Studies on Amino-hexoses. X. A Synthesis of *p*-Acetaminophenyl *N*-Acetyl-β-muramide

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Lysozyme, the enzyme which exerts a bacteriolytic influence, has been found to split the bacterial cell-wall mucopeptides at the site of the glycosidic bond of the *N*-acetyl muramic acid component.[1] However, the substrate specificity of the enzyme is not very clear because of the complexity of the chemical structure of the mucopeptides. Lysozyme is also known to split the glycosidic bonds of chitin[2] and glycol chitin[3], both of which have no *N*-acetyl muramic acid as a component. The present work has been undertaken as a part of the project for the elucidation of the substrate specificity of lysozyme. The course of the synthesis of the model substrate (Fig. 1) followed basically the method reported by one of the present authors.[4] *p*-Nitrophenyl *N*-acetyl-β-D-glucosaminide (I)[5] was reduced to the *p*-aminophenyl compound (II), which was converted to the *p*-acetaminophenyl compound (IV) by acetylation and *O*-deacetylation. The benzylidene derivative of IV was condensed with L-α-chloropropionic acid, and the product, after mild acid hydrolysis, was the desired *p*-acetaminophenyl *N*-acetyl-β-muramide. The methyl ester was obtained by methylation with diazomethane. Egg-white lysozyme revealed no activity on either of these model substrates.

Experimental

p-Aminophenyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (II).—I (4.4 g.) and 32 g. of ferrous sulfate hydrate ($FeSO_4 \cdot 7H_2O$) were suspended in 600 ml. of water, and then 2 ml. of 2 N hydrochloric acid was added. The suspension was stirred and heated to 60°C. Eight milliliters of concentrated ammonium hydroxide (ca. 28%) was added in one portion, and then three 3 ml. portions of ammonium hydroxide were added at two-minute intervals. After the mixture had been stirred for fifteen minutes, the precipitates were removed by filtration and the filtrates were concentrated in vacuo. The concentrate was extracted with methanol, evaporated, and desalted by passing it through a column of Dowex-1 (OH^- form). The eluate was concentrated in vacuo, and the residue was recrystallized from ethanol, giving 2.7 g. (67%) of colorless crystals which melted at 225—228°C (uncorr.). $[\alpha]_D^{16}$ +7.6° (*c* 0.92, water).

Found: C, 53.87; H, 6.80; N, 8.80. Calcd. for $C_{14}H_{20}O_6N_2$: C, 53.84; H, 6.45; N, 8.97%.

p-Acetaminophenyl 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (III).—II (5.8 g.) was dissolved in a mixture of 60 ml. of acetic anhydride and 60 ml. of pyridine. The temperature of the reaction mixture was raised to 50°C in ten minutes, and then the mixture was poured into ice water. The crystalline product was collected by filtration and was recrystallized from ethanol. The colorless crystals thus obtained weighed 5.6 g. (63%) and melted at 245°C (uncorr.). $[\alpha]_D^{16}$ −40.3° (*c* 0.92, pyridine).

Found: C, 55.04; H, 5.83; N, 5.79. Calcd. for $C_{22}H_{28}O_{10}N_2$: C, 54.99; H, 5.87; N, 5.83%.

p-Acetaminophenyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (IV).—III (3 g.) was dissolved in 75 ml. of absolute methanol. After the addition of 3 ml. of 1 N sodium methoxide, the solution was shaken for several minutes. A de-*O*-acetylated product separated and was collected and recrystallized from methanol, giving 1.5 g. (68%) of colorless crystals which melted at 236—237°C (uncorr.). $[\alpha]_D^{16}$ +6.1° (*c* 1.02, water).

Found: C, 54.01; H, 6.13; N, 7.79. Calcd. for $C_{16}H_{22}O_7N_2$: C, 54.23; H, 6.26; N, 7.91%.

p-Acetaminophenyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (V).—IV (3.2 g.), 40 ml. of freshly-distilled benzaldehyde and 3.2 g. of anhydrous zinc chloride were shaken together in a closed bottle for 24 hr. The reaction mixture was then poured into ice water, and the insoluble material was separated from the water layer by decantation. The product gave, after it had been washed with petroleum ether and recrystallized from ethanol, 2.8 g. (70%) of colorless crystals which melted at 258—262°C (uncorr.). $[\alpha]_D^{16}$ −10° (*c* 1.83, dimethylformamide).

