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Studies on Amino-hexoses. IX. The *N*-Deacetylation of Methyl *N*-Acetyl-β-D-glucosaminide, Methyl *N*-Acetyl-3-*O*-methyl-α- and β-D-glucosaminide, and Methyl *N*-Acetyl-4, 6-*O*-ethylidene-α-D-glcosaminide

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The efficiency of hydrazine as a mild *N*-deacetylating reagent has been studied in the reaction of hydrazine with four *N*-acetylaminosugars. This reagent has been shown to be of great use in the *N*-deacetylation of methyl *N*-acetyl-β-D-glucosaminide and methyl *N*-acetyl-4, 6-*O*-ethylidene-α-D-glucosaminide. It may be noted that the 3-*O*-substitution of the sugars makes the deacetylation reaction reluctant. The synthesis of methyl *N*-acetyl-4, 6-*O*-ethylidene-α-D-glucosaminide and the isolation of crystalline methyl β-D-glucosaminide and methyl 4, 6-*O*-ethylidene-α-D-glucosaminide in the free-base form have been described.

The hydrazine treatment of methyl *N*-acetyl-α D-glucosaminide (Ia) was described in a previous paper of this series.[1] The *N*-acetyl group in Ia was there shown to be well removed by the hydrazine treatment, and methyl α-D-glucosaminide (IVa) was isolated in a fairly good yield as the deacetylated product. It was also shown that both anhydrous and hydrated hydrazine had a minimal influence on the glycosidic linkage of Ia.

The present paper will describe the hydrazine treatment of four glucosamine derivatives: methyl *N*-acetyl-β-D-glucosaminide (Ib), methyl *N*-acetyl-3-*O*-methyl-α-D-glucosaminide (IIa), methyl *N*-acetyl-3-*O*-methyl-β-D-glucosaminide (IIb) and methyl *N*-acetyl-4, 6-*O*-ethylidene-α-D-glucosaminide (III). Anomerically-pure Ib was obtained, together with pure Ia by the chromatography on Dowex 1×3 (OH^- form), as has been described by Matsushima, Miyazaki and Park.[3] A pure 3-*O*-methyl α-compound (IIa) was prepared from Ia according to the method of Neuberger.[3] The β-anomer (IIb) was prepared from Ib by the known methods.[2-5] In the preparation of IIb, the physical data of the intermediate compounds were in good agreement with those described by Roth and Pigman,[5] whose data were different from those of Masamune, Okuyama and Sinohara.[4] The ethylidene compound (III) was newly synthesized by a slight modification of the method of Helferich and Appel.[6] The structure of III was verified by leading it to methyl *N*-carbobenzyloxy-4, 6-*O*-ethylidene-α-D-glucosaminide (VII), which had been prepared by Akiya and Osawa,[7] and by the periodate oxidation of the *N*-deacetylated product of III, methyl 4, 6-*O*-ethylidene-α-D-glucosaminide (VI). The deacetylation yield was assayed by the Van Slyke method in the case of Ib and by the micro

1) M. Fujinaga and Y. Matsushima, This Bulletin, **37**, 468 (1964).

2) Y. Matsushima, T. Miyazaki and J. T. Park, *J. Biochem.*, **54**, 109 (1963).

3) A. Neuberger, *J. Chem. Soc.*, **1941**, 50.

4) H. Masamune, T. Okuyama and H. Shinohara, *Tohoku J. Exp. Med.*, **68**, 181 (1958).

5) W. Roth and W. Pigman, *J. Am. Chem. Soc.*, **82**, 4608 (1960).

6) B. Helferich and H. Appel, *Ber.*, **64**, 1841 (1931).

7) S. Akiya and T. Osawa, *Yakugaku Zasshi*, **76**, 1276 (1956).

