyl group of the ribose moiety in lividomycin A. Moreover, results of pmr decoupling experiments indicate that C-2 position in the mannose moiety (2''''-H: δ 4.43, J=1.5, 3.0 Hz) and the C-3 position in the neosamine B moiety (2'''-H: δ 4.7, 3'''-H: δ 4.05) are not phosphorylated.

Proton magnetic resonance spectrum of the inactivated lividomycin A in D_2O (tetramethylsilane as the external reference, $\delta=0$) showed four anomeric protons at δ 5.50 (J=1.5 Hz, ribose), δ 5.53 (J=1.5 Hz, mannose), δ 5.81 (J=3.0 Hz, neosamine B) and δ 5.82 (J=3.5 Hz, 3-deoxyparomose). These assignments of anomeric protons were made by decoupling experiments and comparisons with spectra of paromomycin, methyl lividotriosaminide and methyl lividotriosaminide phosphate. Irradiation of the anomeric proton of ribose moiety at δ 5.50 caused the reduction of the signal at δ 3.55 (2''-H) to a doublet (J=3.0 Hz). From chemical shift values and coupling constants, it was concluded that the C-2 hydroxyl of ribose moiety is not phosphorylated. Irradiation of 2''-H at δ 3.55 collapsed the signal at δ 4.6~4.7. Irradiation at δ 4.7 caused a reduction of the signal at δ 4.98 (4''-H, triplet) and that at δ 3.55 (2''-H) to a singlet and a doublet (J=3.0 Hz), respectively. Thus, the signals of 3''-H and 5''-H in ribose moiety were confirmed to be present at δ 4.6~4.7 region. As reported in a previous paper[5], the signal of 5-H of ribose in adenylyl moiety of 3',4'-dideoxykanamycin B 2''-adenylate[5] appeared at δ 4.6.

The results of these decoupling experiments indicate that the primary hydroxyl group at C-5 of the ribose moiety in lividomycin A is phosphorylated. As reported in the next paper[6], this structure was confirmed

by the synthesis of lividomycin A 5''-phosphate.

Lividomycin A inactivated by 100,000 g supernatant of disrupted cells of *E. coli* K-12 ML1410 R-81 was isolated by the procedure described above and the identity of the inactivated products was confirmed by infrared spectrum, pmr spectrum, identification of methanolysis products and behavior on paper electrophoresis.

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