Studies on the *N*-Glucosaminide

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Received August 19, 1955

The *N*-glucosaminide has been studied with *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide as a model compound for its properties and structure. This componnd is prepared in quite the same way as that for usual *N*-glycoside with good yield. Upon acetylation in acetic anhydride and pyridine, it is converted into a pentaacetyl derivative with open chain structure, and upon methylation it gives a cyclic trimethyl derivative. Reaction of pentaacetyl glucosamines with *p*-toluidine gives cyclic tetraacetyl derivatives which upon acetylation in acetic anhydride and pyridine are also converted into the same pentaacetyl compound, indicating that the opening of the lactol ring takes place in these acetylating conditions. These structural behaviors have been discussed in reference to the structure of the sugar osazone, which has never been finally established due to similar behaviors, and it has been suggested that these behaviors are due to their common 1,2-diamino sugar structure.

1. Introduction

Carbohydrates combine with amines to give *N*-glycosides. This reaction proceeds in some cases nearly spontaneously, but heating, addition of adequate catalyst, or some other condition is usually required for the preparation. Since the *N*-glycosidic linkage is involved in the nucleosides and in some vitamins, it has attracted considerable attention of organic and biochemists in the last two decades. A body of knowledge on the chemical behaviors of synthetic and natural *N*-glycosides is available from such background of study[1], but the biochemical significance of this type of linkage is still unknown. Of many classes of carbohydrate compounds, the *N*-glycosides of various monosaccharides including D-fructose and some of the disaccharides have been prepared and their properties described. They show quite unique behaviors as compared with the corresponding *O*-glycosides.

The *N*-glycoside from glucosamine and an amine, or the *N*-glucosaminide, has scarecely attracted attention hitherto. Glucosamine (2-amino-2-deoxy-glucose) is widely distributed in nature, in microörganisms and in animals, as a constituent of the so-called aminopolysaccharides, mucoproteins, immunological substances, some

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The materials contained in this paper are reported in Japanese in *J. Agr. Chem. Soc. Japan* in three parts (Part 1, **29**, 139 ; Part 2, **29**, 143 ; Part 3, in press (1955)). The present paper contains some more detailed discussion of the results than those Japanese papers.

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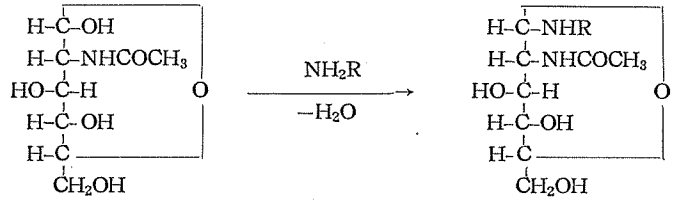
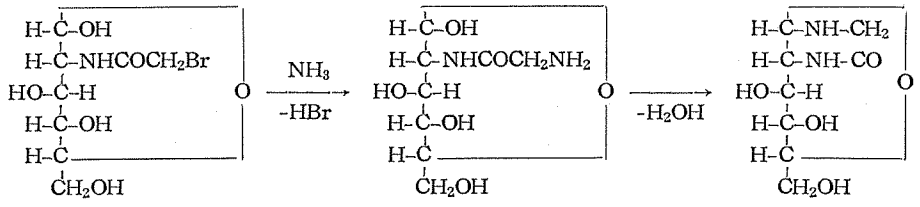
lipoids, and also of usual proteins. This amino sugar, together with another naturally occurring 2-amino hexose, galactosamine, appearently has been gathering profound interests of biochemists as well as well as of biologists because of the important biochemical functions concerned[2]. Since glucosamine is frequently found with amino acids or peptides in some unknown combination, the *N*-glucosaminide can be one of the possible types of compound formed from glucosamine and amino acid. No compound has appeared to be isolated from biological materials, which is definitely established to consist of an amino sugar and an amino acid or peptide. The *N*-glucosaminide may also offer considerable interests to the organic chemists in the carbohydrate field, for it is characterized to be a 1,2-diamino sugar, a new group of sugar derivatives ever investigated. Some aspects of its unique properties have been revealed in the present study.



