Studies on Amino-hexoses. VII. The N-Deacetylation of Methyl N-Acetyl α-D-Glucosaminide

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One of the present authors previously presented a method which might be of use for elucidating aminopolysaccharide structures.[1] The method consisted basically of two procedures, the deacetylation of the *N*-acetyl group in *N*-acetyl aminosugar components and the deamination by nitrous acid of the free amino group thus formed. Anhydrous hydrazine, when used as a deacetylating reagent, had a minimum influence on glycosidic linkages but gave not very satisfactory results. As was shown in the original report,[1] hydrazine treatment of chondroitin sulfate caused considerable degradation of the molecule, and only about 10% of the starting material was recovered as a 90% *N*-deacetylated non-dialysable specimen. Wolfrom and Juliano[2] reported the recovery of a 59~68% *N*-deacetylated specimen in about a 50% yield. Yosizawa and Sato[3-5] observed the formation of 2, 5-anhydrohexose and pentose beside the extensive degradation of the molecule in the hydrazine treatment of the blood group aminopolysaccharide.

The above-mentioned results, obtained in several laboratories, show that more fundamental research into a method is desirable; the present authors chose methyl *N*-acetyl α-D-glucosaminide as the simplest model compound. This compound was treated both with anhydrous and hydrated hydrazine. The deacetylation yield was assayed by Van Slyke nitrogen determination, and the starting compound which remained was assayed by isolation. The results are shown in Table I.

The resultant methyl α-D-glucosaminide showed a marked stability against these reagents in the thin layer chromatography of the reaction mixture, which revealed no spots other than those of the starting compound and the deacetylated product. Refluxing with barium hydroxide for several hours did not give even a trace of any colored substance, though it did give crystalline methyl α-D-glucosaminide in a good yield. Since a convenient method for separating methyl *N*-acetyl-D-glucosaminide anomers has been established,[6] these deacetylation methods afford a conveniet

TABLE I. HYDRAZINOLYSIS OF METHYL *N*-ACETYL α-D-GLUCOSAMINIDE

Reaction time	Reagent	Starting compound[a] mg.	Yield of the deacetylated product, %	Recovery of the starting compound				Total recovery %
				mg.	%	$[\alpha]_D$	m. p., C°	
10 hr.	Hydrazine hydrate (90%)	490.9	70.1	99.5	20.3	+134.8°[b]	189~190	90.4
	Anhydrous hydrazine	512.3	54.2	206.1	40.2	+135.2°[c]	184~186	94.4
20 hr.	Hydrazine hydrate (90%)	498.4	93.2	7.6	1.5	—[e]	—[e]	94.7
	Anhydrous hydrazine	500.1	86.1	60.9	12.2	+134.3°[d]	185~186	98.3

a) The specimen had $[\alpha]_D^{29}$ +136.9° (*c* 1.45, in water) and melted at 188~189°C.
b) *c*=0.920 in water at 28°C.
c) *c*=1.03 in water at 28°C.
d) *c*=0.752 in water at 27°C.
e) Identified by thin layer chromatography.

1) Y. Matsushima and N. Fujii, This Bulletin, **30**, 48 (1957).
2) M. L. Wolfrom and B. O. Juliano, *J. Am. Chem. Soc.*, **82**, 2588 (1960).
3) Z. Yosizawa and T. Sato, *Biochim. Biophys. Acta*, **52**, 591 (1961).
4) Z. Yosizawa and T. Sato, *J. Biochem.*, **51**, 155 (1962).
5) Z. Yosizawa and T. Sato, *Tohoku J. Exp. Med.*, **76**, 100 (1962).
6) Y. Matsushima, T. Miyazaki and J. T. Park, *J. Biochem.*, **54**, 109 (1963).

way to obtain anomerically-pure methyl D-glucosaminides.

Experimental

Hydrazine Treatment and Assay of the Reaction Mixture.—About 500 mg. of methyl *N*-acetyl α-D-glucosaminide and 1.5 ml. of hydrazine were kept in a sealed tube at 100°C for 10 or 20 hr. The reaction mixture was then evaporated in vacuo, and the residue was dissolved in 25 ml. of methanol. In order to remove the acethydrazide which had formed, 8 ml. of benzaldehyde was added and the mixture was refluxed for 15 min. and evaporated in vacuo. Twenty milliliters of water was added to the residue, and the mixture was, after having been washed twice each with petroleum ether and ethyl ether, subjected to Van Slyke nitrogen determination.

The hydrazine treated specimen was, after the removal of the excess hydrazine described above, dissolved in water and passed through a column of Dowex 50 (2×10 cm., H^+ form) and a column of Dowex 1 (2×5 cm., OH^- form). About 200 ml. of the solution thus obtained was evaporated in vacuo to dryness. The crystalline residue was weighed. The methyl *N*-acetyl α-D-glucosaminide thus recovered was identified by melting point determination, specific rotation measurement and thin layer chromatography. The results are shown in Table I and in Fig. 1. A thin layer chromatogram of the recovered substance gave only one spot identical with that of a standard sample of methyl *N*-acetyl α-D-glucosaminide in every run.

The Preparation of Methyl α-D-Glucosaminide and Its Hydrochloride.—*With Hydrazine Hydrate.*—One gram of methyl *N*-acetyl-α-D-glucosaminide and 3 ml. of hydrazine hydrate (90%) were kept in a sealed tube at 100°C for 10 hr. After hydrazine had been removed in vacuo, and after a large amount of chloroform had been added to the residue, the mixture was refluxed for an hour. The chloroform solution was concentrated to a small volume in vacuo and kept in a refrigerator. The jelly product thus obtained was filtered and dried in a desiccator. The yield was 0.54 g. (65.9%). The colorless crystals obtained from absolute ethanol melted at 154~160°C $[\alpha]_D^{30}$ +158.5° (*c* 1.06, in water).