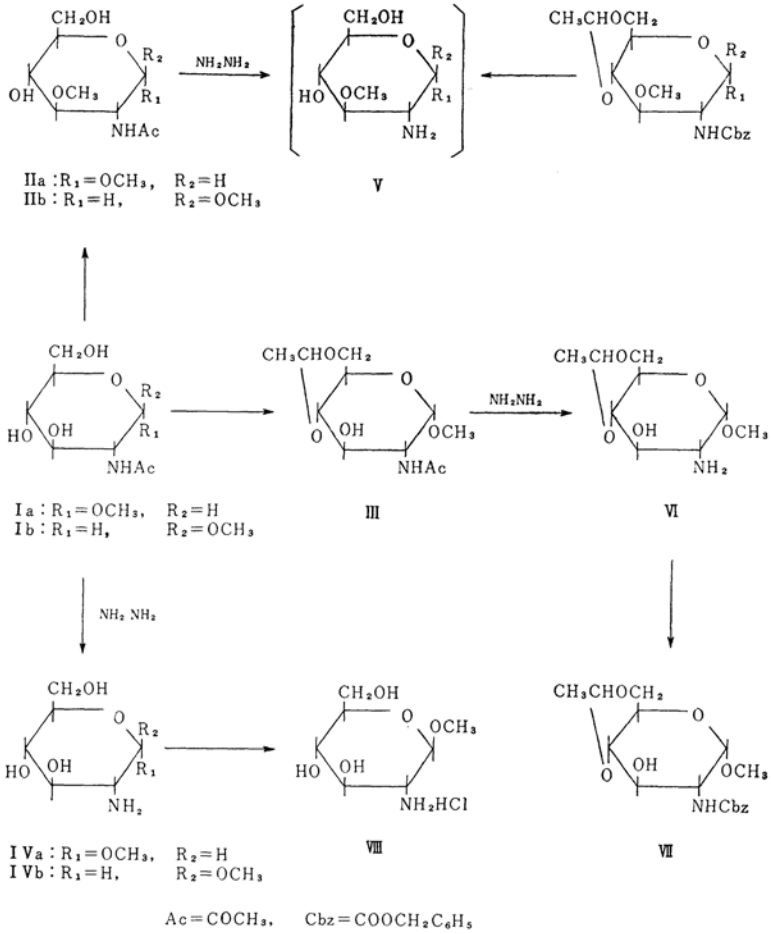

Chart 1.

Kjeldahl method in the case of IIa, IIb and III. A Hitachi amino acid autoanalyzer was also used to determine the deacetylation yield in the case of III. The starting compound which remained was assayed by isolation in every case. The results are shown in Tables I-IV. Methyl N-acetyl-β-D-glucosaminide (Ib) was well deacetylated, as in the case of the α-anomer (Ia);[1] the ethylidene compound (III) was also well deacetylated by the treatment with hydrated hydrazine, while the 3-*O*-methyl compounds (IIa and IIb) were both deacetylated in an unexpectedly low yield. These findings may give some suggestions about the reaction mechanism.

The reaction courses followed in this investigation are shown in Chart 1.

Experimental

Methyl β-D-Glucosaminide (IVb).—The starting methyl *N*-acetyl-β-D-glucosaminide (Ib)[2] (Chart 2) had a m. p. of 202—203°C and $[\alpha]_D^{29}$ −39.7° (*c* 2.890, water). A mixture of Ib (1 g.) and hydrazine (3 ml.) was kept in a sealed tube at 100°C for 20 hr.; it was then treated in a manner similar to that described in a previous paper.[1] The colorless crystals obtained from ethanol had a m. p. of 132—133°C and $[\alpha]_D^{14}$ −41.2° (*c* 1.608, water). Yield, 0.45 g. (55%).

Found: C, 43.56; H, 7.93, N, 7.26. Calcd. for $C_7H_{15}O_5N$: C, 43.52; H, 7.83; N, 7.25%.

The infrared spectrum of IVb is shown in Chart 2.

