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Studies on D-Glucosamine Derivatives. XIII.*1 The Synthesis of *N*-Acetyl-1-*O*-aminoacyl-D-glucosamines

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For the purpose of examining the properties of the glycosidic ester linkage of sugars to amino acids, *N*-acetyl-1-*O*-aminoacyl-D-glucosamines were synthesized. *N*-Acetyl-4, 6-*O*-benzylidene-D-glucosamine was condensed with an equimolar amount of *N*-benzyloxycarbonyl amino acids by the dicyclohexylcarbodiimide method, followed by the hydrolytic and hydrogenolytic removal of the protecting groups. *N*-Acetyl-3-*O*-acetyl-D-glucosamine was also newly prepared as a contrast compound.

Though the presence of glycosidic ester lingkage between *N*-acetyl-D-galactosamine and L-glutamic acid in ovine submaxillary gland mucoprotein, reported by Gottschalk et al.,[1] is at present doubted by several workers,[2] it seems to be of interest to examine the properties of such a possible linkage.

The homologues, in which amino acids are linked to C-6, C-4, or C-3 positions of *N*-acetyl-D-glucosamine, were synthesized in previous experiments[3,4]; the present paper will describe the selective synthesis of *N*-acetyl-1-*O*-aminoacyl-D-glucosamines.

Results and Discussion

The glycosidic hydroxyl group in a sugar moiety is generally accepted to be much more reactive than the other groups, but few examples of the selective acylation of this hydroxyl group are known. Zervas[5] has reported the selective synthesis of 1-*O*-benzoyl-β-D-glucose by condensing sodium salt of 4, 6-*O*-benzylidene-D-glucose with an equimolar amount of benzoyl chloride, followed by hydrogenolysis.

In a similiar manner 1-*O*-glycyl-D-glucose sulfate[6] has been obtained from the sugar and *N*-benzyloxycarbonyl-glycyl chloride. On the other hand, it is known that the primary hydroxyl group of unprotected D-glucose or *N*-acetyl-D-glucosamine is directly acylated with an equimolar amount of *N*-Z*2-amino acids by the dicyclohexylcarbodiimide (DCC) method.[3,7]

In view of the facts mentioned above, *N*-acetyl-4, 6-*O*-benzylidene-D-glucosamine (I) was used as the starting material, in which the primary hydroxyl group is protected. The compound I was condensed with an equimolar amount of *N*-Z-amino acids (a, *N*-Z-glycine; b, *N*-Z-L-alanine; c, β-benzyl *N*-Z-L-aspartate) by DCC in pyridine or in a mixture of pyridine and dimethylformamide (DMF) to give *N*-acetyl-1-*O*-(*N*-Z-aminoacyl)-4,6-*O*-benzylidene-D-glucosamines (II) in a 40—50% yield. In order to remove the benzylidene group, II was then hydrolyzed with 70% acetic acid at 100°C for half an hour; *N*-acetyl-1-*O*-(*N*-Z-aminoacyl)-D-glucosamines (IIIa and IIIb) were isolated in ca. a 20% yield. Although the complete hydrolysis of *N*-Z-aminoacyl group was observed in the case of IIc, the catalytic hydrogenolysis of IIc in glacial acetic acid with palladium-charcoal (10%) could be directly carried out to give *N*-acetyl-1-*O*-(L-α-aspartyl)-D-glucosamine (IVc) in a 46% yield. The removal of the benzyloxycarbonyl group from IIIa and IIIb was also performed by palladium-catalyzed hydrogenolysis in the presence of oxalic acid, and *N*-acetyl-1-*O*-aminoacyl-D-glucosamines (IVa and were isolated as labile oxalates.

The infrared spectra of II and III showed the absorption band of ester carbonyl at 1730—1750 cm^{-1} and that of urethane carbonyl at 1690—1710 cm^{-1}. These compounds gave one spot in paper chromatography and showed no mutarotation, which indicates that the position of the ester linkage is at C-1. The anomeric structure of these compounds, which seems likely to depend on the solvent in the first condensation reaction, as will

*1 Part XII; This Bulletin, to be published.

1) A. Gottshalk and W. H. Murphy, *Biochem. Biophys. Acta*, **46**, 81 (1961).