N-Acetylglucosamine　　*N*-(*N'*-acetyl)glucosaminide

In the early history of the study of glucosamine, Weizmann and Hopwood[3] reported a reaction of *N*-α-halogenacyl glucosamine with ammonia with the purpose to prepare *N*-aminoacyl glucosamine. In this reaction, they obtained anhydrides of the desired *N*-aminoacyl derivatives. The structures of these compounds have never been pursued thereafter. It is of our opinion that in this reaction an intramolecular *N*-glycosidic linkage is formed after the formation of *N*-aminoacylglucosamine as shown in the following formula :



Syntheses of some arylamine-*N*-(*N'*-acetyl) glucosaminides have been previously performed in our laboratory.[4] Since then Bertho and Revesz[5] prepared some 1,2-diamino derivatives of glucose through glucosamine-1-azide, and Kuhn and Brossmer[6] mentioned the color reaction of some of the arylamine-*N*-(*N'*-acetyl)glucosaminide. An *N*-glycoside of amino sugar was first found in an antibiotic puromycin which contains *N*-3-amino-riboside.[7] In the course of study of this new antibiotic, preparation of purine-*N*-glucosaminide has been carried out.[8]

The present paper treats solely of *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide as a

model compound of the *N*-glucosaminide and contains results obtained concerning its properties and its structural behaviors. From the standpoint of both chemical and biochemical views, this group of gugar derivatives having the adjacent amino functions seems to be of great interest.

2. Preparation of *p*-Toluidine-*N*-(*N'*-acetyl)glucosaminide

In the condensation of *p*-toluidine and *N*-acetylglucosamine, the usual methods of preparing *N*-glycosides were found to be wholly available. Thus, heating both the starting materials in equimolar ratio in a boiling water bath with the addition of a small volume of water and a small amount of acidic catalyst completed the condensation in a few minutes, or refluxing both in alcoholic media for 1-3 hours gave the same product, *p*-toluidine-*N*-(*N'*-acetyl) glucosaminide (I). In the latter case of reaction, employment of methanol or 98 % ethanol as the solvent required no catalyst for the condensation, while in absolute ethanol absence of the catalyst effected to retard the reaction greatly. The *N*-glucosaminide thus prepared was obtained in solvated form and the solvent of crystallization was driven off after drying in vacuum at high temperature for considerably long hours. The thoroughly dried preparation showed m.p. 184° and $[\alpha]_D^{21} - 33°$ (*c* 0.24 pyridine).

3. The Color Reaction

N-Acetylglucosamine is conveniently characterized by the Morgan and Elson reaction[9] with the Ehrlich reagent (*p*-dimethylaminobenzaldehyde) which gives a reddish-purple color after treatment of the sugar in dilute alkali. It has been found that *p*-toluidine-*N*-(*N'*-acetyl) glucosaminide does not give the reddish color but it produces a yellow color. As shown in the Fig. 1, this color is due to absorption at 4400 Å which is also obtained by the reaction of *p*-toluidine and the Ehrlich reagent. This finding indicates that *p*-toluidne is split off, at least partially, to produce a yellow color with the Ehrlich eagent, and that *N*-acetylglucosamine with at the same time should be split off does not give the ordinary reddish color. The curve IV in the Fig. 1 shows that severe conditions of the color reaction produce a slight reddish-purple color, the absorption maxima being identical to those obtained in the usual *N*-acetylglucosamine-Ehrlich reagent reaction. These results apparently contradict to those reported recently by Kuhn and Brossmer[6] that *p*-toluidine and *p*-phenetidine-*N*-(*N'*-acetyl)glucosaminides are positive to the Morgan and Elson reaction.

The present examination of the Morgan and Elson color reaction also indicates that the reaction gives upon N-acetylglucosamine three absorption maxima, 5100, 5450, and 5850 Å (Fig. 1, Curve I). According to Schloss,[10] this reaction gives two or three absorption maxima, one of the latter being due to some volatile com-

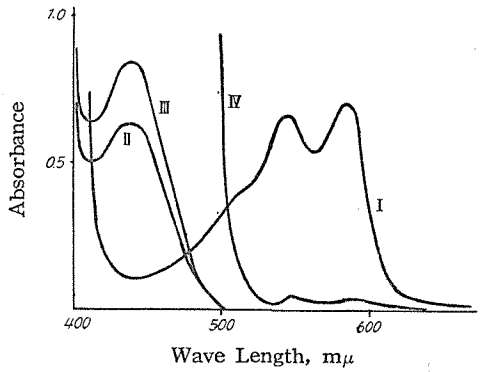


Fig. 1. The Absorption Curves of *N*-Acetylglucosamine, *p*-Toluidine-*N*-(*N'*-acetyl) glucosaminide, and *p*-Toluidine in the Morgan and Elson Color Reaction.

I. *N*-Acetylglucosamine. 5 cc. of 2.5 mg./cc. solution was added with 0.1 cc. of 5 *N* Na_2CO_3 solution and boiled for 5 minuites.

