added to 74.9 g. (0.534 mole) of the phosphonate during a 5-hr. addition period while refluxing. The condenser was attached to a Dry Ice trap, which, after a total of 24 hr. of refluxing had condensed 51.7 g. (1.02 moles) of methyl chloride. Fractionation of the reaction mixture gave acetic anhydride and 26.4 g. (0.13 mole) of a liquid, b.p. 100–101.5° (0.4 mm.), which was identified as dimethyl dimethylpyrophosphonate. The infrared spectrum showed P–CH_3 at 1325 and 900, P–OCH_3 at 1190 and 1050, P–O–P at 960, and P=O at 1265 cm.$^{-1}$.

Anal. Calcd. for $C_4H_{12}O_5P_2$: C, 23.7; H, 5.93. Found: C, 24.2; H, 6.25.

The residue in the distillation flask was a brown tacky immobile material which reacted rapidly with water.

Acknowledgment.—We wish to thank Drs. Joseph Pellon and Richard W. Young for their helpful suggestions and interest.

The Ammonolysis of 1,6-Anhydro-2,4-di-*O*-*p*-tolylsulfonyl-β-D-glucopyranose and the Synthesis of 2,4-Diamino-2,4-dideoxy-D-glucose[1]

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Received April 15, 1963

Ammonolysis of 1,6-anhydro-2,4-di-*O*-*p*-tolylsulfonyl-β-D-glucopyranose afforded one diamino and two monoamino derivatives. The structure of the first product was established as a 2,4-diamino-2,4-dideoxy derivative of D-glucose by its independent synthesis from 2-acetamido-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-*O*-methylsulfonyl-β-D-galactopyranose. One of the two monoamino compounds is presumably a derivative of 4-amino-4-deoxy-D-glucose.

Numerous diamino derivatives of hexoses have been isolated from antibiotics in the last few years. In all these compounds, the amino groups are located at positions C-2 or C-3 and at position C-6 of the carbon chain. The recent isolation by Sharon and Jeanloz,[2] from a polysaccharide of *Bacillus subtilis*, of a diamino hexose in which the two amino groups are probably located at positions C-2 and C-4 of the carbon chain has aroused interest in this type of derivative. The synthesis of 2,4-diamino-2,4-dideoxy-D-glucose was, therefore, investigated.

Numerous studies on the opening of epoxide rings of carbohydrates with ammonia have shown that the transdiaxal conformation was greatly favored when the spacial configuration of the starting material was stabilized by the presence of a 1,6-anhydro or a 4,6-*O*-benzylidene ring. During ammonolysis of 1,6-anhydro-2,4-di-*O*-*p*-tolylsulfonyl-β-D-glucopyranose (II), transdiaxal opening resulted in the preponderant formation of 2,4-diamino-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose isolated as the fully acetylated derivative VII. The formation of this diamino product VII could proceed *via* either monoepoxide intermediate, 1,6;3,4-dianhydro-2-*O*-*p*-tolylsulfonyl-β-D-galactopyranose (I) or 1,6;2,3-dianhydro-4-*O*-*p*-tolylsulfonyl-β-D-mannopyranose (III). The presence of traces of water could result in the hydrolytic splitting of the *p*-tolylsulfonyl groups. Since the newly formed hydroxyl groups would be in *trans* position to the epoxide groups, a second nucleophilic displacement would result in the formation of 1,6;2,3-dianhydro-β-D-gulopyranose (V) from I and 1,6;3,4-dianhydro-β-D-altropyranose (IV) from III, respectively.[3] Formation of the epoxide III seems, however, not to be favored, since reaction of II with sodium methoxide, even for prolonged periods of time, gives a monotosyl–monoepoxide product, which has been shown by Černý and Pacák[4] to be 1,6;3,4-dianhydro-2-*O*-*p*-tolylsulfonyl-β-D-galactopyranose (I). Additional evidence for the stability of I in the presence of alkali is shown by its formation during the reaction of 1,6-anhydro-2,3,4-tri-*O*-*p*-tolylsulfonyl-β-D-glucopyranose with a very large excess of barium hydroxide.[5]

In order to ascertain the *gluco* configuration of the diacetamido derivative VII, 2-acetamido-1,6-anhydro-3-*O*-benzoyl-2-deoxy-4-*O*-methylsulfonyl-β-D-galactopyranose (IX)[6] was treated with sodium azide, and the resulting azido compound VIII was hydrogenated and acetylated to give a compound VII inentical to the one obtained by ammonolysis of II. The displacement by an azide group of a sulfonyloxy group located in a pyranose ring in vicinal position to a *cis* hydroxyl group, with concomittant Walden inversion, has been reported recently.[7–9] Saponification of the 3-*O*-acetyl group gave crystalline VI.

Attempts to obtain 2,4-diamino-2,4-dideoxy-D-glucose as the dihydrochloride derivative by direct hydrolysis of VI were not successful. As had already been observed by Sharon and Jeanloz with the diamino sugar isolated from *Bacillus subtilis*[2] and more recently by Reist, *et al.*,[7] with derivatives of 4-amino-4-deoxy-D-glucose, hydrolysis of 4-amino-4-deoxy sugars results in extensive degradation. The hydrolysis of VI also was accompanied by much degradation and the hydrolyzate gave multiple spots on paper chromatograms. These

(1) Amino Sugars XXXV. This is publication no. 339 of The Robert W. Lovett Memorial Unit for the Study of Crippling Disease, Harvard Medical School at the Massachusetts General Hospital, Boston 14, Mass. This investigation has been supported by research grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service (Grant A-3564). This work was presented before the Division of Carbohydrate Chemistry, at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1962.