2) W. Pigmann, *Exp. Ann. de Biochimie Medical*, **24**, 67 (1962); S. Harbon, G. Hermann Boussier and H. Clauser, *Bull. Soc. Chim. Biol.*, **45**, 1279 (1693).

3) J. Yoshimura, M. Funabashi, S. Ishige and T. Sato, *J. Chem. Soc. Japan, Pure Chem. Sect. (Nippon Kagaku Zasshi)*, **85**, 511 (1964).

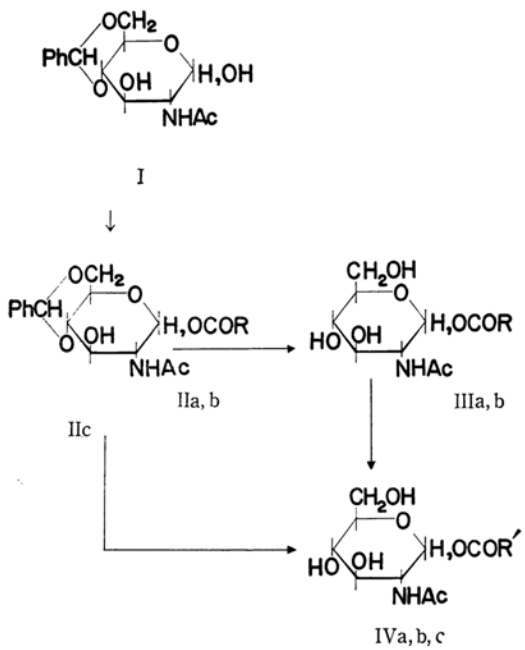
4) Part XII.

5) L. Zervas, *Ber.*, **64**, 2289 (1931).

6) M. Bergmann, L. Zervas and J. Overhoff, *Z. Physiol. Chem.*, **224**, 52 (1934).

*2 The benzyloxycarbonyl group is abbreviated to Z hereafter.

7) K. Kochetkov, V. A. Derevitskaya, L. M. Linkhosherstov, N. V. Molodtsov and S. G. Kara-Murza, *Tetrahedron*, **18**, 273 (1962).



a) R=-CH_2NHZ
R'=-$CH_2NH_2 \cdot 1/2(COOH)_2 \cdot H_2O$
b) R=-$CH(CH_3)NHZ$
R'=-$CH(CH_3)NH_2 \cdot 1/2(COOH)_2 \cdot 2H_2O$
c) R=-$CHCH_2COOCH_2Ph$ (NHZ)
R'=-$CHCH_2COOH \cdot H_2O$ (NH_2)
(Z=-$COOCH_2Ph$)

be described in the Experimental Section, was deduced from the rotational values and the infrared spectra.

In order to compare the properties with III, *N*-acetyl-3-*O*-acetyl-D-glucosamine (V) was newly prepared by the hydrolytic and hydrogenolytic removal of benzylidene and benzyl groups from benzyl *N*-acetyl-3-*O*-acetyl-4, 6-*O*-benzylidene-β-D-glucosaminide.[4] In contrast with III, V showed mutarotation in water ($[\alpha]_{578}^{14}$ $+89°$—$+44°$). In the periodate oxidation of these compounds in an acetate buffer (pH 5.0) for 20 hr., overoxidation was observed in each case; for example, β-IIIa and V consumed, respectively, 5.0 and 3.4 mol. of periodates, as is shown in Fig. 1. Therefore, the periodate oxidation would not be adopted for determining the structure of *O*-acyl derivatives of *N*-acetyl-D-glucosamine as it is known in other amino sugar derivatives.[8]

However, the position of the ester linkage in II-IV is concluded to be at C-1 from the facts that the compounds do not show mutarotation