II. *p*-Toluidine-*N*-(*N'*-acetyl)glucosaminide. 5 cc. of 0.5 mg./cc. solution was added with 0.1 cc. of 5 *N* Na_2CO_3 solution and boiled for 5 minutes.

III. *p*-Toluidine. 5 cc. of 0.25 mg./cc. solution.

IV. *p*-Toluidine-*N*-(*N'*acetyl)glucosaminide. 5 cc. of 2.5 mg./cc. solution was added with 0.2 cc. of 5 *N* Na_2CO_3 solution and boiled for 30 minutes. These solutions developed celor on addition of *p*-dimethylaminobenzaldehyde solution prepared by dissolving 2 g. of it in 100 cc. glacial acetic acid containing 5 cc. HCl and diluting to 9/10.

pound. Our present data differ from those by Schloss in the positions of the maximum peaks, possibly due to the difference in the reaction conditions, particularly of the solvent employed. The present data are obtained by following the conditions directed by Morgan and Elson.

4. Acetylation

p-Toluidine-*N*-(*N'*-acetyl) glucosaminide was submitted to acetylation in usual manner, in pyridine and acetic anhydride. Acetylation at low and high (75°) temperatures has given an identical product with the m.p. 181° and $[\alpha]_D^{24} - 49°$ (*c* 1.74 pyridine). Upon elementary analyses and the determination of acetyl by the method of Bredereck,[11] it has been found that the acetylated product contains five acetyl groups in the molecule and that one of them is an *N*-acetyl group and the remaining four *O*-acetyl groups. This indicates that the product has an open-chain structure (II).

Attempts have been made to deaglyconate the acetylated product by treating it under acidic conditions to find that it hardly splits off the aglycone under usual conditions. The unacetylated *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide readily splits the aglycone under the same conditions.

Treatment of the acetylated product with Na methoxide relieves the acetyls combined to the OH groups of glucosamine to give a *p*-toluidine-*N*-(*N'*-acetyl)glucosa-

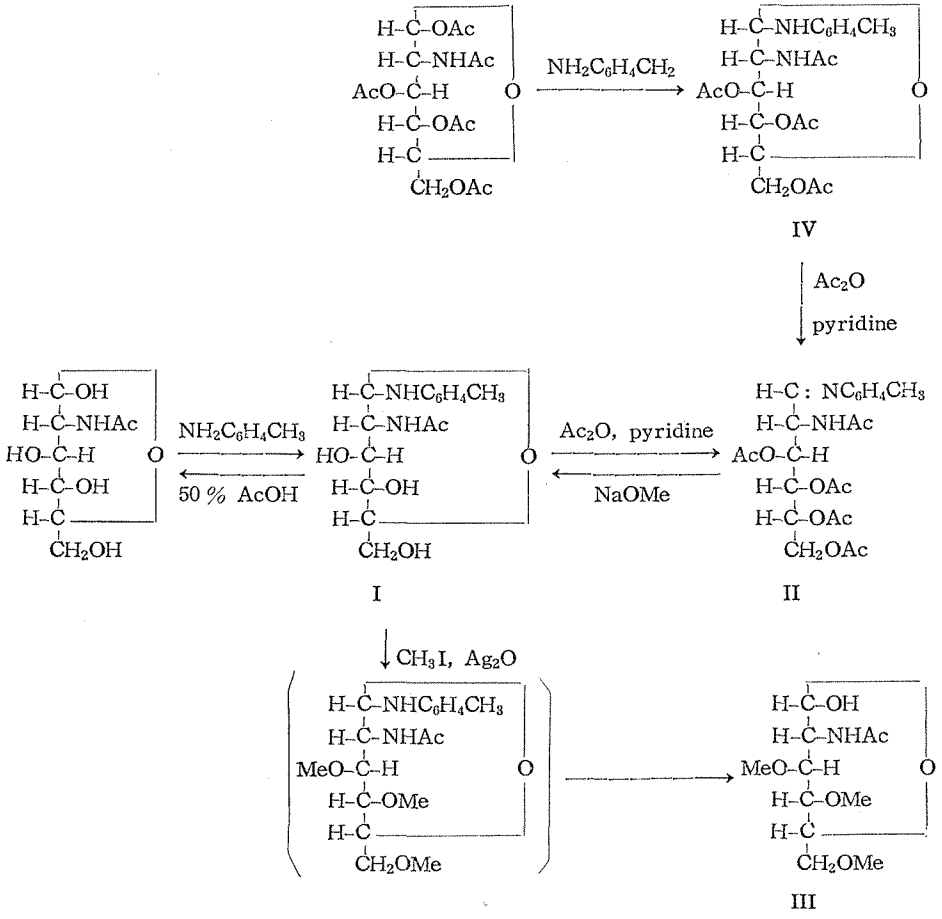
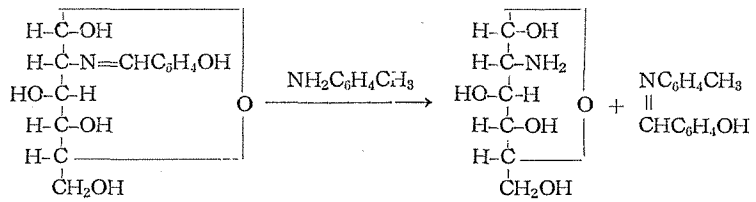