Methyl β-D-Glucosaminide Hydrochloride (VIII).—The treatment of IVb with 1 N hydrochloric acid gave a hydrochloride which ahd a m. p. of 187—188°C and $[\alpha]_D^{14}$ −23.6° (*c* 1.824, water) after recrystallization from ethanol.

Found: C, 35.81; H, 7.00; N, 6.04; Cl, 15.49. Calcd. for $C_7H_{16}O_5NCl$: C, 36.61; H, 7.02; N, 6.10; Cl, 15.44%.

Methyl *N*-Acetyl-4, 6-*O*-Benzylidene-β-D-glucosaminide.—A mixture of Ib, benzaldehyde and zinc chloride was shaken for 16 hr. The resulting benzylidene compound was then isolated by the usual method. Recrystallization from methanol gave the pure substance. Yield, 80%. M. p. 270—274°C. $[\alpha]_D^{18}$ −17.4° (*c* 0.432, dimethylsulfoxide).

Found: C, 59.39; H, 6.54; N, 4.28. Calcd. for $C_{16}H_{21}O_6N$: C, 59.43; H, 6.55; N, 4.33%.

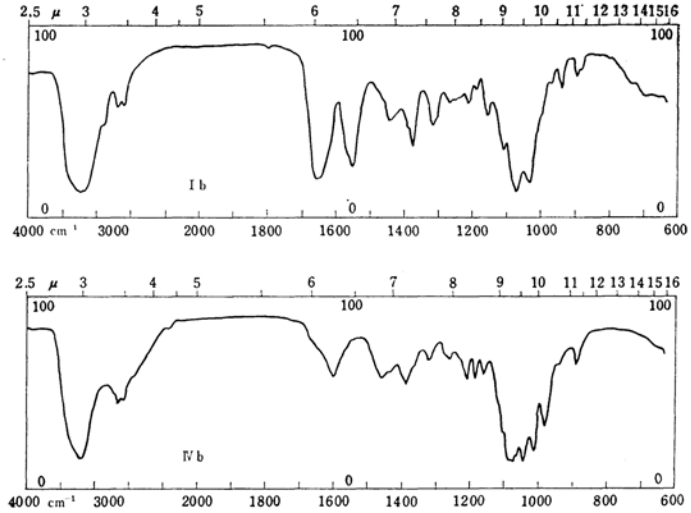

Chart 2. Infrared spectra of methyl *N*-acetyl-β-D-glucosaminide (Ib) and methyl β-D-glucosaminide (IVb).

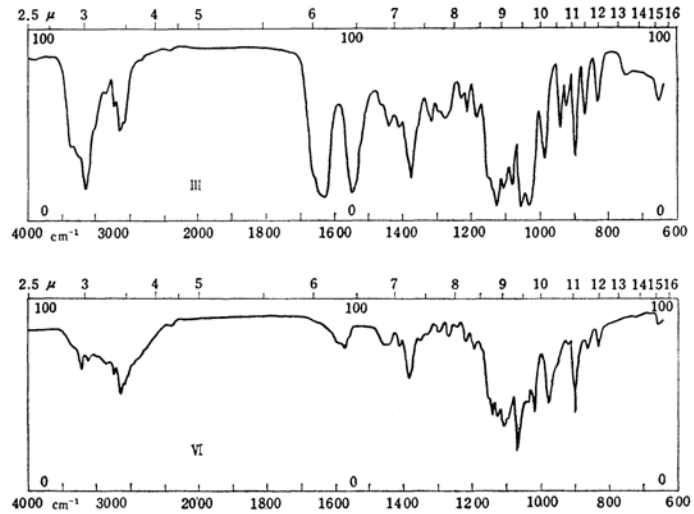

Chart 3. Infrared spectra of methyl *N*-acetyl-4,6-*O*-ethylidene-α-D-glucosaminide (III) and methyl 4,6-*O*-ethylidene-α-D-glucosaminide (VI).

