AMINO SUGAR ANTIBIOTIC AS PHOSPHOAMIDE FROM *STREPTOMYCES FRADIAE*

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Although various phosphate esters related to amino sugar antibiotics have been isolated from different microorganisms: streptidine phosphate and amidino-*scyllo*-inosamine phosphate from *Streptomyces griseus*[1~3], inosamine phosphate, amidino-inosamine phosphate, amidino-streptamine phosphate and streptidine phosphate from *Streptomyces bikiniensis*[4]; phosphorylated streptomycin from *Streptomyces griseus*[5]; monophosphorylated kanamycin as an inactive product from the phosphorylation of kanamycin by enzymes from *Pseudomonas aeruginosa*[6]; but there is no report about the isolation of any phosphoamidoamino sugar compound. A preliminary study on the amino sugar phosphoamide has been described here.

The organism used was *Streptomyces fradiae* 3535[7]. A well sporulated slant culture (grown for 7 days on potato-yeast extract agar slant) was washed with 5 ml of sterile water, and 0.5 ml was used to inoculate 500 ml Erlenmeyer flask containing 200 ml medium of the following composition: maltose, 15 g; glycine, 4.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.04 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; $ZnSO_4 \cdot 7H_2O$, 0.0005 g; water 1,000 ml; pH was 7.5 ± 1[8]. On the 6 th day of fermentation, 10 liters broth were filtered to remove mycelium and then passed through an Amberlite IRC 50 (NH_4^+ form, 100~200 mesh) column (dimension 3.5 (i. d.) × 8 cm) at a flow rate of 700 ml/hr at 6°C; column was washed, organic phosphate fractions eluted with 1 N NH_4OH and then concentrated under reduced pressure at a low temperature. This product was dissolved in 3 ml of water adding minimum amount of NH_4OH and the solution was passed through Amberlite IRC 50 (NH_4^+ form, 200~400 mesh) column (dimension 1.4 cm (i. d.) × 54.5 cm). Elution of the column with dilute NH_4OH solution gave three fractions of which middle fraction (chromatographically pure) was concentrated, treated with absolute ethanol and kept at 4°C overnight. The precipitate was centrifuged and washed with alcohol, then dried at 4°C over pohsphorus pentoxide, to give hygroscopic white substance. Biological activity study indicated that 30 μg of the compound had the same inhibitory effect on growth of *Bacillus subtilis* (B_3) as 0.6 μg of neomycin B. The activity of the compound, however, increased 30 times after treatment with alkaline phosphatase (calf intestinal mucosa).

The compound showed no absorption in ultraviolet region. It gave positive MOLISCH and carbazole test for carbohydrate, ammonium molybdate test for phosphate, ninhydrin test for amino group and a negative ELSON-MORGAN test. It did not reduce FEHLING's or TOLLENS' reagents. Acid hydrolysate (6 N HCl, 100°C, 18 hrs) of the compound showed absence of amino acids in paper chromatogram and gave positive ELSON-MORGAN test[9]. On the paper electrophoregram (140 volt, 2.5 hrs, 4°C) in different buffer solutions (formic acid-ammonium hydroxide 0.2 M) at pH 3.5, 7.0 and 9.0 it migrated towards the cathode at distance of 7.6 cm, 3.1 cm and 0.8 cm respectively showing its basic characteristic and homogeneity.

The compound was N-acetylated with acetic anhydride in presence of methanol and sodium bicarbonate at 5°C, completion of reaction was tested with ninhydrin negativity. N-Acetylation of the compound after alkaline phosphatase treatment and phosphatase hydrolysed N-acetylated compound gave different migration in the paper chromatogram (Table 1).

The low migration of N-acetylated product after alkaline phosphatase hydrolysis might be due to one or more amino groups becoming free by the action of enzyme. Table 1 also indicates that the dephosphorylated compound after N-acetylation showed mig-

Table 1. Paper chromatography of N-acetylated derivative*

Treatment	Migration from origin (cm)	
	n-Butanol – water – piperidine (84 : 16 : 2, v/v/v)**	*n*-Butanol – water – pyridine (6 : 3 : 4, v/v/v)***
Pretreatment of the compound with phosphatase and then N-acetylated[10]	7.6	11.3
Compound N-acetylated and then treated with phosphatase	3.0	6.3
N-Acetyl neomycin C	7.6	11.3

* Detected by method of MAJUMDAR and MAJUMDAR[10]
** Whatman No. 4, descending, 28°C, 48 hours
*** Whatman No. 1, descending, 28°C, 24 hours

ration same as N-acetylneomycin C (neomycin C is also elaborated by this strain). The formula of the compound from elemental analysis is $C_{23}H_{48}N_6O_{19}P_2 \cdot 3H_2O$, which means that the compound contains 2 molecules of phosphoric acid and 3 molecules of water in addition to neomycin C. Moreover, the infrared absorption spectrum of the compound was very much similar to neomycins except for the absorptions in the regions 1639 cm^{-1}, 900~950 cm^{-1}, which might be due to bound water and phosphate group respectively.

The N-acetylated compound after phosphatase treatment was isolated by Amberlite IRC 50 (NH_4^+ form) chromatography. This gave ninhydrin positive test. According to MORTON[11] calf intestinal mucosa phosphatase could hydrolyse phosphoamides such as creatine phosphate. Besides, at 37°C the compound underwent complete dephosphorylation with 1 N HCl acid in 10 minutes. These sorts of extreme unstability towards acid and stability to alkali are the characteristics of N-P linked compounds. All these findings suggested the compound to be a phosphoamido-amino sugar antibiotic (possibly neomycin C).

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