Fig. 2.

minide, m.p. 187°. Identification of this compound with the previous preparation with the m.p. 184° has not been accomplished.

5. Reaction of *p*-Toluidine with Salicylidene Glucosamine

Salicylidene glucosamine did not give an *N*-glucosaminide on the reaction with *p*-toluidine, but it split into glucosamine and salicylaldehyde, the latter of which immediately combined with *p*-toluidine to form salicylidene-*p*-toluidine.



6. Methylation

Methylation of *p*-toluidine-*N*-(*N'*-acetyl) glucosaminide was performed by susped-ing it in methyl iodide and refluxing with addition of silver oxide for about 50 hours. Methylating reagents were added newly once more in the middle of the reaction. The reaction mixture was filtered and treated with various solvents. A small amount of colorless needles has been obtained, the m.p. being 235°. Elementary analyses accorded with the calculated values for 3,4,6-trimethyl-*N*-acetylglucosamine (III), whose m.p. has been reported to be 234° by white.[12] Identification has thus been made on these bases. Formation of trimethylated product indicates that the methylation gives rise to a cyclic derivative. In another experiment. a white powder has been obtained which upon ignition left ash suggesting that it is a Ag-glucosamine complex.

7. Cyclic Acetyl Derivatives

Frèrejacque[13] reported that pentaacetylglucoses reacted with amines to give the arylamine-*N*-glucoside tetraacetates. This principle was employed for preparation of cyclic tetraacetyl derivatives of *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide. Thus, previous fractionation of α- and β-pentaacetylglucosamines by recrystallization followed by reaction with *p*-toluidine yielded α and β-tetraacetates of *p*-toluidine-*N*-glucosaminides (2-*N*-acetyl-3,4,6-tri-*O*-acetyl *p*-toluidine-glucosaminide) (IV). These cyclic tetraacetates have given the above open-chain pentaacetate on further acetylation in pyridine and acetic anhydride. This result indicates that the opening of the lactol ring of the *N*-glucosaminide takes place during the acetylation reaction in pyridine and acetic anhydride.

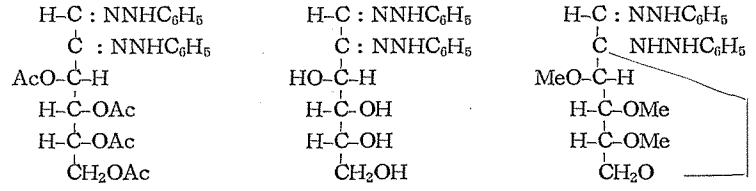
8. Discussion

The properties of *N*-glycosides have been well investigated mainly on arylamine *N*-glycosides and particularly their liability to interchange the optical forms (α and β forms) and the lactol rings (pyranoside and furanoside) and to convert into ketose isomers (isoglycosamines) has been thoroughly studied.[1] The present studies have not covered these properties, but they chiefly concern unique bahaviors in the lactol ring structure of the *N*-glucosaminide. It is very interesting that *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide gives an open-chain pentaacetyl componund on acetylation and a trimethyl derivative with cyclic structure on methylation.

Glucosamine is afforded to show unique properties by the presence of an amino group in the C_2 position, or adjacent to the carbonyl group of glucose. It is postulated that the amino group strongly affects the carbonyl group. Difficulty of *O*-glycosidation of *N*-acetylglucosamine in usual methods, for example, has been explained on this basis.[14] The difficulty is so large that a real chemical combina-

tion between the C_1 and C_2 of glucosamine has been believed to exist by some investigators.[15] Ready formation of *N*-glucosaminide is noted in comparison with the *O*-glucosaminide. The two amino groups at C_1 and C_2 of the *N*-glucosaminide may be responsible for the behaviors described above.