Methyl *N*-Acetyl-4, 6-*O*-benzylidene-3-*O*-methyl-β-D-glucosami-nide.—Methyl *N*-acetyl-4, 6-*O*-benzylidene-β-D-glucosaminide was treated with dimethylsulfate and 30% sodium hydroxide in a large amount of dioxane. The reaction mixture was then poured into water, and the precipitates were collected and recrystallized from methanol. Yield, 57%. M. p. 276—280°C; $[\alpha]_D^{18}$ −64.8 (*c* 0.432, dimethylsulfoxide).

Found: C, 60.45; H, 6.70; N, 4.13. Calcd. for $C_{17}H_{23}O_6N$: C, 60.52; H, 6.87; N, 4.15%.

Methyl *N*-Acetyl-3-*O*-Methyl-β-D-Glucosaminide (IIb).—Methyl *N*-acetyl-4, 6-*O*-benzylidene-3-*O*-methyl-β-D-glucosaminide was heated with 60% acetic acid for 15 min. The clear solution was then evaporated in vacuo, and the residue was recrystallized from ethanol. Yield, 55%. M. p. 220—22°C; $[\alpha]_D^{14}$ −50.1 (*c* 2.176, water).

Found: C, 48.40; H, 7.71; N, 5.65. Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68; N, 5.62%.

Methyl *N*-Acetyl-4, 6-*O*-ethylidene-α-D-glucosaminide (III).—Into a cooled suspension of Ia (4g.) in freshly-distilled paraldehyde (80 ml.), two drops of concentrated sulfuric acid were mechanically stirred. After the mixtrue had then been warmed at 60°C for 4 hr., a large amount of chloroform was added. The insoluble materials were then filtered off, and the filtrate was treated with solid potassium carbonate and then evaporated in vacuo. The crystalline residue was recrystallized twice from ethyl acetate. Yield, 3.6 g. (80%). M. p. 237—238°C; $[\alpha]_D^{12}$ +118.9° (*c* 1.968, ethanol).

Found: C, 50.31; H, 7.33; N, 5.34. Calcd. for $C_{11}H_{19}O_6N$: C. 50.56; H, 7.33; N, 5.36%.

The infrared spectrum of III is shown in Chart 3.

TABLE I. HYDRAZINOLYSIS OF METHYL *N*-ACETYL-β-D-GLUCOSAMINIDE (Ib)

Reagent	Starting compound mg.	Yield of the deacetylated product, %	Recovery of the starting compound			Total recovery %
			mg.	%	m. p.,* °C	
Hydrazine hydrate (90%)	500.4	99.1	5.2	1.0	148—150	100.1
Anhydrous hydrazine	500.2	74.1	135.5	27.1	163—164	101.2

* Before recrystallization

TABLE II. HYDRAZINOLYSIS OF METHYL *N*-ACETYL-3-*O*-METHYL-α-D-GLUCOSAMINIDE (IIa)

Reagent	Starting compound mg.	Yield of the deacetylation product, %	Recovery of the starting compound			Total recovery %
			mg.	%	m. p.,* °C	
Hydrazine hydrate (90%)	1001.2	27.0	716.6	71.6	205—207	98.6
Anhydrous hydrazine	1000.4	10.4	869.7	86.6	210—211	97.0

* Before recrystallization

Methyl 4, 6-*O*-Ethylidene-α-D-glucosaminide (VI).—A mixture of III (1.0 g.) and hydrazine hydrate (6 ml.) was kept, with occasional shaking, in a sealed tube at 100°C for 20 hr. The crystalline residue obtained by the evaporation of the reaction mixture was dissolved in hot cyclohexane, and then the solution was cooled gradually. The soft colorless needles thus obtained had a m. p. of 134—135°C ($[\alpha]_D^{18}$ +110.8° (*c* 2.048, water)) and 6.49% nitrogen. Yield, 0.7 g. (83%). Recrystallization from cyclohexane gave a pure VI, m. p. 135—136°C, $[\alpha]_D^{18}$ +111.1° (*c* 1.980, water).