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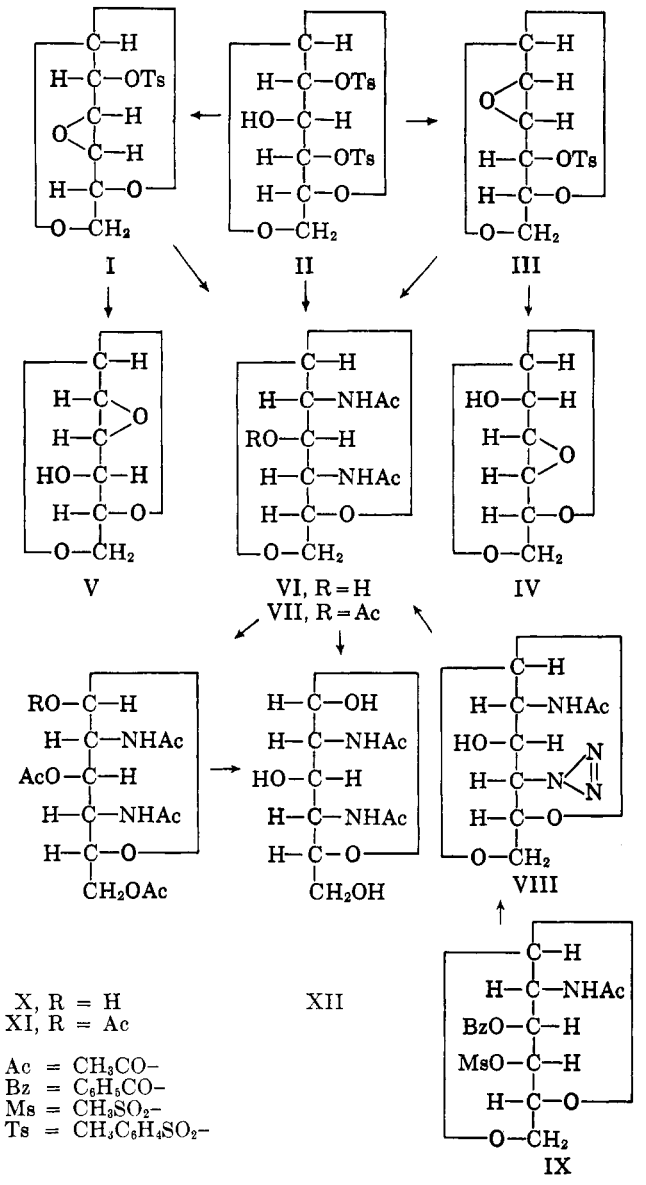
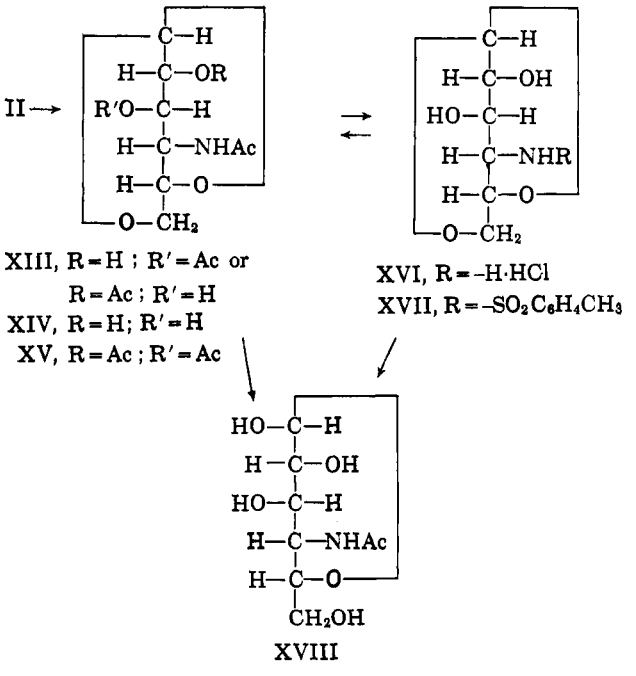


spots correspond to the various possible intermediates resulting from partial deacetylation at positions 2 and 4 with or without opening of the 1,6-anhydro ring.

Opening of the 1,6-anhydro ring of VI was effected by acetolysis. Crystalline mixtures were obtained, but these could not be resolved by crystallization or by chromatography. During purification, partial deacetylation had evidently occurred, giving a 3,6-di-*O*-acetyl derivative X as shown by mutarotation, elemental analysis, and color formation with the Morgan and Elson reagent. Reacetylation of X with acetic anhydride and pyridine gave in very small yield crystalline 2,4-diacetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-β-D-glucose (XI). De-*O*-acetylation of mixtures of X and XI and of their anomers gave the crystalline 2,4-diacetamido-2,4-dideoxy-α-D-glucopyranose (XII). The same product was obtained in 16% yield by direct hydrolysis of VI, followed by *N*-acetylation and chromatographic separation of the resulting mixture of products. Hydrolysis of the diacetamido derivative XII was studied by paper chromatography, and the presence of a monoacetamido–monoamino hydrochloride derivative and of a diamino dihydrochloride derivative could be ascertained. On a preparative scale, however, all attempts to obtain these products in crystalline form failed.

Chromatographic separation of the mixture resulting from the ammonolysis of 1,6-anhydro-2,4-di-*O*-*p*-tolylsulfonyl-β-D-glucopyranose (II) followed by acetylation gave an additional crystalline product, corresponding to a monoamino derivative. From the mode of preparation and from the course of the reaction most likely to take place, it is probable that this monoamino derivative resulted from traces of water, and that it corresponds to the 4-amino-4-deoxy derivative XIII. The very small yield of the compound, and the fact that the presence of a second monoamino derivative could be detected among the products of ammonolysis, raises some doubt about the validity of this structure. Various derivatives were prepared in order to confirm the proposed structure. The crystalline product XIII contained only one *O*-acetyl group at position 2 or 3 and could be de-*O*-acetylated into 4-acetamido-1,6-anhydro-4-deoxy-β-D-glucopyranose (XIV), which in turn could be fully acetylated to give XV. Attempts to hydrolyze XIV gave as the only crystalline product 4-amino-1,6-anhydro-4-deoxy-β-D-glucopyranose hydrochloride (XVI). *N*-Tosylation of this material gave a crystalline product LXVII different from 1,6-anhydro-2-deoxy-2-*p*-tolylsulfamido-β-D-glucopyranose prepared previously by Micheel and Michaelis,[10] thus establishing conclusively that, if the derivative XVI had the *gluco* configuration, the amino group could not be located at C-2. Acetolysis of XIV followed by de-*O*-acetylation gave in a 40% over-all yield the crystalline 4-acetamido-4-deoxy-β-D-glucose (XVIII), which showed no reaction in the Morgan and Elson test, a proof that it was not a 2-acetamido-2-deoxy sugar. Since the physical properties of XVIII or of 1,6-anhydro derivative XIV differ from those of seven of the 3-acetamido-3-deoxyhexoses or of their 1,6-anhydro derivatives[11-16]