The analogous structural behaviors are found in the sugar osazones. This group of compounds occupies in the sugar chemistry a special position because of its importance for the use of identifaction of individual sugar structures. The role played by it in the progress of sugar chemistry since its first employment by Fischer[16] in that field can never be exaggerated. The structure of this important sugar derivatives, however, is still in debate, because of its peculiar behaviors quite similar to those found in the *N*-glucosaminide. In 1935, Percival and Percival[17] methylated phenylglucosazone and obtained 3,4,5-trimethyl phenylglucosazone. Upon this result, Percival[18] has insisted on the cyclic structure of the sugar osazones. On the other hand, Wolfrom and colaborators[19] found that glucose and galactose phenylosazones gave tetraacetyl products of open-chain structure. Many other reports have appeared since then in favor of either of them, but the final conclusion as to whether the osazone has the open-chain structure or the ring structure, or the explanation why it gives these two types of structure according to the conditions has never been achieved. Very recently Akiya and Tejima[20] found an open-chain structure in the acetone-condensed derivative of glucosazone.



Phenylglucosazone

(Wolfrom) (Fischer's formula) (Percival)

The sugar osazones and the *N*-glucosaminide share common structure in having two adjacent nitrogen atoms in the positions of C_1 and C_2. If the ring-retaining and ring-opening of the *N*-glucosaminide depending methylating and acetylating conditions can be explained by this particular arrangement of functional groups, the same may be said in the structural behaviors of the sugar osazones. Thus, the findings on the *N*-glucosaminide appear to offer means for elucidation of the structure of the osazones. If things are quite same with the *N*-glucosaminide, the sugar osazones open the lactol ring in the usual acetylating conditions or similar ones. Methylation does not affect the lactol ring. It is, therefore, of our opinion that the sugar osazones are of ring structure and open the ring in pyridine and acetic anhydride to give the acyclic acetates. Opening of the lactol ring in these reagents has been confirmed in the *N*-glucosaminide by acetylating α- and β-tetraacetyl *p*-toluidine-*N*-glucosaminide.

EXPERIMENTAL

1. *N*-Acetylglucosamine. The preparation was made by the method of White[12]. After twice recrystallization, it showed a m.p. 204-5°. This m.p. is higher than that reported by White, but Roseman[21] has found m.p. 210° on his purest preparation.

2. *p*-Toluidine-*N*-(*N'*-acetyl)glucosaminide. a). Condensation in aqueous medium: *N*-Acetylglucosamine 5.8 g., *p*-toluidine 2.9 g., glacial acetic acid 0.5 cc., and water 2 cc. were heated in a boiling water bath. The reaction mixture was immediately dissolved to give a homogeneous solution, which upon leaving give crystals. They were dissolved in 25 cc. of ethanol and allowed to stand overnight in an ice-box. The crystals that appeared were collected, washed with small volumes of ethanol and ether, and dried over concentrated sulfuric acid. The yield was 4.5 g. Addition of ether into the mother solution followed by leaving gave further yield of *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide. Upon recrystallization from ethanol, the m.p. was 184° (decompn.). $[\alpha]_D^{21} - 33°$ (*c* 0.24 pyridine). The preparation was obtained in solvated from, and the following analytical dara were obtained after drying at 100° for 3 hours over P_2O_5.

Anal. Found: C 58.19, 58.37, H 7.34, 7.19, N 8.79, 8.70; Calcd. for $C_{15}H_{22}O_5N_2$: C 58.03, H 7.15, N 9.03.

b) Condensation in methanol: *N*-Acetylglucosamine 5 g., *p*-toluidine 2.5 g. were refluxed in 30 cc. of methanol. The reaction solution gradually gained a brownish color and usually within 2-3 hours *N*-acetylglucosamine dissolved. Addition of NH_4Cl initially or in the course of the reaction influenced slightly the reaction time. After dissolution completed, it was additionally heated for 20 minutes and filtered. Upon standing in an ice-box overnight it gave crystals which were collected and washed with alcohol and ether. The yield was 5 g. after drying. It was recrystallized from very dilute ethanol to give large needles. The m. p. was 184° (decompn., browned at about 175°). After drying over P_2O_5 for 30 minutes, it gave the following analytical data.

Anal. Found: C 57.75, 57.81, H 6.75, 6.96, N 8.26, 8.34; Calcd. for $C_{15}H_{22}O_6N_2$: C 58.05, H 7.15, N 9.03.

c) Condensation in anhydrous ethanol: *N*-Acetylglucosamine 5g. and *p*-toluidine 3g. were refluxed in 50 cc. of absolute ethanol under exclusion of moisture. After 2 hours, the mixture was slightly colored with slight dissolution of *N*-acetylglucosa mine. Ammonium chloride 0.3 g. was then added as a catalyst and the reaction completed in additional 1 hour. Leaving overnight in an ice-box gave rise to crystallization. The crystals after collecting, washing with absolute ethanol and ether, and drying were in the yield of 6.5 g. Recrystallization from absolute ethanol showed the m.p. 183°.