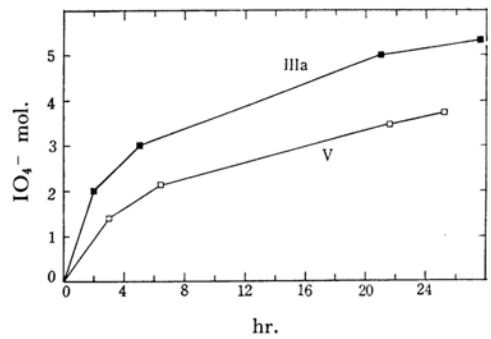


Fig. 1. Periodate Oxidation of *N*-acetyl-1-*O*-(*N*-benzyloxyloxycarbonylglycyl)-β-D-glucosamine (IIIa) and *N*-acetyl-3-*O*-acetyl-α-D-glucosamine (V).

and that 1-*O*-(*N*-Z-glycycl)-D-glucose, prepared by the procedure mentioned above, also showed no mutarotation and consumed 2 mol. of periodate, an amount consistent with the supposed structure.[9]

Experimental

All melting points are uncorrected. The optical rotations were measured at 578 mμ using a Carl-Zeiss polarimeter. Paper chromatography was carried out on Toyo Roshi No. 50 filter paper using the following solvent systems: A) isobutanol saturated with water, and B) *n*-butanol, acetic acid and water (4 : 1 : 5). An aqueous solution of 1% potassium iodide – 1% starch,[10] and a 0.1% ninhydrin solution in *n*-butanol were used as the spray reagents.

***N*-Acetyl-1-*O*-(*N*-benzyloxycarbonyl-glycyl)-4, 6-*O*-benzylidene-β-D-glucosamine (IIa).**—A solution of *N*-acetyl-4, 6-*O*-benzylidene-D-glucosamine (9.3 g.; 0.03 mol.) dissolved in a mixture of hot dry pyridine (80 ml.) and DMF (30 ml.) was cooled to room temperature. *N*-Z-Glycine (6.3 g.; 0.03 ml.) and DCC (8.0 g.; 0.039 mol.) were then added, and the mixture was allowed to stand overnight in a refrigerator. The precipitate of dicyclohexylurea was filtered off and washed with toluene. The filtrate and the washings were then concentrated to half their volume under reduced pressure and poured into ice water (300 ml.). The precipitate was filtered and washed several times with water.

The fractional recrystallization of the dry crude product was repeated several times from methanol in order to remove the unchanged starting material, I, which was recovered in ca. 30% yield. The final recrystallization from a mixture of methanol and pyridine (10 : 1) gave 4.1 g. (40%) of IIa, m. p. 180—182°C (decomp.), $[\alpha]_{578}^{20}$ $-36°$ (*c* 0.49, pyridine).

Found: C, 60.04; H, 5.81; N, 5.36, Calcd. for $C_{25}H_{28}O_9N_2$ (500): C, 59.99; H, 5.64; N, 5.60%.

In another experiment, only dry pyridine (150 ml.)

8) A. B. Foster and D. Horton, "*Advances in Carbohydrate Chemistry*," XIV, Academic Press, New York (1959) p. 258.

9) Private communication from N. Muramatsu (Central Research Laboratory of Ajinomoto Co. Ltd.).

10) H. N. Rydon and P. W. Smith, *Nature*, **169**, 922 (1952).

was used as the solvent, and the filtrate was concentrated under reduced pressure to a gelatinous residue, which was then washed with ether or ethyl acetate.

After fractional recrystallization, 5.1 g. (49%) of the product was obtained. M. p. 178—181°C (decomp.); $[\alpha]_{578}^{15}$ −20° (*c* 0.51, pyridine).

Found: C, 59.93; H, 5.36; N, 5.48%.

The rotational value and the infrared spectrum indicate the product to be contaminated with the α-anomer.

N-Acetyl-1-O-(N-benzyloxycarbonyl-L-alanyl)-4, 6-O-benzylidene-α, β-D-glucosamine (IIb). — *N*-Benzyloxycarbonyl-L-alanine (6.1 g.) was condensed with I (4.5 g.) by DCC (4.8 g.) in dry pyridine (120 ml.). It was subsequently treated in the manner described above. Yield, 3.1 g.; m. p. 172—175°C (decomp.); $[\alpha]_{578}^{17}$ +23° (*c* 0.59, pyridine).

Found: C, 60.13; H, 6.29; N, 5.51. Calcd. for $C_{26}H_{30}O_9N_2$ (514): C, 60.69; H, 5.88; N, 5.45%.