Found: C, 49.13; H, 7.69; N, 6.34. Calcd. for $C_9H_{17}O_5N$: C, 49.30; H, 7.82; N, 6.39%.

The infrared spectrum of VI is given in Chart 3.

Methyl *N*-Carbobenzyloxy-4, 6-*O*-ethylidene-α-D-glucosaminide (VII).—Sodium bicarbonate (0.5 g.) and carbobenzyloxychloride (1 ml.) were stirred into an aqueous solution of VI (0.5 g.) at room temperature. After the stirring had been continued for an hour, the precipitates were filtered and washed with water. Recrystallization from ethanol gave fine needles; m. p. 176—177°C, $[\alpha]_D^{18}$ +67.5° (*c* 2.058, chloroform). Yield, 0.42 g. (52%).

Found: C, 57.69; H, 6.39; N, 3.96. Calcd. for $C_{17}H_{23}O_7N$: C, 57.78; H, 6.56; N, 3.96%.

No depression of the melting point was observed when VII was mixed with an authentic specimen which has been prepared according to the method of Akiya and Osawa,[7] and it had a m. p. of 176—177°C; $[\alpha]_D^{17}$ +67.4° (*c* 1.720, chloroform).

The Hydrazine Treatment of Methyl *N*-Acetyl-β-D-glucosaminide (Ib).—An about 500 mg. portion of Ib was treated with hydrazine (1.5 ml.) at 100°C for 20 hr. in a sealed tube, and then it was treated in a manner similar to that described in the previous paper.[1] The recovered substance obtained by passing the reaction mixture through a column of Dowex 50×4 (H^+ form) was characterized as the starting compound, Ib, by chromatographic examination and by a study of the infrared spectra, though a slight difference in melting point was observed. The results are shown in Table I.

The Hydrazine Treatment of Methyl *N*-Acetyl-3-*O*-methyl-α-D-glucosaminide (IIa).—The starting methyl *N*-acetyl-3-*O*-methyl-α-D-glucosaminide (IIa)[3] had a m. p. of 212—213°C; $[\alpha]_D^{12}$ +113.6° (*c* 1.690, water). In a sealed tube, IIa (1 g.) and hydrazine (3 ml.) were kept at 100°C for 20 hr. After it had then cooled, the reaction mixture was dissolved in absolute ethanol and concentrated in vacuo on a boiling water bath. An aqueous solution of the crystalline residue was passed through a column of Dowex 50×4 (2×18 cm., H^+ form), and then washed with water (300 ml.). The effluent and washings were combined and concentrated in vacuo to dryness, and the final crystalline residue was weighed. The compound thus recovered was identified as the starting compound, IIa, by a melting point determination, a study of its infrared spectra, and thin-layer chromatography.

The column of Dowex 50×4 was eluted with 0.5 N hydrochloric acid. The effluent solution was collected in about 6 ml. fractions, which were then checked with ninhydrin and *p*-dimethylaminobenzaldehyde - hydrochloric acid reagents. Hydrazine and hydrazide react with the latter reagent to form an intensely-yellow to orange-colored substance and a yellow fluorescent product respectively.[8,9] The fractions which were positive with the ninhydrin and negative with the *p*-dimethylaminobenzaldehyde reagent were collected, passed through a column of Dowex 1×4 (2.2×25 cm., OH^- form), and then washed with water. The effluent, combined with washings, was concentrated in vacuo to a syrup, which was then dissolvend in an appropriate amount of water. The aqueous solution thus obtained was subjected to micro Kjeldahl nitrogen determination, from which the yield of the deacetylated product was calculated. The results are shown in Table II.

The Hydrazine Treatment of Methyl *N*-Acetyl-3-*O*-methyl-β-D-glucosaminide (IIb).—One gram of IIb was treated with hydrazine as in the case of IIa. The results are shown in Table III.