3. The Color Reaction with the Ehrlich Reagent. The reagents were prepared by faithfully following the directions by Morgan and Elson[9]. The test solution of *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide was prepared by dissolving 0.5 g. of it in 50 cc. of water, and diluted appropriately at the test. Detailed conditions of the reaction are given in the legend of Fig. 1.

4. Acid Hydrolysis of *p*-Toluidine-*N*-(*N'*-acetyl)glucosaminide. *p*-Toluidine-*N*-(-*N'*-acetyl)glucosaminide (m.p. 184°) 2 g. was suspended in 12 cc. of methanol and added with 5 cc. of 50 % acetic acid. The larger part of crystals dissolved on the instance. Shaking it at room temperature for 3 hours produced a complete solution with yellowish color. Into this a large volume of ether was added to produce turbidity and it was left in an ice-box overnight to give 0.7 g. of crystals, which melted at 188-90°. When recrystallized from water-ethanol by adding ether it showed a m.p. 208°, thus indicating it to be *N*-acetylglucosamine. It was positive to the Morgan and Elson reaction under the standard conditions.

5. Acetylation. a). *p*-Toluidine-*N*-(*N'*-acetyl)glucosaminide 2.9 g. was dissolved in 10 cc. of pyridine at room temperature, and added with 10 cc. of acetic anhydride with cooling. After leaving for 48 hours, it was added with 20 cc. of ethanol and condensed at 30-40° under reduced pressure. Ethanol was repeatedly added before pyridine and acetic anhydride were completely driven off. The crystals that appeared were finally recrystallized from ethanol, giving an yield of 3.4 g., m.p. 177°. Repeated recrystallization from ethanol gave m.p. 181°, $[\alpha]_D^{21}$-49° (*c* 1.74 pyridine). Analyses were performed after drying at 100° over P_2O_5 for 30 minutes.

Anal. Found: C 58.16, 57.99, H 6.27, 6.49, N 5.62; Calcd. for $C_{23}H_{30}O_9N_2$; C 57.7, H 6.8, N 5.86.

The determination of acetyl was made with a sample in alcoholated form by the directions of Brederecк. Found: total Ac 42.1, O-Ac 32.3 %; Calcd. for $C_{23}H_{30}O_9N_2$-C_2H_5OH: 5 Ac 41.0, 4 O-Ac 32.8 %.

b) *p*-Toluidine-*N*-(*N'*-acetyl)glucosaminide 3.7 g. was dissolved in 20 cc. of pyridine and warmed to 75° and gradually added with 10 cc. of acetic anhydride warmed at 75°. The reaction mixture was allowed to stand at 28° for 25 hours. Crystallization was performed by using a large volume of ethanol in the same way as described above. The m.p. was 168° and the yield was 3.5 g. Upon recrystallization the preparation showed m.p. 179-80°. The analytical data after drying at 100° for 30 minutes over P_2O_5 were as follows:

Anal. Found: C 57.81, 57.94, H 6.27, 6.39, N 5.38, 5.39; Calcd. for $C_{23}H_{30}O_9N_2$: C 57.7, H 6.28, N 5.86.

When this preparation of the pentaacetate, about 5 mg., was dissolved in 5 cc. of 50 % ethanol, added with 0.2 cc. of 0.5 *N* Na_2CO_3 and heated for 5 minutes in boiling water-bath, cooled immediately, and added with 2 cc. of *p*-dimethylaminobenzaldehyde-90 % acetic acid, a yellow color was developed.

6. Treatment of Pentaacetylglucosamine-1-*N*-*p*-toluidide with Acid. a) Pentaacetylglucosamine-1-*N*-*p*-toluidide 0.5 g. was suspended in 15 cc. of methanol and added with 2 cc. of 50 % acetic acid. This was shaken at room temperature for two hours and for additional one hour with further addition of 1.5 cc. of 50 % acetic acid. The reaction mixture was cooled with ice-water and neutralized with 0.5 *N* Na_2CO_3 with the use of phenolphthalein as an indicator to give crystals which were collected by filtration and washed with, successively, 50 % ethanol, ethanol, and ether. The yield was 0.45 g. This showed m.p. 180° and from the following analytical data which were obtained after drying over P_2O_5 for 30 minutes at 100° it was identified to be the original pentaacetylglucosamine-1-*N*-*p*-toluidide.