N-Acetyl-1-O-(β-benzyl N-benzyloxycarbonyl-L-aspartyl)-4, 6-O-benzylidene-α, β-D-glucosamine (IIc).—The compound I (9.3 g.; 0.03 mol.) was condensed with β-benzyl *N*-benzyloxycarbonyl-L-aspartate (10.6 g.; 0.03 mol.) by DCC (8.0 g.; 0.039 mol.) in pyridine (150 ml.). Yield, 4.9 g.; m. p. 183—185°C (decomp.); $[\alpha]_{578}^{20}$ −9.8° (*c* 0.05, pyridine).

Found: C, 62.78; H, 5.49; N, 4.52. Calcd. for $C_{34}H_{36}O_{11}N_2$(648): C, 62.95; H, 5.59; N, 4.33%.

N-Acetyl-1-O-(N-benzyloxycarbonyl-glycyl)-β-D-glucosamine (IIIa).—A suspension of IIa (2.5 g.) in 70% acetic acid (60 ml.) was heated on a boiling water bath for 30 min. while being stirred. The solution was then concentrated under reduced pressure to a gelatinous residue, which was redissolved in water and extracted twice with ether. The aqueous layer was then extracted five times with *n*-butanol (10 ml.). The *n*-butanol extract was evaporated in vacuo to a crystalline residue. The recrystallization from ethanol and ether gave a compound IIIa with a m. p. of 178—180°C (decomp.). R_f 0.65 (Solvent A) and $[\alpha]_{578}^{16}$ −20° (*c* 0.2, water).

Found: C, 52.15; H, 5.92; N, 7.02. Calcd. for $C_{18}H_{24}O_9N_2$ (412): C, 52.42; H, 5.87; N, 6.79%.

The compound IIa which was condensed in pyridine was treated in the same manner to give a product with a m. p. of 153—155°C (decomp.) and a $[\alpha]_{578}^{23}$ −11° (*c* 0.41, water).

N-Acetyl-1-O-(N-benzyloxycarbonyl-L-alanyl)-α-D-glucosamine (IIIb).—A suspension of the crude IIb (4.2 g.) in 70% acetic acid (70 ml.) was heated on a boiling water bath for 30 min. while being stirred. The solution was diluted with water to 500 ml. and neutralized with sodium bicarbonate. The precipitate was filtered off, and the filtrate was extracted with ether (10 ml.×3). The aqueous layer was then extracetd with *n*-butanol (20 ml.×5), and the extract was concentrated in vacuo to a crystailline residue. Recrystallization from ethanol gave 0.43 g. of white needles with a m. p. of 165—167°C (decomp.). R_f 0.71 (Solvent A), $[\alpha]_{578}^{13}$ +80° (*c* 0.23, water).

Found: C, 53.75; H, 6.28; N, 6.53. Calcd. for $C_{19}H_{26}O_9N_2$ (426): C, 53.51; H, 6.15; N, 6.57%.

N-Acetyl-1-O-glycyl-β-D-glucosamine Oxalate (IVa).—A mixture of IIIa (620 mg.), oxalic acid dihydrate (190 mg.) and 0.6 g. of Pd-C (10%) in 70% methanol (10 ml.) was shaken with hydrogen until the absorption had substantially ceased.

After the catalyst had then been removed, ether was added to the filtrate and centrifugation was performed to collect a precipitate. For the purification the precipitated solid was dissolved in a few drops of water, reprecipitated with ethanol, centrifugated, and dried in vacuo. The amorphous hygroscopic powder, which is gradually decomposed with browning in moist air, gave a single ninhydrin positive spot on paper chromatography (R_f 0.19, solvent B). Yield, 310 mg. (60%); it gradually melts at ca. 82°C with decomposition. $[\alpha]_{578}^{15}$ −7.6° (*c* 0.53, water).

Found: C, 38.93; H, 7.08; N, 7.45. Calcd. for $C_{10}H_{18}O_7N_2 \cdot 1/2(COOH)_2 \cdot H_2O$: C, 38.71; H, 6.20; N, 8.21%.