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it can be safely assumed that it is a 4-acetamido-4-deoxy sugar and the *gluco* configuration seems to be the most logical one.[17] Attempts to obtain 4-amino-4-deoxy-D-glucose hydrochloride in crystalline form have failed up to now thus rendering impossible a comparison with the product prepared by Reist and associates.[8]

During the purification of XIII a sirupy fraction was isolated, which gave after de-*O*-acetylation a second crystalline 1,6-anhydromonoacetamidomonodeoxy-β-D-hexopyranose. Splitting of the acetamido group gave a crystalline free amino hydrochloride, but all attempts to open the 1,6-anhydro ring or to obtain other crystalline derivatives failed, and no structure can, therefore, be proposed for this product at the present time.

A 4-amino-4-deoxy sugar recently has been isolated by Wheat and associates[18] from *Chromobacterium violaceum*. This is the first time that a monoamino sugar with the amino group at position 4 has been isolated from a natural source, which points to the possible biological importance of this type of compound.

Experimental

Melting points were taken on a hot stage, equipped with a microscope, and correspond to "corrected melting point." Rotations were determined in semimicro or micro (for amounts smaller than 3 mg.) tubes with lengths of 100 or 200 mm., using a Rudolph photoelectric polarimeter attachment, Model 200; the chloroform used was A.R. grade and contained approximately 0.75% of ethanol. Infrared spectra were determined on a Perkin-Elmer spectrophotometer Model 237. Chromatograms were carried out with the flowing method on "Silica Gel Davison," from the Davison Co., Baltimore 3, Md. (grade 950, 60–200 mesh) used without pretreatment. The sequence of eluants was hexane, benzene or chloroform, ether, ethyl acetate, acetone, and methanol individually or in binary mixtures. The proportion of weight of substance to be absorbed to weight of adsorbent was 1 to 50–100. The proportion of weight of substance in grams to volume of fraction of eluent in milliliters was 1 to 100. The ratio of diameter to length of the column was 1 to 20. Evaporations were carried out *in vacuo*, with an outside bath temperature kept below 45°. Amounts of volatile solvent smaller than 20 ml. were evaporated under a stream of dry nitrogen. The microanalyses were done by Dr. M. Manser, Zürich, Switzerland.

Descending paper chromatograms were developed with a mixture of pyridine, ethyl acetate, acetic acid, water, 5:5:1:3,[19] on sheets of Whatman No. 1 and Whatman No. 54 paper, and the compounds were subsequently detected with ninhydrin or with aniline phthalate on no. 1 papers, and with the alkaline silver reagent on no. 54 papers.

Ammonolysis of 1,6-Anhydro-2,4-di-*O*-p-tolylsulfonyl-β-D-glucopyranose (II).—A solution of 5 g. of II[20] in 320 ml. of methanol was saturated with ammonia gas at 0°, in tubes which were sealed, and then heated for 48 hr. at 100°. After cooling, the reaction mixture was evaporated to dryness, and the residue was acetylated for 24 hr. at room temperature with 40 ml. of acetic anhydride and 120 ml. of anhydrous pyridine. The excess of anhydride was destroyed by adding methanol while cooling in ice, and the solution was evaporated, the last traces of pyridine being removed by codistillation with ethanol and toluene. The residue was dissolved in chloroform and the solution was chromatographed on 500 g. of silica gel. Mixtures of various concentrations of chloroform and ethyl acetate, and pure ethyl acetate eluted 1.34 g. of partially crystalline material which, upon de-*O*-acetylation with sodium methoxide, gave sirups from which no crystalline material could be isolated.

Mixtures of ethyl acetate and acetone, 9:1 and 4:1, eluted a partially crystalline material, which was purified by a second chromatography and recrystallized from a mixture of methanol, ethyl acetate, and pentane to give 140 mg. (5%) of **4-acetamido-1,6-anhydro-4-deoxy-2- or 3-*O*-acetyl-β-D-glucopyranose (XIII)**, m.p. 195–205°. After two recrystallizations, thin, rectangular plates, m.p. 203–204°, with sublimation from 190°, were obtained (the melting point varies with the method of crystallization and the speed of heating); $[\alpha]^{23}D$ −95° (*c* 1.24 in methanol).

Anal. Calcd. for $C_{10}H_{15}O_6N$: C, 48.97; H, 6.17; N, 5.71. Found: C, 48.91; H, 6.11; N, 5.72.

During the purification of XIII, a second monoacetamido compound was isolated. The study of this product is described at the end of this paper.

Elution of the original silica gel column with mixtures of ethyl acetate and acetone, 2:1, 3:2, and 1:1, gave 1.66 g. of partially crystalline material which, after two recrystallizations from a mixture of methanol, ethyl acetate, and pentane, gave 0.90 g. (30%) of **2,4-diacetamido-3-*O*-acetyl-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose (VII)** as prismatic needles, m.p. 226–228°, with sublimation about 200°; $[\alpha]^{22}D$ −43° (*c* 1.04 in methanol).[21]

Anal. Calcd. for $C_{12}H_{18}O_6N_2$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.46; H, 6.31; N, 9.84.

Further elution of the silica gel column with pure acetone and with methanol gave 5.87 g. of side products which were not further investigated.

In another experiment where 4 g. of the ditosyl ester was treated as previously described, the yield of XIII was 35% for the crude material and 11% after recrystallization (m.p. 203–206°), whereas 52% of crude VII was obtained, giving 26% of pure material (m.p. 225–227°) after recrystallization.

