Studies on Amino-hexoses. VIII. The Fischer Synthesis of Methyl D-Glucosaminides

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The Fischer synthesis of methyl glycosides using methanolic hydrogen chloride(1) seems to have failed in the case of glucosamine(2) because the positive charge on the amino-sugar repels the catalyst proton. Some of the known ways of glucosaminide synthesis detour to *N*-benzyloxycarbonyl- or *N*-acetylglucosamine, which do not have a cationic nature. The fission of the benzyloxycarbonyl group by catalytic hydrogenation(2) or by reduction with sodium in liquid ammonia(3) after the Fischer glycosidation regenerates the free amino group. *N*-Deacetylation by hydrazinolysis of methyl *N*-acethylglucosaminide also provides a way to methyl glucosaminide.(4)

As has been mentioned above, the glycosidation reaction of the amino-sugar may be slow under the usual conditions of the Fischer method, but the reaction will be appreciably accelerated when the reactants and the catalyst are brought to a satisfactorily high concentration. As one of way of realizing the idea, the authors treated glucosamine and excess methanol with an excess amount of a strong cation exchanger, which combines glucosamine through the salt linkage and limits the site of the reaction to the surface of the resin only, thus bringing the reactants and the catalyst close to one another. As will be shown in the Experimental section below, D-glucosamine hydrochloride was converted, in a considerably high yield, into a methyl glucosaminide mixture, of the components of which methyl 2-amino-2-deoxy-α- and β-D-glucopyranoside were unambiguously identified after separation by the recently-developed column chromatography using Dowex-1 X 4 free base resin.(5) The probable formation of the two furanosides was inferred from paper chromatographic and other physical data.

Experimental

Glycosidation.—A mixture of 15 g. D-glucosamine hydrochloride, 150 g. of dried Amberlite IR-120 (H^+-form, 200—400 mesh) and 1500 ml. of absolute methanol was refluxed while being mechanically stirred for 10 hr. The reaction mixture was then poured into a glass tube 5.5 cm. in diameter, and the methanol was sucked off. After the resin had been washed with absolute methanol, the glycoside mixture was eluted with methanolic ammonia. The evaporation in vacuo of the eluted solution (1600 ml.) gave 13.1 g. of a brown syrup. It was found by the Somogyi method(6) that the syrup contained reducing sugar, corresponding to 2.3 g. of glucosamine hydrochloride, which had escaped the glycosidation.

The Separation of the Glycoside Mixture by Column Chromatography.—Thirteen grams of the above-mentioned syrup in 30 ml. of water was placed in a Dowex-1 X 4 column (OH^--form, 200—400 mesh) 55 cm. high by 3.7 cm. in diameter and was eluted with water. Each 10 ml. of the fraction was then collected, and the elution was traced by optical rotation measurements. After the elution pattern had been established, the appropriate fractions were combined and evaporated in vacuo, and the specific rotation of each combined fraction, A to I (as shown in Fig. 1), was measured. The recovery of the glycosides was 7.8 g. This corresponded to about 70% of the total non-reducing material obtained. The reducing sugar which escaped the

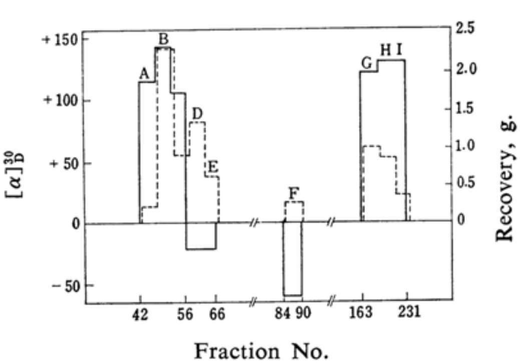

Fig. 1. The separation of the glycosidation products by the column chromatography using Dowex-1 X 4 (OH^--form) resin.
Solid line: specific rotation; broken line: recovery in grams

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

3) A. B. Foster, D. Horton and M. Stacey, ibid., **1957**, 81.

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

5) Y. Matsushima, T. Miyazaki and J. T. Park, *J. Biochem.*, **54**, 109 (1963); P. W. Austin, F. E. Hardy, J. G. Buchanan and J. Baddiley, *J. Chem. Soc.*, **1963**, 5350; Y. Matsushima and T. Miyazaki, *J. Biochem.*, **55**, 464 (1964).

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glycosidation was supposed to have changed to some acidic substance during contact with the strong base resin[7] and to have been retained in the column. Of fractions A to I, only fraction B was obtained as a crystalline material; the others were all a brown syrup.

Methyl 2-Amino-2-deoxy-α-D-glucopyranoside.—The raw crystalline material (2.1 g) obtained from fraction B was recrystallized using absolute ethanol. The colorless crystals obtained weighed 1.6 g. and melted at 155—159°C alone or at 154—159°C on admixture with an authentic specimen[4]. $[\alpha]_D^{30}$ +159.8 (*c* 1.01, water).