Anal. Found : C 57.70, 57.54, H 6.27, 6.42, N 5.49, 5.46, ; Calcd. for $C_{23}H_{30}O_9N_2$: C 57.7, H 6.28, N 5.86.

This preparation also gave a yellow color in the Morgan and Elson reaction.

b) Pentaacetylglucosamine-1-*N*-*p*-toluidide 2 g. in 15 cc. chloroform was added with 5 cc. of methanol, to which 0.4 g. oxalic acid had been dissolved, and 0.1 cc. of water added. Leaving this mixture at 28° for 1 hour precipitated crystals in the yield 0.4 g. Upon recrystallization from ethanol, it showed a m.p. 173°. This was identified to be an addition-product composed of each one molecule of oxalic acid and *p*-toluidine.

Anal. Found : N 7.30, 7.34; Calce. for $C_9H_{11}O_4N$: N 7.11.

Upon condensation of the filtrate it gave considerable amount of crystals which from the analytical data were identified to be unreacted pentaacetylglucosamine-1-*N*-*p*-toluidide.

Anal. Found. : C 57.3, 57.55, H 6.44, 6.42, N 5.54, 5.52; Calcd. for $C_{23}H_{30}O_9N_2$: C 57.7, H 6.28, N 5.86.

The above results indicate that pentaacetylglucosamine-1-*N*-*p*-toluidide partially splits upon treatment with oxalic acid the aglycone, but the larger part remains unattacked.

7. Deacetylation of Pentaacetylglucosamine-1-*N*-*p*-toluidide by sodium methoxide. Pentaacetylglucosamine-1-*N*-*p*-toluidide 0.5 g. in 20 cc. of anhydrous methnol was added with approximately 2 mg. of sodium and left at room temperature for 2 days. Upon condensation under reduced pressure, it gave a *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide, melting at 187° after recrystallization from ethanol.

Anal. Found : C 57.66, 57.56, H 7.06, 7.16 ; Calcd. for $C_{15}H_{22}O_5N_2$: C 58.03, H 7.15.

Determination of acetyl by the Bredereck method indicated the presence of an *N*-acetyl.

8. An attempt for condensation of salicylidene glucosamine with *p*-toluidine. Salicylidene glucosamine 6.1 g. and *p*-toluidine 2.5 g. in 50 cc. of methanol were refluxed for 1 hour and left for spontaneous cooling to give an ether soluble yellow-

ish crystals. After recrystallization from methanol it showed m.p. 97-8°. When ether was added into the mother solution, glucosamine appeared in visid mass. Leaving salicylidene glucosamine and *p*-toluidine overnight in cold methanol also gave the identical procuct, melting at 97°. It was identified to be salicylidene-*p*-toluidine. *Anal.* Found: C 80.29, 80.28, H 6.40, 6.22; Calcd. for $C_{14}H_{13}ON$: C 79.62, H 6.16. Acetylation in acetic anhydride and pyridine gave acetylsalicylidene-*p*-toluidine. The m.p. was 106°.

Anal. Found: C 75.96, 75.84, H 6.38, 6.08; Calcd. for $C_{16}H_{15}O_2N$: C 75.89, H 5.92.

9. Methylation of *p*-Toludine-*N*-(*N'*-acetyl)glucosaminide. a) *p*-Toluidne-*N*-(*N'*-acetyl)glucosaminide 0.8 g. and Ag_2O in 20 cc. of CH_3I were refluxed under exclusion of moisture for 46 hours. In the middle of the reaction, Ag_2O 2 g. and CH_3I 20 cc. were newly added into the reaction mixture. After the reaction completed, the mixture was filtered and the residual Ag salts were washed thoroughly with aceton. The filtrate was combined with the washings and condensed under reduced pressure. The condensed solution was left in a desiccator for a time to give solid which was extracted with ether and the residue was taken in methanol. Some insoluble matter was filtered off. The methanolic extract upon leaving for spontaneous evapration produced needles in a yield of approximately 20 mg. After two recrystallizations from ethanol, it showed mp. 235°, identical with 3, 4, 6-trimethyl-*N*-acetylglucosamine.