2,4-Diacetamido-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose (VI) from VII.—To a solution of 200 mg. of VII, in 13 ml. of methanol, cooled at 0°, was added 0.80 mmole (1.14 mole equivalent) of cold sodium methoxide, and the solution was kept at 0° for 3 days. After evaporation of the solvents, the residue was dissolved in 4 ml. of water, and deionized by passage through a short column of Dowex 50 (H^+ form). The effluent was concentrated to give a sirup which crystallized spontaneously. Recrystallization from a mixture of methanol, acetone, and ether gave 164 mg. (96%) of elongated prisms, m.p. 246–248° (with sublimation from 243°); $[\alpha]^{23}D$ −46° (*c* 0.96 in methanol).[21]

Anal. Calcd. for $C_{10}H_{16}O_5N_2$: C, 49.17; H, 6.60. Found: C, 49.17; H, 6.66.

2,4-Diacetamido-3-*O*-acetyl-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose (VII) from IX.—A solution of 270 mg. of 2-acetamido-1,6-anhydro-3-*O*-benzoyl-4-*O*-methylsulfonyl-β-D-galactopyranose (IX)[5,22] and 150 mg. of sodium azide in 25 ml. of dimethylformamide was refluxed for 24 hr. The solution was then evaporated to dryness, and the residue was dissolved in 10 ml. of methanol. To the solution cooled at 0° was added 0.5 ml. of 1.75 *N* barium methoxide. After standing overnight at 0°, the solution was diluted with methanol and filtered through a double layer of Celite and Darco G-60. It was evaporated to dryness and the residue, dissolved in water, was passed through a column of Dowex 50 (H^+ form). After evaporation, the residue weighed 106 mg. and showed in the infrared spectra the typical adsorption at 2120 cm.$^{-1}$ for the azide group. The material was dissolved in 20 ml. of ethanol and the solution was filtered through Darco G-60 and hydrogenated in the presence of 20 mg. of platinum oxide under a slight pressure of hydrogen for 4 to 5 hr. After filtration, the solution was evaporated and the residue was acetylated overnight with 0.5 ml. of anhydrous pyridine and 0.3 ml. of acetic anhydride. The excess of anhydride was decomposed by addition of ice, and the solution was evaporated to dryness. The residue was dissolved in chloroform and chromatographed on silica gel. Elution with pure acetone gave 84 mg. of crystalline material which afforded, after recrystallization from a mixture of methanol, acetone and ether, 70 mg. (35%) of prismatic needles VII, m.p. 228–230°; $[\alpha]^{21}D$ −44° (*c* 0.94 in methanol). The product showed no depression of the melting point in admixture with the product prepared by ammonolysis of II, and the infrared spectra of both samples were identical.

Anal. Calcd. for $C_{12}H_{18}O_6N_2$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.25; H, 6.40; N, 9.54.

(17) The possibility of the formation of the *allo* configuration is very remote.

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(21) The first preparation of this product was carried out in this laboratory by Dr. S. Hakomori.

(22) On large crystals and rapid heating, m.p. 221–222° dec., was observed.

Acetolysis of VI.—A solution of 50 mg. of VI in a mixture containing 3 ml. of acetic anhydride, 0.05 ml. of concentrated sulfuric acid, and 1.95 ml. of glacial acetic acid was kept at room temperature for 5 days, during which time the optical rotation changed from $[\alpha]^{28}D$ −37° (after 8 min.) to $[\alpha]^{28}D$ +125° (after 24 hr.), and $[\alpha]^{23}D$ +117° (after 5 days). The excess of acetic anhydride was decomposed by addition of a small piece of ice, and the reaction mixture was neutralized with 230 mg. of anhydrous barium acetate. The salts were eliminated by filtration through a double layer of Darco G-60 and Celite and, after evaporation of the solvents, the residue was dissolved in chloroform, and the solution was chromatographed on silica gel. Elution with pure ethyl acetate and mixtures of ethyl acetate and acetone, 7:1 and 3:1, eluted 58 mg. of a nearly colorless sirup. Crystallization of this sirup from a mixture of methanol and ether gave 24 mg. of microcrystals, m.p. 183–200°. Both crystals and mother liquor gave two spots on paper chromatography (Whatman No. 54), with $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.28 and $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.51. Complex mutarotations were observed in both cases; with the microcrystals, there was a change from $[\alpha]^{26}D$ +52° (after 12 min.) to $[\alpha]^{26}D$ +71° (after 60 min.) and $[\alpha]^{26}D$ +63° (after 24 hr., *c* 0.91 in methanol), whereas the mother liquor mutarotated from $[\alpha]^{27}D$ +79° (after 6 min.) to $[\alpha]^{27}D$ +85° (after 30 min.) and $[\alpha]^{27}D$ +65° (after 24 hr., *c* 0.74 in methanol).

Acetylation of 12 mg. of impure crystalline material was carried out with 0.3 ml. of acetic anhydride and 3 ml. of anhydrous pyridine in the usual manner to give 18 mg. of a brown sirup which was purified by chromatography on silica gel. Elution with mixtures of ethyl acetate and acetone, 3:1 and 3:2, gave 11 mg. of **2,4-diacetamido-3,6-di-*O*-acetyl-2,4-dideoxyglucose (X)**, a colorless sirup which could not be crystallized, but which gave only one spot on paper chromatography (Whatman No. 54), R_f 0.91, $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.75.

Anal. Calcd. for $C_{14}H_{22}O_8N_2$: C, 48.55; H, 6.40. Found: C, 48.63; H, 6.45.

Acetylation of 21 mg. of the microcrystals obtained initially gave 14 mg. of the same tetraacetate X.

In another acetolysis experiment carried out on 165 mg. of VI, the colorless sirup isolated in the first chromatogram was immediately acetylated with acetic anhydride and pyridine, and care was taken to avoid moisture. This gave 120 mg. of partially crystalline material, which was purified by chromatography on silicic acid. Mixtures of ethyl acetate and acetone (4:1, 3:2, and 1:1) eluted 43 mg. of partially crystalline material which, after crystallization from a mixture of methanol, ethyl acetate, and ether, gave 15 mg. (7%) of **2,4-diacetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-β-D-glucopyranose (XI)**, transparent platelets, m.p. 263–265°; $[\alpha]^{22}D$ −8° (*c* 0.89 in methanol); no mutarotation was observed.

Anal. Calcd. for $C_{16}H_{24}O_9N_2$: C, 49.48; H, 6.23; N, 7.21. Found: C, 49.37; H, 6.24; N, 7.32.