Found: C, 62.01; H, 6.08; N, 6.26. Calcd. for $C_{23}H_{26}O_7N_2$: C, 62.43; H, 5.92; N, 6.33%.

p-Acetaminophenyl 2-Acetamido-4,6-O-benzylidene-3-O-(D-1'-carboxyethyl)-2-deoxy-β-D-glucopyranoside (VI).—V (2.7 g.) was dissolved in 105 ml. of freshly-distilled dimethylformamide, and the mixture was heated to 30°C. Sodium hydride (0.7 g., suspended in mineral oil at ca. a 50% concentration) was added, and the mixture was stirred for one hour. After the reaction temperature had been raised to 40°C, 3.8 g. of L-α-chloropropionic acid was added, and then 2.8 g. more of sodium hydride was added after 30 min. Stirring was continued overnight without applying any exteranl heating. Water was added in order to decompose the excess sodium hydride, and the mixture was concentrated in vacuo. The concentrate was dissolved in water, and the solution, after it had been washed with chloroform, was made acidic (pH

1) M. R. J. Salton and J. M. Ghuysen, *Biochim. Biophys. Acta*, **36**, 552 (1959); **45**, 355 (1960).
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3) K. Hamaguchi and M. Funatsu, *J. Biochem.*, **46**, 1695 (1959).
4) Y. Matsushima and J. T. Park, *J. Org. Chem.*, **27**, 3581 (1962).
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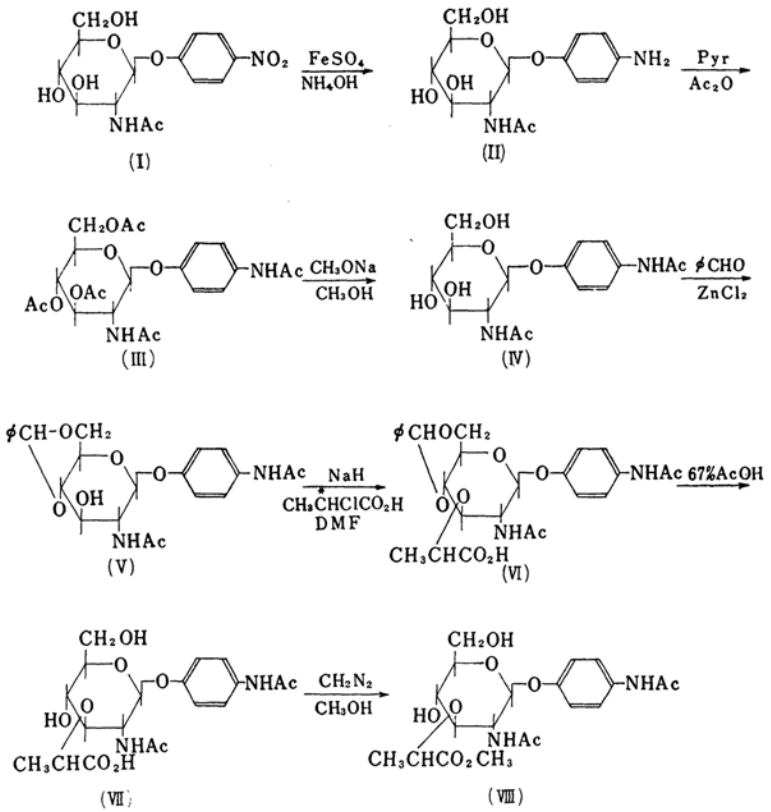

Fig. 1. The reaction sequence of the synthesis.

2.8) by adding hydrochloric acid while the mixture was kept at the temperature of ice water. The precipitates were collected and recrystallized from ethanol, giving 1.4 g. (43%) of colorless crystals which melted at 248—250°C (uncorr.). $[\alpha]_D^{16}$ +15.4° (*c* 0.72, dimethylformamide).

Found: C, 58.73; H, 6.30; N, 5.05. Calcd. for $C_{26}H_{30}O_9N_2 \cdot H_2O$: C, 58.64; H, 6.06; N, 5.26%.

The hydrochloric acid hydrolysis of VI gave a product which showed a spot identical to that of muramic acid on a paper chromatogram with the solvent system of phenol-water (75 : 25). VI showed an absorption band at 1740 cm^{-1} in the infrared spectrum, a band due to carboxyl carbonyl.

***p*-Acetaminophenyl 2-Acetamido-3-*O*-(D-1'-carboxyethyl)-2-deoxy-β-D-glucopyranoside (VII).**—In the 67% acetic acid, VI was boiled for ten minutes. After the solution had then been evaporated in vacuo, there remained a syrup which failed to crystallized.

***p*-Acetaminophenyl 2-Acetamido-3-*O*-(methyl-D-1'-ethylcarboxylate)-2-deoxy-β-D-glucopyranoside (VIII).**—An ethereal solution of diazomethane was dropped into the methanol solution of VII until a persistent yellow color remained. After evaporation in vacuo, the residual syrup was dissolved in a small amount of methanol; the mixture was then allowed to stand at −20°C. The crystals thus obtained were recrystallized from methanol, giving a specimen which melted at 210—213°C (uncorr.). $[\alpha]_D^{16}$ +17° (*c* 0.94, methanol).

Found: C, 54.03; H, 6.54; N, 5.93. Calcd. for $C_{20}H_{28}O_9N_2$: C, 54.54; H, 6.41; N, 6.36%.

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