*Studies on Amino-hexoses. VI. The Synthesis of Phenyl 2-Acetamido-3-O-(D-1′-carboxyethyl)-2-deoxy-α- and β-D-Glucopyranoside and Their Methyl Esters**

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A new aminosugar named muramic acid has been discovered in uridine pyrophosphate peptide complexes in *Staphylococcus aureus*[1] and was determined to be 2-amino-3-*O*-(D-1′-carboxyethyl)-2-deoxy-D-glucose.[2] It has been found to be distributed among the cell walls of a number of bacteria. Lysozyme, the enzyme which exerts a bacteriolytic action, has been found to split the cell-wall polysaccharide-peptide complex called muco-peptide at the site of the glycosidic bond of the *N*-acetylmuramic acid component.[3] However, the substrate specificity of lysozyme is not very clear because of the complexity of the chemical structure of the mucopeptide.

One of the present authors has reported on a convenient method for synthesizing *N*-acetylmuramic acid glycosides.[4] The present paper will describe the synthesis of phenyl *N*-acetyl-α- and β-muramide and their methyl esters by this method and will also describe the action of lysozyme on these model substrates.

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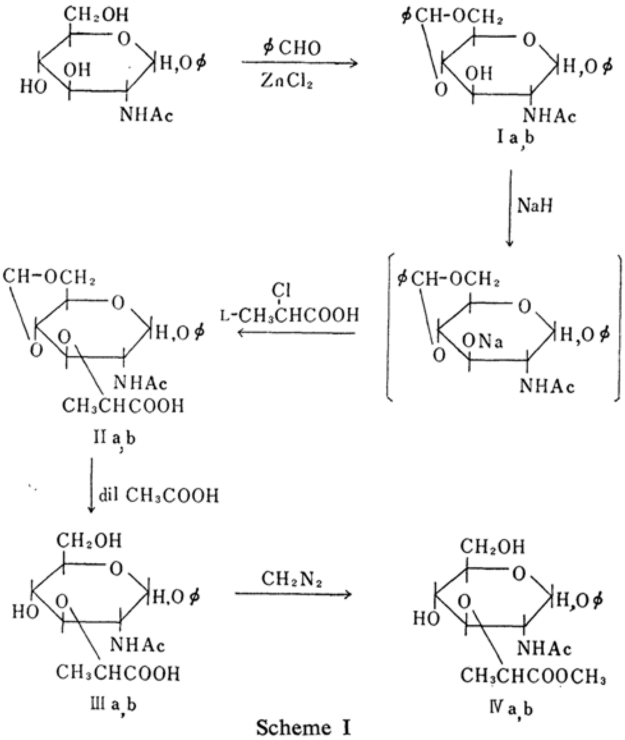
[1] J. T. Park. *J. Biol. Chem.*, **194**, 885 (1952).

[2] R. E. Strange and L. H. Kent, *Biochem. J.*, **71**, 333 (1959).

[3] L. R. Berger and R. S. Weiser, *Biochim. Biophys. Acta*, **26**, 517 (1957); W. Brumfitt, A. C. Wardlan and J. T. Park, *Nature*, **181**, 1783 (1958); M. R. Salton and J. M. Ghuysen, *Biochim. Biophys. Acta*, **36**, 552 (1959); H. Pelzer, ibid., **63**, 229 (1962).

[4] Y. Matsushima and J. T. Park, *J. Org. Chem.*, **27**, 3581 (1962).



Scheme I

Phenyl α- and β-D-glucosaminide were prepared according to the methods cited.[5,6] These phenyl glycosides were converted by the usual method into 4, 6-*O*-benzylidene derivatives, and the latter were condensed with L-α-chloropropionic acid, as has been reported.[4] The debenzylation with dilute acetic acid of these condensation products yielded phenyl *N*-acetyl-α- and β-muramide repectively. Methylation with diazomethane gave the respective methyl esters. The procedure of the synthesis is shown in Scheme I.