The pentaacetate gave a weak Morgan–Elson reaction which intensified on standing. In paper chromatography, the sugar moved with R_f of 0.89 and 0.94, and $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.74 and 2.1 (on Whatman papers No. 1 and 54, respectively); it reacted very weakly with the alkaline silver reagent and gave a light pink spot with aniline phthalate. Further elution of the initial silica gel column with acetone and with methanol gave 177 mg. of sirup, giving a faintly positive Morgan–Elson reaction, and moving on paper chromatography with $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.06 and 1.35 (Whatman No. 54). This material could not be further purified, and it was de-*O*-acetylated as described in the next paragraph.

2,4-Diacetamido-2,4-dideoxy-α-D-glucopyranose (XII) from X and XI.—De-*O*-acetylation was carried out on a mixture of several more or less acetylated products obtained in the acetolysis experiment previously described; 210 mg. of sirupy material was dissolved in 2 ml. of methanol, and to this cooled solution was added 1.92 mmoles of cold sodium methoxide. The solution was kept at 0° overnight, then evaporated to dryness. The residue was dissolved in 1 ml. of water and deionized by passage through a short column of Dowex 50 (H^+ form). After concentration 126 mg. of sirup was obtained, which was chromatographed on silica gel. A mixture of ethyl acetate and acetone, 1:1, and pure acetone eluted 81 mg. of a sirupy product which, on paper chromatograms, could be detected neither with aniline phthalate nor with the silver reagent, and which is probably a 1,6-anhydro derivative. Further elution of the silica gel column with mixtures of acetone and methanol (19:1, 9:1, and 4:1) gave 50 mg. of sirup which, after two recrystallizations from a mixture of methanol, ethyl acetate, and ether, gave 10 mg. of XII, small prisms, decomposing without melting at about 233° (with partial transformation to needles at 210°). This compound was shown by paper chromatography (R_f 0.53, $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.04, on Whatman No. 1 and 54) to be identical with XII obtained by hydrolysis of VI followed by *N*-acetylation as described subsequently. Compound XII showed mutarotation in water, from $[\alpha]^{24}D$ +88° (after 7 min.) to $[\alpha]^{24}D$ +61° (after 3 hr. and after 24 hr., *c* 1.15).

2,4-Diacetamido-2,4-dideoxy-α-D-glucopyranose (XII) from VI.—A mixture of 82 mg. of VI and 1 ml. of 0.5 *N* hydrochloric acid was heated for 1 hr. at 100°, after which a large amount of absolute ethanol was added, and the solution evaporated, the last traces of acid being removed by codistillation with ethanol and toluene. A dark-colored sirup was obtained (107 mg.) which was shown by paper chromatography (Whatman No. 54) to contain at least 4 components: $R_{2\text{-amino-2-deoxyglucose}}$ 0.61, $R_{2\text{-amino-2-deoxyglucose}}$ 1.00, $R_{2\text{-amino-2-deoxyglucose}}$ 1.39, and $R_{2\text{-amino-2-deoxyglucose}}$ 1.96. No attempt was made to separate these products, and the crude hydrolysate was directly *N*-acetylated, by dissolving it in 2.5 ml. of methanol, and adding 284 mg. of silver acetate and 1.5 ml. of acetic anhydride to the solution. After standing overnight at room temperature, the mixture was refluxed for 5 min. and filtered while hot through a double layer of Celite and Darco G-60, and the salts were successively washed with hot methanol and hot water. To the filtrate was added 6 drops of 2 *N* hydrochloric acid, and, after 2 hr., the silver chloride precipitate was removed by filtration and washed with methanol. The filtrate was evaporated in the presence of toluene and absolute ethanol to give 104 mg. of dark brown sirup. It was chromatographed on silica gel, and pure acetone eluted 19 mg. (23%) of the starting material VI. Pure acetone, and a mixture of acetone and methanol, 19:1, eluted 17 mg. of a sirup showing an R_f 0.73 and an $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.26 on Whatman No. 1 paper; this material had a positive ninhydrin reaction but also gave a faint color in the Morgan–Elson reaction. The sirup could not be crystallized. Further elution of the silicic acid column with mixtures of acetone and methanol (19:1, 9:1, and 4:1) gave 32 mg. (36%) of partially crystalline material, which gave a very faint ninhydrin reaction. In the Morgan–Elson test, it gave a weak color which intensified on standing. Crystallization from a mixture of 95% ethanol, ethyl acetate, and ether gave 14 mg. (16%) of XII, small prisms, decomposing without melting at about 230°. This product had the same mobility on paper chromatograms as that obtained from XI (R_f 0.53, $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.04), and a mutarotation in water from $[\alpha]^{21}D$ +84° (after 30 min.) to $[\alpha]^{22}D$ +69° (after 2.5 hr. and 24 hr., *c* 0.32).

Anal. Calcd. for $C_{10}H_{18}O_6N_2$: C, 45.80; H, 6.92. Found: C, 45.86; H, 6.93.

Hydrolysis of VI.—Examination of the products resulting from the hydrolysis of VI with concentration of hydrochloric acid varying from 0.5 *N* to 3 *N* and time of reaction varying from 30 min. to 24 hr. was made by paper chromatography. It showed, in cases where hydrolyses had been carried out for 30 min. with 2 *N* or 3 *N* acid, and for 30 min., 1 hr., and 3 hr. with 0.5 *N* acid, the presence of five different products. The R_f values and properties of these products were as follows: R_f 0.19 ($R_{2\text{-amino-2-deoxyglucose}}$ 0.66), giving a purple spot with ninhydrin, a brown spot with aniline phthalate, and reducing silver nitrate; R_f 0.22 ($R_{2\text{-amino-2-deoxyglucose}}$ 0.76), blue with ninhydrin and not detected by the other two reagents; R_f 0.29 ($R_{2\text{-amino-2-deoxyglucose}}$ 1.00), purple with ninhydrin, brown with aniline phthalate, and reducing silver nitrate; R_f 0.37 ($R_{2\text{-amino-2-deoxyglucose}}$ 1.28), brownish yellow with ninhydrin, pink with aniline phthalate, and reducing silver nitrate; R_f 0.44 ($R_{2\text{-amino-2-deoxyglucose}}$ 1.52), purple with ninhydrin, but not detected with either aniline phthalate or silver nitrate. In addition, a pink spot of R_f 0.52 ($R_{2\text{-amino-2-deoxyglucose}}$ 2.00, $R_{2\text{-acetamido-2-deoxyglucose}}$ 1.01) was observed with aniline phthalate, but not with ninhydrin; this last named material must, therefore, correspond to the diacetamido glucose XII previously described. The five spots of ninhydrin positive material probably correspond to the various possible combinations of compounds having the 1,6-anhydro ring either present (compounds not reacting with silver nitrate and aniline phthalate) or opened (reducing compounds), and having either or both of the amino groups free or acetylated. All hydrolysates were found to be composed of complex mixtures and, even with strong acid and prolonged hydrolysis, the presumed 1,6-anhydro compounds were always found present.