Anal. Found: C 49.31, H 8.14, N5.23, 5.34; Calcd. for $C_{11}H_{21}O_6N$: C 50.2, H 8.0, N 5.3.

b) *p*-Toluidine-*N*-(*N'*-acetyl)glucosaminide 3.2 g., methyl iodide 50g., and Ag_2O 5g. were refluxed for 48 hours with further addition of Ag_2O 2g. and CH_3I 20cc. in the middle of the reaction. The reaction mixture was filtered and washed successively with acetone, ether, and methanol and each filtrate was colleted separately. The methanol washing was combined with the acetonic washing and added with considerably large volume of methanol to give a white precipitate which was removed by filtration. The filtrate was condensed under reduced pressure to dryness. Extraction of this powder with methanol followed by condensation gave a white powder, melting at 202°. Ignition left ash indicating that it is some complex of Ag. Thee structure of this cmplex is not known. The analytical data are as follows: C 43.7 43.3, H 7.2, 7.0, N 5.6.

10. Pentaacetylglucosamines. Glucosamine hydrochloride 10 g. was placed in a boiling mixture of acetic anhydride and NaOAc, and the boiling continued for additional 5 minutes. Upon cooling some volume of ethanol was added and left at rome temperature to make a large amount of NaOAc appear. The crystals were removed by filtration and the filtrate was concentrated to yield a larger amount of of NaOAc. The same procedure with addition of ethanol was repeated several times and NaOAc was removed as perfectly as possible. The deep reddish syrup

thus obtained yielded crude β-pentaacetylglucosamine in needles upon standing overnight. Addition of ether into the mother solution yielded crude α-pentaacetylglucosamine in crystalline powder. The latter was cosiderably soluble in water and alcohol. The yieldes were α isomer 2 g. and β isomer 5g. After recrystallization from ethanol the physical properties of both isomers were as follows:

	m.p.	$[\alpha]_D$ ($CHCl_3$)	*Anal.* Found		
			C	H	N
α	134-6°	+ 86°	49.32	5.84	3.45
β	180-1°	+ 2°	49.37	5.98	3.49
			Calcd. for $C_{16}H_{23}O_{10}N$		
			49.36	5.96	3.61

11. *p*-Toluidine-*N*-α-glucosaminide tetraacetate. α-pentaacetylglucosamine 3.1 g., *p*-toluidine 2 g., and acetic acid 0.5 cc. were refluxed in 50 cc. of ethanol. The yellowish reaction solution was left for 2 days at room temperature and concentrated under reduced pressure to give a thick syrup, which was dissolved in ether and was made turbid by adding a small volume of ligroin. Upon leaving in desiccator crystallization occurred. The crystals were collected and washed with ethanol and ether. Recrystallization from a small volume of ethanol gave 0.4 g. needles; m. p, 117-18°. $[\alpha]_D^{16}$+62°(*c* 1 $CHCl_3$)

12. *p*-Toluidine-*N*-β-glucosaminide tetraacetate. β-Pentaacetylglucosamine 2 g. and *p*-toluidine 1.3 g. were refluxed in 60 cc. of ethanol containing 0.5 cc. acetic acid, and, the reaction solution was left for 2 days to give crystals. After washing with ethanol and ether the m. p. was 174°; the yield was 2.1 g. Recrystallization from ethanol gave m. p. 174-5°. $[\alpha]_D^{17}$−3°(*c* 2 $CHCl_3$).

The preparation gave yellow color on boiling in Na_2CO_3 solution containing ethanol for a few minutes and adding with the Ehrlich reagent.

13. Deacetylation of *p*-Toluidine-*N*-glucosaminide tetraaectate. *p*-Toluidine-*N*-glucosaminide tetraacetate(α,β-mixture) 1g. was thrown in 30cc methanol and added with approximately 3 mg. of sodium. The solution was completed by frequent shaking and left at room temperature for 2 days. Condensation under reduced pressure followed by recrystallization of the crystals that appeared gave *p*-toluidine-*N*-(*N'*-acetyl)glucosaminide, m.p. 185°.

14. Acetylation of *p*-Toluidine-*N*-glucosaminide tetraacetate. *p*-Toluidine-*N*-β-glucosaminide 1 g. in a mixture of 10 cc. pyridine and 8 cc. acetic anhydride was heated for 1 hour at 50-60°. The reaction solution was slightly colored. The small amount of insoluble matter was filtered off and left at room temperature for 2 days. The solution was added with a large volume of ethanol and repeatedly concentrated with new addition of ethanol to drive off pyridine and acetic anhydride. The final thick syrup upon standing in an ice-box produced pentaacetylglucosamine-

1-*N*-*p*-toluidide ; m.p. was 181° after recrystallization from ethanol.

Anal. Found : C 57.92, 57.96, H 6.33, 6.38: Caled. for $C_{23}H_{30}O_9N_2$: C 57.7, H 6.28.

The *α*-isomer of the tetraacetate also gave the identical product upon acetylation under the same conditions. The pentaacetate melted at 180-1°.

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