Lysozyme action[7] was tested on these acids as well as on methyl esters over a range of pH values. It was found that no appreciable cleavage of the phenyl group occurred in either α- or β-anomer.

Experimental

Phenyl 2-Acetamido-4, 6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (Ia).—Four grams of phenyl 2-acetamido-2-deoxy-α-D-glucopyranoside,[5] 40 g. of freshly distilled benzaldehyde, and 4.0 g. of anhydrous zinc chloride were kept in a tightly closed polyethylene bottle while being mechanically shaken at room temperature for 24 hr. The reaction mixture was then poured into 1000 ml. of cold water, and the water layer was separated by decantation from the oily product. This product was washed three times with petroleum ether, at which the product turned into a fine powder which was recrystallized from methanol. The crystals melted at 282~284°C. $[\alpha]_D^{18}$ +182.7 (*c* 0.684, pyridine). The yield was 4.3 g. (82.9%).

Found: C, 62.39; H, 6.24; N, 3.61. Calcd. for $C_{21}H_{23}O_6N\cdot H_2O$: C, 62.52; H, 6.25; N, 3.47%.

Phenyl 2-Acetamido-4, 6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (Ib). — Two grams of phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside[6] were treated as in the case of the α-anomer (Ia). After recrystallization from methanol, the product melted at 254°C with decomposition. $[\alpha]_D^{24}$ −27.1 (*c* 5.10, pyridine). The yield was 2.3 g. (90%).

Found: C, 62.27; H, 6.31; N, 3.47. Calcd. for $C_{21}H_{23}O_6N\cdot H_2O$: C, 62.52; H, 6.25; N, 3.47%.

Phenyl 2-Acetamido-4, 6-*O*-benzylidene-3-*O*-(D-1′ carboxyethyl)-2-deoxy-α-D-glucopyranoside (IIa). —Five grams of Ia were dissolved in dry hot dioxane (450 ml.) under mechanical stirring; after the addition of 2.5 g. of sodium hydride (a fine powder dispersed in mineral oil at a ca. 50% concentration), the mixture was kept at 90°C for 150 min. Eight grams of L-α-chloropropionic acid[8] were added when the temperature had gone down to 60°C. The mixture became a heavy paste and was vigorously stirred for 50 min. 5.5 g. of sodium hydride were again added, where-upon the mixture became thin. The temperature was again raised

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[5] S. Roseman and A. Dorfman, *J. Biol. Chem.*, **191**, 607 (1951).

[6] B. H. Leaback and P. G. Walker, *J. Chem. Soc.*, **1957**, 4754.

[7] Crystalline egg-white lysozyme was used. Cf. J. M. Ghuysen and M. R. J. Salton, *Biochim. Biophys. Acta*, **40**, 462 (1960).

[8] Shou-Cheng J. Fu, S. M. Birnbaum and J. P. Greenstein, *J. Am. Chem. Soc.*, **76**, 6054 (1954).

to 90°C for a short time, and then the mixture was allowed to stand overnight at room temperature. After the cautious addition of 100 ml. of water to decompose the excess sodium hydride, concentration in vacuo gave a thick yellow brown syrup, which was then washed with petroleum ether and dried in vacuo. The aqueous solution (1000 ml.) of the product, after it had once been filtered from a trace of insoluble matter, was made acidic (pH=2.0~2.2) by adding, drop by drop, 6 N hydrochloric acid while the solution was cooled by ice water. The slightly yellow crystals which precipitated were filtered, washed with water, and recrystallized from ethanol. The colorless crystals melted at 243~244°C. $[\alpha]^{20}_{D}$ +185.6 (*c* 0.388, pyridine). The yield was 4.5 g. (75.9%).

Found: C, 62.48; H, 5.91; N, 3.27. Calcd. for $C_{24}H_{27}O_8N$: C, 63.01; H, 5.95; N, 3.06%.