Hydrolysis of XII.—Samples of XII were hydrolyzed with 0.5 *N* and with 2 *N* hydrochloric acid for 30 min., 2 hr., 6 hr., and 24 hr., and the hydrolysates were examined by chromatography on paper. Paper chromatography on Whatman No. 1 paper showed that the starting material (R_f 0.50 and $R_{2\text{-amino-2-deoxyglucose}}$ 1.82) had completely disappeared in less than 30 min. with 2 *N* acid, and in more than 2 hr. with 0.5 *N* acid. Two spots of ninhydrin positive material were observed in all cases: one with an R_f 0.17 and $R_{2\text{-amino-2-deoxyglucose}}$ 0.68, and a second having R_f 0.28 and $R_{2\text{-amino-2-deoxyglucose}}$ 1.12. The slower moving component seemed to be present in slightly larger amounts, and it was in the 2-hr. and the 6-hr. hydrolysates that the color obtained with ninhydrin was most intense. Since both compounds must be amino sugar hydrochlorides, it was assumed that one of them, probably the faster moving component, was **4-(or 2)-acetamido-2-(or 4)-amino-2,4-dideoxy-D-glucose hydrochloride** and the other was **2,4-di-amino-2,4-dideoxy-D-glucose dihydrochloride.**

Several attempts were made to hydrolyze XII on a preparative scale and to isolate the products, either by crystallization, or by elution from a Dowex-50 column (H^+ form) with 0.3 *N*, 0.5 *N*, and 1 *N* hydrochloric acid, according to the method described by Gardell[23] for the separation of glucosamine and galactosamine. In no case, however, could a good separation be obtained, nor any crystalline material be isolated.

4-Acetamido-1,6-anhydro-4-deoxy-β-D-glucopyranose (XIV).—To a cold solution of 102 mg. of XIII in 2 ml. of absolute methanol was added 1.15 mole equivalent of sodium methoxide. After standing overnight at 0°, the solution was evaporated to dryness and the residue dissolved in water, then deionized by passage through a short column of Dowex 50 (H^+ form). After evaporation, the crystalline residue was recrystallized from a mixture of methanol and ether to give 73 mg. (87%) of small prisms, m.p. 212–213° (with sublimation starting at 190°); $[\alpha]^{27}D$ −118° (*c* 0.73 in methanol).

Anal. Calcd. for $C_8H_{13}O_5N$: C, 47.29; H, 6.45. Found: C, 47.35; H, 6.63.

4-Acetamido-2,3-di-*O*-acetyl-1,6-anhydro-4-deoxy-β-D-glucopyranose (XV).—Acetylation of 24 mg. of XIV with acetic anhydride in pyridine solution in the usual manner gave, after crystallization from a mixture of methanol and ether, 25 mg. (73%) of small prisms, m.p. 181.5–182.5°; $[\alpha]^{27}D$ −62° (*c* 0.83 in methanol).

Anal. Calcd. for $C_{12}H_{17}O_7N$: C, 50.17; H, 5.96; CH_3CO, 44.95. Found: C, 50.10; H, 6.08; CH_3CO, 44.95.

The same product was obtained in 34% yield by acetylation of XIII. On paper chromatography (Whatman No. 54) XV had an R_f of 0.93, and $R_{2\text{ acetamido 2 deoxyglucose}}$ 1.71, giving a weak spot with the alkaline silver reagent.

4-Acetamido-4-deoxy-β-D-glucopyranose (XVIII).—A solution of 90 mg. of XIV in a mixture of 3 ml. of acetic anhydride, 0.05 ml. of concentrated sulfuric acid, and 1.95 ml. of glacial acetic acid was kept for 24 hr. at room temperature, during which time the optical rotation was observed to shift from $[\alpha]^{23}D$ +89° (23 min.) to $[\alpha]^{21}D$ +146° (19 hr. and 24 hr.). The acetolysis mixture was poured onto 10 ml. of crushed ice and then extracted with 200 ml. of chloroform. The organic layer was washed three times with saturated sodium bicarbonate and three times with water, then dried over sodium sulfate. The solution was evaporated to dryness, to give 164 mg. of a pale yellow sirup which could not be crystallized. It was directly de-*O*-acetylated by dissolving it in 2 ml. of absolute methanol, and adding to the solution cooled at 0°, 1.92 mmoles of cold sodium methoxide. The mixture was kept overnight at 0°, then evaporated to dryness, and the residue was dissolved in water and passed through a short column of Dowex 50 (H^+ form). The effluent was concentrated to give 100 mg. of yellow sirup. It was dissolved in a mixture of ethyl acetate and acetone, and the solution was chromatographed on silica gel. Mixtures of acetone and methanol (19:1, 9:1, and 4:1) eluted 97 mg. of partially crystalline material which, after two recrystallizations from a mixture of ethanol and ether, gave 37 mg. (40%) of very fine needles, m.p. 171–174° dec. The compound showed mutarotation from $[\alpha]^{23}D$ +12.5° (after 15 min.) to $[\alpha]^{26}D$ +19° (after 44 hr., at equilibrium) (*c* 0.79 in water).

Anal. Calcd. for $C_8H_{15}O_6N$: C, 43.42; H, 6.84. Found: C, 43.48; H, 6.81.

