Note

ISOLATION OF PAROMAMINE INACTIVATED BY *PSEUDOMONAS AERUGINOSA*

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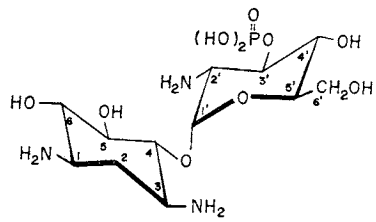
(Received for publication May 21, 1968)

As reported by UMEZAWA *et al.*[1~5], enzymes acetylating the amino group of the 6-amino-6-deoxy-D-glucose moiety in kanamycin, phosphorylating the C-3 hydroxyl group of the same moiety in kanamycin, or phosphorylating the C-3 hydroxyl group of the D-glucosamine moiety in paromamine are formed in cells of *E. coli* carrying the R factor. It has been also reported by UMEZAWA *et al.*[6] that an enzyme solution of *Pseudomonas aeruginosa* inactivated kanamycin in the presence of ATP and that the inactivated product was identical with the phosphorylated kanamycin produced by the enzyme of *E. coli* carrying R factor.

In this paper, we report on the inactivation of paromamine by an enzyme solution obtained from *Pseudomonas aeruginosa*, and the isolation and stereochemistry of the inactivated paromamine, which is identical with the phosphorylated paromamine produced by an enzyme solution prepared from *E. coli* ML 1629 carrying R factor.

An enzyme solution was prepared from the growing cells of *Pseudomonas aeruginosa* strain NIHJ B-328 by the same procedure reported by UMEZAWA *et al.*[6] Paromamine was inactivated at 37°C for 24 hours or 5.5 hours in the following reaction medium (total volume, 79 ml); enzyme solution from *Pseudomonas aeruginosa* (9 ml), paromamine trihydrochloride (435 mg, 0.001 moles), disodium ATP (1.64 g, 0.003 moles), sodium bicarbonate (415 mg, 0.005 moles) and ten-fold-concentrated modified TMK solution (0.6 M KCl, 0.1 M $MgCl_2$ and 0.6 M 2-mercaptoethanol in 1 M tris-buffer, pH 7.8) (27 ml). Complete inactivation was found after 5 hours' reaction.



After the reaction, the mixture was passed through a column of Amberlite IRC-50 resin (400 ml of Na^+ form). After washing with 850 ml of water, the inactivated paromamine on the column was eluted with 1% aqueous ammonia. The eluate (160 ml), which showed positive reaction to ninhydrin and to HANES reagent[7] for phosphorous, was concentrated to dryness under reduced pressure. The crude powder (650 mg) thus obtained was dissolved in 10 ml of water and subjected to column chromatography on Amberlite CG-50 resin (50 ml of NH_4^+ form) developed with water (250 ml). The ninhydrin positive effluent (40 ml) was concentrated to about 3 ml, and addition of 0.3 ml methanol yielded white crystals of the inactivated paromamine (160 mg), which were recrystallized from hot water.

The inactivated paromamine has the following properties: darkening at 260~290°C, but not melting at 300°C; Anal., calcd. for $C_{12}H_{24}N_3O_7 \cdot PO(OH)_2 \cdot 2H_2O$: C 32.80, H 6.88, N 9.56, O 43.70, P 7.05; found: C 32.40, H 7.09, N 9.00, O 44.17, P 6.37; positive ninhydrin reaction and positive test for phosphorous by HANES reagent; having no maximum in the ultraviolet absorption spectrum. The inactivated product is a monophosphorylated paromamine as demonstrated by these analytical result and by its behavior on paper electrophoresis. It moved 9.8 cm toward the cathode during high-voltage paper electrophoresis, using acetic acid - formic acid - water (75 : 25 : 900, v/v), 3,500 V for 15 minutes. Paromamine moved

14.8 cm toward the cathode. The inactivated product was converted to paromamine by heating at 80°C for 20 hours in 0.4 M $HClO_4$ adjusted to pH 4.0 with sodium hydroxide. The inactivated paromamine consumed 2.0 moles of periodate at pH 4.05 in 20 hours, while paromamine trihydrochloride consumed 3.9 moles.

From the foregoing results, it is concluded that the inactivated paromamine is identical with phosphorylated paromamine (paromamine-3′-phosphate)[5] obtained by treatment with an enzyme solution from *E. coli* ML 1629 carrying R factor.

In the n.m.r. spectrum (D_2O, 100 Mc) of the inactivated paromamine, the triplet ($J=8$ cps) at 4.3δ is assigned to an axial proton at C-3 position of the D-glucosamine moiety. The retention of configuration at C-3′ during phosphorylation by the enzyme from *E. coli* ML 1629 and *Pseudomonas* was confirmed.

References

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