Phenyl 2-Acetamido-4, 6-*O*-benzylidene-3-*O*-(D-1′-carboxyethyl)-2-deoxy-β-D-glucopyranoside (IIb).—2.4 g. of Ib were treated as in the case of IIa. The crystals from ethanol melted at 248~249°C with decomposition. $[\alpha]^{28}_{D}$ −15.0 (*c* 2.93, pyridine). The yield was 2.3 g. (81%).

Found: C, 60.72; H, 6.15; N, 2.97. Calcd. for $C_{24}H_{27}O_8N\cdot H_2O$: C, 60.62; H, 6.15; N, 2.95%.

Phenyl-2-Acetamido-3-*O*-(D-1′-carboxyethyl)-2-deoxy-α-D-glucopyranoside (IIIa).—Four grams of IIa were boiled for exactly 10 min. in 820 ml. of 67% acetic acid. After evaporation of the mixture in vacuo, the repeated addition of a small amount of water, and evaporation in vacuo until no odor of acetic acid remained, there remained an amorphous residue which failed to crystallize. $[\alpha]^{20}_{D}$ +164.5 (*c* 0.106, pyridine).

Phenyl 2-Acetamido-3-*O*-(D-1′-carboxyethyl)-2-deoxy-β-D-glucopyranoside (IIIb).—One gram of IIb was treated as in the case of IIIa. The product was recrystallized from water. The crystals melted at 192~193°C with decomposition. $[\alpha]^{19}_{D}$ −4.1 (*c* 3.83, pyridine).

Found: C, 55.77; H, 6.02; N, 3.84. Calcd. for $C_{17}H_{23}O_8N$: C, 55.28; H, 6.28; N, 3.79%.

Phenyl 2-Acetamido-3-*O*-(methyl D-1′-ethylcarboxylate)-2-deoxy-α-D-glucopyranoside (IVa).—An ethereal solution of diazomethane was dropped into a solution of IIIa in 50 ml. of methanol which had been cooled with ice water. The reagent was added until a consistent yellow color remained. The residue which was obtained by evaporating the solution in vacuo was recrystallized from a mixture of ethyl acetate and petroleum ether. The colorless crystals melted at 170~175°C with decomposition. $[\alpha]^{20}_{D}$ +169.6 (*c* 1.256, ethyl acetate). The yield was 2.2 g. (65.7%).

Found: C, 56.73; H, 6.76; N, 3.75. Calcd. for $C_{18}H_{25}O_8N$: C, 56.39; H, 6.57; N, 3.65%.

Phenyl 2-Acetamido-3-*O*-(methyl D-1′-ethylcarboxylate)-2-deoxy-β-D-glucopyranoside (IVb).—One gram of a crude specimen of IIIb was treated with diazomethane as in the case of IVa. The crystalline product obtained melted at 216~217°C with decomposition. $[\alpha]^{14}_{D}$ +5.3 (*c* 3.90, pyridine). The yield was 0.7 g. (83%).

Found: C, 56.47; H, 6.57; N, 3.65. Calcd. for $C_{18}H_{25}O_8N$: C, 56.39; H, 6.56; N, 3.65%.

The Hydrolysis of IIa and IIb to 2-Amino-3-*O*-(D-1′-carboxyethyl)-2-deoxy-D-glucose (Muramic Acid).—IIa and IIb both gave a single spot of muramic acid on a papergram after hydrolysis with 2 N hydrochloric acid at 100°C for 4~5 hr. Runs were made along with an authentic muramic acid specimen in a solvent system of phenol-water (75 : 25); the R_f value of the spot developed with ninhydrin was about 0.51. These findings confirm the previously-assigned structures II—V.

Summary

A convenient method for the synthesis of anomeric phenyl glycosides of *N*-acetyl muramic acid and their methyl esters has been described. The test of lysozyme action on these model substrates was negative.

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