The product gave no color with the Morgan–Elson reagent. On paper chromatography (Whatman No.1) it migrated with an R_f of 0.44 ($R_{2\text{-amino-2-deoxyglucose}}$ 1.37, $R_{2\text{-acetamido-2-deoxyglucose}}$ 0.90), giving a reddish purple spot with aniline phthalate. When 3-acetamido-3-deoxy-β-D-glucose (*N*-acetylkanosamine) was chromatographed in the same solvent system, it moved with an R_f of 0.49, $R_{2\text{-amino-2-deoxyglucose}}$ 1.45 and $R_{2\text{-acetamido 2-deoxyglucose}}$ 0.95, giving a brownish red color with aniline phthalate.

Hydrolysis of 4-Acetamido-4-deoxy-β-D-glucopyranose (XVIII).—Samples of XVIII and 3-acetamido-3-deoxy-β-D-glucopyranose for comparison were heated with 1 *N* hydrochloric acid in sealed tubes at 100° for 2 hr. In the Elson–Morgan test the hydrolysate of XVIII gave no color, whereas that of *N*-acetylkanosamine gave a positive reaction. On paper chromatography (Whatman No. 1) the two hydrolyzates were found to migrate in the same way: R_f 0.32, $R_{2\text{-amino-2-deoxyglucose}}$ 1.07, $R_{2\text{-acetamido-2-deoxyglucose}}$ 0.64; but the hydrolysate of XVIII (4-amino-4-deoxy-D-glucose hydrochloride) gave a brown color with aniline phthalate, whereas 3-amino-3-deoxy-D-glucose hydrochloride gave a red-brown color.

Hydrolysis of XIV.—A solution of 70 mg. of XIV in 3.5 ml. of 0.5 *N* hydrochloric acid was heated for 15 hr. in a sealed tube at 100°. The brown hydrolyzate was evaporated, and the last traces of acid removed by codistillation with absolute ethanol and toluene. This gave 59 mg. of partially crystalline material which, after three recrystallizations from a mixture of aqueous ethanol and ether, gave 24 mg. (35%) of **4-amino-1,6-anhydro-4-deoxy-β-D-glucopyranose hydrochloride (XVI)**, small prisms decomposing without melting between 200 and 220°. The product had $[\alpha]^{26}D$ −103° (*c* 0.84 in water), and showed no mutarotation.

Anal. Calcd. for $C_6H_{12}O_4NCl$: C, 36.46; H, 6.12; Cl, 17.94. Found: C, 36.33; H, 6.31; Cl, 17.28.

On paper chromatography (Whatman No. 1) XVI gave a spot of R_f 0.37 and $R_{2\text{-amino-2-deoxyglucose}}$ 1.28, which could be detected both by ninhydrin and by the silver reagent (the starting material XIV had an $R_{2\text{-amino-2 deoxyglucose}}$ of 2.40). Both the Elson–Morgan and the Morgan–Elson tests were negative with compound XVI.

In another experiment, hydrolysis of XIV was carried out with 3 *N* hydrochloric acid and heating for 2.5 hr. at 100°. In this case a sirup was obtained which could not be crystallized. Paper chromatography (Whatman No. 1) of this sirup showed three spots of ninhydrin positive material, with the following $R_{2\text{ amino-2-deoxyglucose}}$ values: 0.87, 1.03, and 1.25. The fast moving material was the major component. The product with $R_{2\text{-amino-2-deoxyglucose}}$ 1.25 corresponds to XVI, while that with 1.03 is probably **4-amino-4-deoxy-D-glucose hydrochloride.** Acetylation of 13 mg. of the sirup with acetic anhydride in pyridine, in the usual manner, gave 25 mg. of a sirup from which 13 mg. of crystals, m.p. 160–172°, was obtained. After three recrystallizations from a mixture of methanol and ether, 7 mg. (37%) of **4-acetamido-2,3-di-*O*-acetyl-1,6-anhydro-4-deoxy-β-D-glucopyranose (XV)** was obtained; m.p. 179–181°; $[\alpha]^{23}D$ −62° (*c* 0.84 in methanol). The melting point was not depressed by admixture with the previously described XV.

1,6-Anhydro-4-deoxy-4-*p*-tolylsulfonamido-β-D-glucopyranose XVII.—A solution of 28 mg. of XVI, 27 mg. of crystalline sodium bicarbonate and 27 mg. (1.1 mole equivalent) of *p*-toluenesulfonyl chloride in 0.8 ml. of water was shaken for 6 hr. at room temperature. After addition of 2 ml. of water, crystals began to form. The mixture was left overnight at 0°, then poured into a large volume of chloroform, and the organic layer was washed three times with saturated sodium bicarbonate and three times with water, and finally dried over sodium sulfate. After evaporation of the solvent, 31 mg. (69%) of a sirup was obtained which, by crystallization from a mixture of acetone and ether, gave 17 mg. (38%) of fine needles, m.p. 187–188.5°; $[\alpha]^{22}D$ −52° (*c* 1.1 in methanol).

Anal. Calcd. for $C_{13}H_{17}O_6NS$: C, 49.54; H, 5.43; S, 10.17. Found: C, 49.28; H, 5.44; S, 10.00.

Monoamino-1,6-anhydro-monodeoxy-β-D-hexopyranose.—In the purification of XIII (which see), some sirupy material had been eluted from the second silicic acid column by mixtures of chloroform and ethyl acetate, and by acetone, either alone or mixed with methanol (whereas XIII was eluted by mixtures of ethyl acetate and acetone). This sirup was added to the mother liquors of XIII, to give a total of 1.17 g. of sirup which was dissolved in methanol and de-*O*-acetylated by adding 5.28 mmoles of sodium methoxide, and keeping overnight at 0°. The reaction mixture was deionized by dilution with water and passage through a column of Dowex 50 (H^+ form), and the effluent was concentrated to give 0.92 g. of sirup which was chromatographed on silica gel. Mixtures of ethyl acetate and acetone, 1:1, and pure acetone eluted 297 mg. of partially crystalline material from

(23) S. Gardell, *Acta Chem. Scand.*, **7**, 207 (1953).

which 186 mg. of pure XIV, m.p. 209–211°, was obtained after recrystallization from a mixture of methanol and ether.

Further elution of the silica gel column with pure acetone, and with a mixture of acetone and methanol, 19:1, gave 593 mg. of a pale yellow sirup which could not be crystallized; $[\alpha]^{28}D$ −175° (*c* 1.08 in methanol). The elemental analysis corresponded to that of a **monoacetamido-1,6-anhydro-monodeoxy-β-D-hexopyranose** with one mole of methanol added.