N-Acetyl-1-O-(L-alanyl)-α-D-glucosamine Oxalate (IVb).—The compound IIIb (170mg.) was hydrogenated in 70% methanol (10 ml.) with 0.2 g. of Pb-C (10%) in the presence of oxalic acid dihydrate (40 mg.). The subsequent operations were performed in the same manner as with IVa. Yield, 52 mg. (37%); R_f 0.25 (solvent B). It melted gradually at ca. 70°C with decomposition; $[\alpha]_{578}^{15}$ +96° (*c* 0.25, water).

Found: C, 37.62; H, 6.53; N, 8.05. Calcd. for $C_{11}H_{18}O_6N_2 \cdot 1/2(COOH)_2 \cdot 2H_2O$; C, 38.58; H, 7.02; N, 7.88%.

N-Acetyl-1-O-(L-α-aspartyl)-α, β-D-glucosamine (IVc).—A mixture of IIc (1 g.) and 1 g. of Pd-C (10%) in glacial acetic acid (30 ml.) was shaken with hydrogen for 15 hr. After the catalyst had been removed, acetone (220 ml.) and ether (300 ml.) were added to the filtrate and centrifugation was performed to collect a precipitate. This was subsequently treated in the same manner as IVa. Yield, 240 mg. (46%); m. p. 149°C (decomp.); R_f 0.15 (Solvent B), $[\alpha]_{578}^{14}$ +9.4° (*c* 0.53, water).

Found: C, 40.62; H, 7.18; N, 8.03. Calcd. for $C_{12}H_{20}O_9N_2 \cdot H_2O$: C, 40.67; H, 6.21; N, 7.90%.

Benzyl N-Acetyl-3-O-acetyl-β-D-glucosaminide.—A suspension of benzyl *N*-acetyl-3-*O*-acetyl-4, 6-*O*-benzylidene-β-D-glucosaminide[4] (1.0 g.) in 70% acetic acid (40 ml.) was heated on a boiling water bath for half an hour. The solution was then concentrated in vacuo to dryness. The preduct was recrystallized from ethanol (17 ml.). Yield, 0.57 g. (72%); m. p. 201—203°C; R_f 0.72 (Solvent A) and $[\alpha]_{578}^{15}$ −66° (*c* 0.36, methanol).

Found: C, 57.98; H, 6.58; N, 3.85. Calcd. for $C_{17}H_{23}O_7N$(353): C, 57.78; H, 6.56; N, 3.96%.

N-Acetyl-3-O-acetyl-β-D-glucosamine (V). — A mixture of benzyl *N*-acetyl-3-*O*-acetyl-β-D-glucosaminide (0.71 g.), oxalic acid dihydrate (0.15 g.) and 0.7 g. of Pd-C (10%) in 60% methanol (20 ml.) was shaken with hydrogen at room temperature for 5 hr. After the catalyst had been removed, the filtrate was concentrated under reduced pressure to dryness. The paper chromatography of the product gave 3 spots (R_f 0.12, 0.35 and 0.71, solvent A), which correspond to *N*-acetyl-D-glucosamine, V and the starting material respectively. V was then separated with a column of magenesol-cerite (1 : 3) by eluting it with water-saturated isobutanol.

The recrystallization of the crude product from ethanol gave colorless needles with a m. p. of 159—161°C (decomp.). Yield, 0.21 g. (40%); R_f 0.35 (Solvent A); $[\alpha]_{578}^{14}$ +89° (after 5 min.)→ +44° (after 50 hr.) (*c* 0.48, water).

Periodate Oxidation.—The oxidation of *N*-acetyl-1-*O*-(*N*-benzyloxycarbonyl - glycyl) - β - D - glucosamine (IIIa), shown in Fig. 1, will be described as an example.

A solution of IIIa (41.5 mg.) in 20 ml. of an acetate buffer (pH 5.0) and 20 ml. of a sodium periodate solution (445.2 mg./40 ml.) were placed in a measuring flask. The flask was then allowed to stand in a refrigerator at 5°C and the solution was subjected to titration four times during a 30 hr. period. To 10 ml. of the sample there was added a 10 ml. portion of a 5% solution of borax and 0.5 g. of potassium iodide. Titration was performed with a 0.05 N sodium arsenite solution (F=1.050). A blank solution was used for correction.