The Hydrazine Treatment of Methyl *N*-Acetyl-4, 6-*O*-ethylidene-α-D-glucosaminide (III).—A two-hundred-mg. portion of III was treated with hydrazine (1.2 ml.) and then treated in a manner similar to that

8) F. Feigle, "Spot Tests in Organic Analysis," Elsevier Publishing Co., Amsterdam (1960), p. 313.

9) S. Seifter, P. M. Gallop, S. Michaels and E. Meuman, *J. Biol. Chem.*, **235**, 2613 (1960).

TABLE III. HYDRAZINOLYSIS OF METHYL *N*-ACETYL-3-*O*-METHYL-β-D-GLUCOSAMINIDE (IIb)

Reagent	Starting compound mg.	Yield of the deacetylation product, %	Recovery of the starting compound			Total recovery %
			mg.	%	m. p.,* °C	
Hydrazine hydrate (90%)	1001.7	8.2	911.5	91.0	186—187	99.2
Anhydrous hydrazine	1000.2	2.5	963.9	96.4	196—198	98.9

* Before recrystallization

TABLF IV. HYDRAZINOLYSIS OF METHYL *N*-ACETYL-4,6-*O*-ETHYLIDENE-α-D-GLUCOSAMINIDE (III)

Reagent	Starting compound mg.	Yield of the deacetylated product, %	Recovery of the starting compound			Total recovery %
			mg.	%	m. p.,* °C	
Hydrazine hydrate (90%)	204.3	99.8 (97.5)**	6.7	3.3	206—210	103.1
Anhydrous hydrazine	203.2	58.7 (60.3)**	85.8	42.2	220—222	100.9

* Before recrystallization
** Determined by an amino acid autoanalyzer

used in the case of IIa, except that a column of Dowex 50×4 cooled with ice-water was used to avoid the splitting of the ethylidene group. The Hitachi amino acid autoanalyzedr was used to determine the yield of the deacetylated product. An aqueous solution (1 ml.) containing about 0.5 μmol. of the deacetylated product, from which hydrazine and hydrazide had been removed as thoroughly as possible by repeated evaporation, was introduced into an amino acid analyzer (0.9×7 cm. column, pH 5.28, 0.2 N citrate buffer). A small peak due to acthydrazide, a low broad peak due to hydrazine, and a main peak of methyl 4,6-*O*-ethylidene-α-D-glucosaminide (VI) were observed. The results are shown in Table IV.

The Periodate Oxidation of Methyl 4,6-*O*-Ethylidene-α-D-Glucosaminide (VI). — One milliliter of a 0.05 M potassium periodate solution was added to 9 ml. of a 10^{-3} M aqueous solution of VI at 25°C in a dark place, and the optical density at 290 mμ was read at several intervals against the reagent blank.[10] The decrease in the optical density at this wave lengh: after 72 hr. was 0.194, which corresponded to the consumption of one mole of periodate per mole of VI.

Discussion

Methyl β-D-glucosaminide (IVb) has also been obtained, by a different method, by Matsushima and Miyazaki,[11] who gave a m. p. of 130—132°C, $[\alpha]_D^{18}$ −40.7°. As has been described above, the compound isolated from the reaction mixture gave identical physical constants. The hydrochloride of IVb had melting point and optical rotation values consistent with those described by Foster, Horton and Stacey,[12] who prepared the compound via methyl *N*-carbobenzyloxy-β-D-glucosaminide and gave a m. p. of 189—190°C, $[\alpha]_D^{20}$ −24°. The infrared spectrum of IVb (Chart 2) shows the existence of the free amino group. Thin-layer chromatography using a moving phase of buthanol: acetic acid: water=4 : 1 : 5 (upper layer) revealed that the reaction mixture contained only two components, Ib and IVb. These findings together with the data in Table I, show that Ib was well deacetylated by the hydrazine treatment and gave IVb as the sole deacetylated product. The glycosidic linkage in Ib was stable under the hydrazine treatment.