Found: C, 43.69; H, 7.96; N, 7.20. Calcd. for $C_7H_{15}O_5N$: C, 43.51; H, 7.83; N, 7.25%.

The compound was dissolved in water, and the pH of the solution was adjusted to 4.4 with 1 N hydrochloric acid. Evaporation in vacuo gave the hydrochloride as a colorless crystalline powder $[\alpha]_D^{29}$ +131.6° (*c* 1.14, in water).

Found: C, 37.56; H, 6.96; N, 6.88; Cl, 13.97. Calcd. for $C_7H_{16}O_5NCl$: C, 36.61; H, 7.02; N, 6.10; Cl, 15.44%.

With Barium Hydroxide.—The mixture of methyl *N*-acetyl-α-D-glucosaminide (1.0 g.), barium hydroxide (2.5 g., octahydrate) and 18 ml. of water was refluxed for an hour. The mixture was then filtered and saturated with carbon dioxide. After filtration from barium carbonate, the pH of the filtrate was adjusted to 4 by 1 N sulfuric acid. The filtrate free from barium sulfate was passed through a column of Dowex 1 (OH^- form) and the solution was concentrated to dryness in vacuo. The crystalline residue was dissolved in a small volume of hot ethanol and then kept in a refrigerator. The colorless crystals obtained melted at 155~157°C ($[\alpha]_D^{22}$ +157.5°; (*c* 0.838, in water).

Found: C, 43.94; H, 7.98; N, 7.17 (6.94 (Van Slyke)). Calcd. for $C_7H_{15}O_5N$: C, 43.51; H, 7.83; N, 7.25%.

Colorless crystalline hydrochloride ($[\alpha]_{30}^{D}$ +129.2°) (*c* 1.32, in water) was obtained.

Found: C, 36.51; H, 6.99; N, 6.28; Cl, 15.37. Calcd. for $C_7H_{16}O_5NCl$: C, 36.61; H, 7.02; N, 6.10; Cl, 15.44%.

The Identification of the Hydrazinolyzed Product.—As has been described above, the hydrochloride of the deacetylated product gave optical rotation data which were in good agreement with the results obtained by Neuberger and Rivers,[7] who prepared the compound via *N*-carbobenzyloxy-D-glucosamine and gave $[\alpha]_D$ +127°. The melting

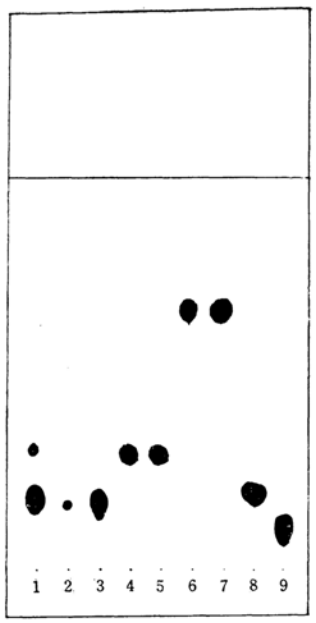

Fig. 1. Thin layer chromatogram of products and authentic specimen.
Moving phase: *n*-BuOH-EtOH-H_2O-NH_4OH (40 : 10 : 49 : 1)
Spray: 5%H_2SO_4
1: Hydrazinolysate freed from acethydrazide
2: Authentic methyl α-D-glucosaminide·HCl[7]
3: Hydrochloride prepared by $Ba(OH)_2$-deacetylation
4: Authentic starting compound
5: Starting compound recovered
6: *N*-Carbobenzyloxy derivative prepared from hydrazinolysate
7: Authentic *N*-carbobenzyloxy compound[7]
8: *N*-Acetyl D-glucosamine
9: D-Glucosamine·HCl

7) A. Neuberger and R. P. Rivers, *J. Chem. Soc.*, **1939**, 122.

point determination of our hydrochloride was difficult because of its hygroscopic nature. While thin layer chromatography (Fig. 1) identified our compounds well, a crystalline *N*-carbobenzyloxy derivative was prepared and compared with an authentic specimen synthesized by the Neuberger and Rivers method.

The syrup obtained by treating methyl *N*-acetyl α-D-glucosaminide with hydrazine hydrate was washed repeatedly with hot chloroform until free from acethydrazide. 0.36 g. of the syrup was then dissolved in 15 ml. of water. 0.5 ml. of carbobenzyloxy chloride and 0.36 g. of sodium bicarbonate were mechanically stirred into the solution, and the stirring was then continued for an hour. After having passed through a column of Dowex 50 (H^+ form), the solution was dried up and the residue was crystallized from water as colorless needles which melted at 159~161°C alone or at 157~160°C when mixed with an authentic specimen. The yield was 0.22 g. (36.1%) $[\alpha]_D^{28}$ +100.0° (*c* 0.84, in pyridine).

Found: C, 54.62; H, 6.60; N, 4.23. Calcd. for $C_{16}H_{21}O_7N$; C, 55.04; H, 6.47; N, 4.28%.

Summary

Methyl *N*-acetyl-α-D-glucosaminide has been found to be well *N*-deacetylated by hydrazine treatment. The methyl α-D-glucosaminide thus produced shows so much stability with regarded to hydrazine and to barium hydroxide that these reagents provide a convenient means of preparing anomerically-pure methyl D-glucosaminide and its hydrochloride. The free base of methyl α-D-glucosaminide has been obtained crystalline for the first time.

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