Anal. Calcd. for $C_8H_{13}O_5N \cdot CH_3OH$: C, 45.95; H, 7.28; N, 5.96. Found: C, 46.12; H, 7.38; N, 6.01.

On paper chromatography (Whatman No. 54) this product moved with an $R_{2\text{-amino-2-deoxyglucose}}$ 2.07 and reacted weakly with the alkaline silver reagent.

A solution of 80 mg. of this sirup in 3.5 ml. of 0.5 *N* hydrochloric acid was heated for 15 hr. at 100° in a sealed tube. The solution was then evaporated to dryness, the last traces of acid being removed by codistillation with ethanol and toluene. This residue was crystallized from a mixture of water, ethanol, and ether to give 34 mg. (51%) of elongated prisms, decomposing at 215–225° without melting. This compound had $[\alpha]^{26}D$ −169° (*c* 1.10 in water), and no mutarotation was observed. The elemental analysis corresponded to a **monoamino-1,6-anhydro-monodeoxy-β-D-hexopyranose hydrochloride.**

Anal. Calcd. for $C_6H_{12}O_4NCl$: C, 36.46; H, 6.12; Cl, 17.94. Found: C, 36.34; H, 6.15; Cl, 18.05.

This hydrochloride gave a positive ninhydrin reaction, but no color in either the Elson–Morgan or the Morgan–Elson test. By paper chromatography (Whatman No. 1 and 54) a single spot was obtained with R_f 0.32 and $R_{2\text{ amino-2 deoxyglucose}}$ 1.10.

Several attempts were made to obtain the free sugar by opening the 1,6-anhydro ring by hydrolysis with 2 *N*, 3 *N*, or 6 *N* hydrochloric acid, but this was always accompanied by much decomposition, as evidenced on paper chromatograms by trailing and multiple spots, and no pure material could be obtained. Attempts to obtain other crystalline derivatives by acetylation, tosylation, or acetolysis were also unsuccessful.

1,6;3,4-Dianhydro-2-O-p-tolylsulfonyl-β-D-galactopyranose (I).[21]—A solution of 300 mg. of II[20] in 10 ml. of methanol and 5.8 ml. of a solution of 0.43 *N* sodium methylate (3.7 moles) was refluxed for 24 hr. After concentration to 3 ml., the sirup was diluted with 30 ml. of water and 76 mg. of crystals were filtered. The filtrate was deionized by passage through columns of Amberlite IR 400 (OH^- form) and Dowex 50 (H^+ form) and evaporated to dryness. The residue was crystallized from methanol to give an additional yield of 9 mg. (total yield, 70%). Recrystallization from a mixture of methanol and pentane raised the melting point to 149–150°; $[\alpha]^{28}D$ −37° (*c* 0.56 in chloroform).[24]

Anal. Calcd. for $C_{13}H_{14}O_6S$: C, 52.34; H, 4.73; S, 10.75. Found: C, 52.42; H, 4.80; S, 10.59.

Acknowledgment.—The authors wish to thank Dr. R. B. Baker for providing before publication the experimental conditions for the replacement of a mesyloxy group with an azide group, and Dr. H. H. Baer for a sample of 3-acetamido-3-deoxy-D-glucose.

(24) Černý, Gut, and Pacák[25] reported m.p. 148–150°; $[\alpha]D$ −40° (*c* 1.4 in chloroform); Hann and Richtmyer[5] observed 149–149.5°; $[\alpha]^{20}D$ −41.7° (*c* 1 in chloroform).

(25) M. Černý, V. Gut, and J. Pacák, *Collection Czech. Chem. Commun.*, **26**, 2542 (1961).

The Synthesis of 2-Acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (*N*-Acetylmuramic Acid) and of Benzyl Glycoside Derivatives of 2-Amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (Muramic Acid)[1]

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Received June 6, 1963

The synthesis of various derivatives of the benzyl α-D-glycoside of 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (muramic acid) and of the disaccharide benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside is described. In addition, crystalline 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (*N*-acetylmuramic acid) has been obtained.

In a previous paper,[3] the synthesis of the acetylated methyl α-glycoside of the disaccharide 2-acetamido-2-deoxy-D-glucopyranosyl-(1 → 6)-*N*-acetylmuramic acid has been described. This disaccharide has been postulated as one of the repeating units of the 2-amino-2-deoxyglucan, which constitutes the backbone of the cell wall of numerous Gram-positive and Gram-negative bacteria.[4–6] Since removal of the protective methyl α-glycosidic group cannot be accomplished without considerable degradation of the disaccharide linkage, the synthesis of the disaccharide was repeated using the protective benzyl α-glycoside group, which can be removed by catalytic hydrogenolysis. Starting from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (I),[7] the synthesis proceeded along a route similar to the one described for the methyl α-glycoside derivative.[3] The various crystalline muramic acid derivatives II to VIII were obtained in yields quite similar to those obtained for the methyl α-glycoside derivatives. The condensation of benzyl 2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methyl carboxylate)ethyl] - α - D - glucopyranoside (VI) with 2-acetamido - 3,4,6 - tri - *O* - acetyl - 2 - deoxy - α - D - glucopyranosyl bromide (XI) proceeded, however, in very low yield, and only 3 to 4% of the desired disaccharide (XII) was obtained. Removal of the benzyl glycoside group of the deacetylated product XII by catalytic hydrogenation gave an amorphous disaccharide; its properties, compared to those of the disaccharide isolated from *Micrococcus lysodeikticus* cell wall, will be described in a forthcoming publication.

Removal of the benzyl glycoside group of benzyl 2-acetamido - 3 - *O* - (D - 1 - carboxyethyl) - 2 - deoxy-

(1) Amino Sugars XXXVII. This is publication no. 341 of The Robert W. Lovett Memorial Unit for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston 14, Mass. This investigation has been supported by research grants from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service (Grant E-4282) and the National Science Foundation (Grant 9-2312). It was presented before the Division of Carbohydrate Chemistry at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1962.

(2) On leave of absence from the Weizmann Institute of Science, Rehovoth, Israel.

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