While methyl 3-*O*-methyl-D-glucosaminides (V) have not been reported yet, their 4,6-*O*-ethylidene and 4,6-*O*-benzylidene compounds have been prepared by Akiya and Osawa.[7] By the method of these authors, 4,6-*O*-ethylidene compounds of IIa and IIb were prepared and the ethylidene group was split with 60% acetic acid. The resultant amorphous substances (both of which we tried to crystallize, but all in vain), were used as the standard methyl 3-*O*-methyl-D-glucosaminides in the chromatographic examinations. The hydrazinolyzed products of IIa and IIb were thus characterized without isolating them.

Tables II and III show that the greater part of the starting compounds, IIa and IIb, were recovered, the *N*-deacetylation occurring in an unexpectedly low yield.

As has been mentioned above, the hydrazinolyzed product of III was characterized as a *N*-carbobenzyloxy derivative, one with a melting point and optical rotation data consistent with those of an authentic specimen prepared by the method of Akiya and Osawa.[7] A thin-layer chromatogram and its infrared spectra showed that VII was identical with the authentic specimen.[7] The infrared spectrum (Chart 3) and the elemental analyses support the structure of VI. These results and the

10) T. Ikenaka, *J. Biochem.*, **54**, 328 (1963).
11) Y. Matsushima and T. Miyazaki, This Bulletin, **38**, 1325 (1965).
12) A. B. Foster, D. Horton and M. Stacey, *J. Chem. Soc.*, **1957**, 81.

periodate oxidation of VI show definitely that an ethylidene group in III or VI is in the 4, 6-position.

Hydrazine hydrate seems to have a greater power to deacetylate than does anhydrous hydrazine (Tables I—IV). Another trait observed is that the compounds with a free hydroxyl group vicinal to the *N*-acetyl group undergo deacetylation more easily. The results obtained in the case of IIa and IIb are consistent with the fact that the hydrazine treatment of *N*-acetylaminopolysaccharides with a substituent of C-3, such as chondroitin sulfate,[13-15] hyaluronic acid[15] and the cell wall mucopolysaccharide of Micrococcus lysodeikticus,[16] resulted in low yields.

The total recovery (Table I—IV) and the chromatographic examinations show that hydrazine treatment caused little cleavage of glycosidic likages. These findings are consistent with the reported fact that the hydrazine treatment of a simple polysaccharide such as soluble starch and glycogen[17] does not cause any extensive cleavage of glycosidic linkages. As regards the hydrazinolysis of mucopolysaccharides,[13-15] which ended in a considerable degradation of the molecule, some specific interpretations will be required.

13) Y. Matsushima and N. Fujii, This Bulletin, **30**, 48 (1957).
14) M. L. Wolfrom and B. O. Juliano, *J. Am. Chem. Soc.*, **82**, 2588 (1960).
15) Z. Yosizawa and T. Sato, *J. Biochem.*, **51**, 155 (1962).
16) S. Hara Y. Matsushima, in preparation.
17) Z. Yosizawa and T. Sato, *Tohoku J. Exp. Med.*, **76**, 100 (1962).

Summary

Methyl *N*-acetyl-β-D-glucosaminide, methyl *N*-acetyl-3-*O*-methyl-α- and β-D-glucosaminide and methyl *N*-acetyl-4, 6-*O*-ethylidene-α-D-glucosaminide have been deacetylated using anhydrous and hydrated hydrazine. It has been found that the 3-*O*-substitution slowed the deacetylation reaction considerably.

Crystalline methyl β-D-glucosaminide (IVb) and methyl 4, 6-*O*-ethylidene-α-D-glucosaminide (VI) have been newly obtained in the free base form from the corresponding *N*-acetyl compounds.

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