Found: C, 43.65; H, 7.92; N, 7.32. Calcd. for $C_7H_{15}O_5N$: C, 43.51; H, 7.82; N, 7.25%.

Methyl 2-Benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside.—A solution of 0.4 g. of methyl 2-amino-2-deoxy-α-D-glucopyranoside in 15 ml. of water was mixed with 0.4 g. of sodium bicarbonate and 0.4 g. of benzyloxycarbonyl chloride. The reaction was continued for 2.5 hr. at room temperature while the mixture was being mechanically stirred. The crystals thus obtained were washed with chloroform. The specimen melted at 158—160°C alone or at 153—158°C on admixture with a sample prepared by the literature method.[2,4] $[\alpha]_D^{30}$ +100.2[8] (*c* 1.04, pyridine)

Found: C, 54.72; H, 6.50; N, 4.25. Calcd. for $C_{16}H_{21}O_7N$: C, 55.04; H, 6.47; N, 4.28%.

Another crop, with essentially the same physical constants, was obtained from the mother solution. The total yield was 0.57 g.

Methyl 2-Amino-2-deoxy-β-D-glucopyranoside.—An absolute ethanol solution of 0.45 g. of a syrup from fraction E gave a crystalline material when left standing for a long time in a refrigerator. The specimen melted at 130—132°C. $[\alpha]_D^{18}$ −40.7 (*c* 1.57, water)

Found: C, 43.31; H, 7.87; N, 7.29. Calcd. for $C_7H_{15}O_5N$: C, 43.51; H, 7.82; N, 7.25%.

The compound, on paper chromatography using two solvent systems, behaved in the exactly same way as the standard specimen[9] which was prepared by the deacetylation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.

Methyl 2-Benzyloxycarbonylamino-β-D-glucopyranoside.—The syrup from fraction D was *N*-benzyloxycarbonylated by a method similar to that used for the corresponding α-isomer, and 1.2 g. of the syrup gave 1.3 g. of a crystalline material which melted at 165—167°C after recrystallization from ethanol. $[\alpha]_D^{30}$ −34.6 (*c* 1.00, pyridine) The melting point and the optical activity data are in good agreement with those in the literature.[2,3]

Found: C, 54.32; H, 6.49; N, 4.24. Calcd. for $C_{16}H_{21}O_7N$: C, 55.04; H, 6.47; N, 4.28%.

Methyl 2-(2′,4′-Dinitrophenyl)amino-2-deoxy-α-D-glucofuranoside.—Ten milliliters of an aqueous solution of 0.3 g. of the syrup from fraction I was mixed with 0.2 g. of sodium carbonate and 0.3 g. of 2,4-dinitrofluorobenzene in 3 ml. of acetone, and then the mixture was shaken overnight at room temperature. The reaction mixture was dried in vacuo, and the crystalline residue was successively washed with water and petroleum ether. Recrystallization from ethanol gave 0.12 g. of yellow crystals melting at 113—167°C. $[\alpha]_D^{27}$ +36.5 (*c* 0.96, acetone)

Found: C, 42.96; H, 4.11; N, 12.44. Calcd. for $C_{13}H_{17}O_9N_3$: C, 43.46; H, 4.78; N, 11.69%.

As the broad range of the melting points shows, the purification of the compound was unsatisfactory. Paper chromatography with *n*-butanol : ethanol : water (4 : 1 : 5) gave two spots, with R_f values of 0.89 and 0.92 respectively. The literature[10] gave the following physical constants: m. p. 166—167°C; $[\alpha]_D^{18}$ +30.6 (*c* 1.0, acetone); R_f 0.88 in the same solvent system.

Methyl 2-Acetamido-2-deoxy-β-D-glucofuranoside.—The syrup (0.22 g.) from fraction F was mixed with 20 ml. of acetic anhydride, 50 ml. of water and Dowex-1 X 4 (OH^--form), and then the mixture was stirred at room temperature overnight. The resin was removed by filtration and washed with water. The filtrate and washing were combined and evaporated in vacuo. The syrup thus obtained was decolorized with a small amount of active charcoal and dried over phosphorus pentoxide. The syrup (0.18 g.) obtained failed to crystallize. $[\alpha]_D^{30}$ −20.9 (*c* 1.24, water). This syrup showed the same R_f value (0.45) in the ascending paper chromatographic system (*n*-butanol : ethanol : water = 4 : 1 : 5) as did the specimen obtained by the literature method.[11]

Summary

The direct synthesis of methyl D-glucosaminides by proton catalysis using a strong cation exchanger has been investigated. The formation of the two anomeric pyranosides has been proved unambiguously. Also, the two anomeric furanosides, which have been obtained in an unsatisfactory purity, have been inferred, according to their physical constants, to have formed in the glycosidation reaction.

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