and $[\alpha]_D$ +92° → +84° in water. *Anal.* Calcd. for $C_{10}H_{20}O_6$: OMe, 52.5. Found: OMe, 52.7.

(b) **2,3,6-Tri-*O*-methyl-D-glucose.**—The sirupy component 2 (Table III, 0.979 g.) crystallized when nucleated with 2,3,6-tri-*O*-methyl-D-glucose and had m.p. 117–118° undepressed when mixed with an authentic specimen after recrystallization from ethyl ether, $[\alpha]^{24}D$ +92° → +68.7° in water (*c* 1.1); lit.[16-18] m.p. 122–123° and $[\alpha]_D$ +90.2° → +70.5° in water.

(c) **2,3,4-Tri-*O*-methyl-D-glucose.**—The sirupy component 3 (Table III, 0.045 g.) had $[\alpha]^{24}D$ +61° in methanol (*c* 0.7) and was chromatographically identical with 2,3,4-tri-*O*-methyl-D-glucose when developed either with butanone:water azeotrope or with benzene:ethanol:water:ammonium hydroxide (200:47:14:1). Treatment of component 3 with aniline in ethanol yielded the crystalline anilide of 2,3,4-tri-*O*-methyl-D-glucose, $[\alpha]^{23}D$ −103° in ethanol (*c* 0.4), and m.p. 150°, undepressed by admixture with an authentic specimen; lit.[19] m.p. 145–146°.

(d) **2,4,6-Tri-*O*-methyl-D-glucose.**—The sirupy product (0.065 g.) recovered from the mother liquors of the 2,3,6-tri-*O*-methyl-D-glucose was allowed to react with 0.5% methanolic hydrogen chloride (10 ml.) at room temperature for 20 hr. until the rotation became constant (final value, $[\alpha]^{23}D$ +20°). Neutralization (Ag_2CO_3), filtration and concentration gave a sirupy product which was resolved by paper chromatography using butanone:water azeotrope into two components. The faster moving component consisted of methyl 2,3,6-tri-*O*-methyl-D-glucofuranoside while the slower component (0.020 g.) proved to be 2,4,6-tri-*O*-methyl-D-glucose, $[\alpha]^{24}D$ +64° in methanol (*c* 0.3). Treatment of this slower moving tri-*O*-methyl-D-glucose with ethanolic aniline in the usual way afforded N-phenyl-D-glucopyranosylamine 2,4,6-tri-*O*-methyl ether, m.p. and mixed m.p. 156–158°, lit.[20] m.p. 162–166°.

(e) **2,3-Di-*O*-methyl-D-glucose.**—Component 5 (Table III, 0.101 g.) yielded crystalline 2,3-di-*O*-methyl-D-glucose when nucleated with an authentic specimen. After recrystallization from ethyl acetate it had m.p. and mixed m.p. 117–119°, $[\alpha]^{26}D$ +51.3° in acetone (*c* 0.8); lit.[21] m.p. 110°, $[\alpha]_D$ +50.9° in acetone. Treatment of the 2,3-di-*O*-methyl-D-glucose with aniline in ethanol yielded the crystalline anilide of 2,3-di-*O*-methyl-D-glucose, m.p. 133–135°; lit.[22] m.p. 134°.

(f) **2,6-Di-*O*-methyl-D-glucose.**—The sirupy component 6 (Table III, 0.160 g.) which had $[\alpha]^{23}D$ +65.5° in water (*c* 1.2)[23] was chromatographically identical with 2,6-di-*O*-methyl-D-glucose; lit.[22] $[\alpha]_D$ +63.3° in water. Treatment of the sirup with *p*-phenylazobenzoyl chloride in pyridine gave 2,6-di-*O*-methyl-D-glucose 1,3,4-tri-azobenzoate, m.p. 206–208°, $[\alpha]^{25}D$ −341° (*c* 0.4 in chloroform), after recrystallization from ethyl acetate–light petroleum ether. The mixed melting point with an authentic specimen (m.p. 200°, $[\alpha]^{25}D$ −329° in chloroform) was 205°; lit.[24] m.p. 205–207°.

(g) **3,6-Di-*O*-methyl-D-glucose.**—Component 7 (Table III, 0.052 g.) was chromatographically identical with 3,6-di-*O*-methyl-D-glucose. When a portion (0.017 g.) of it was treated with methanolic hydrogen chloride (0.5%) at 20°, the rotation changed from $[\alpha]_D$ +75° to +22° in 2 hr. indicating furanoside formation and hence the presence of a free hydroxyl group at C_4 in the di-*O*-methyl-D-glucose. Component 7 crystallized completely when nucleated with an authentic specimen[25] of 3,6-di-*O*-methyl-D-glucose. After recrystallization from ethyl acetate, 3,6-di-*O*-methyl-α-D-glucose was obtained, m.p. 118–119°, $[\alpha]^{24}D$ +60° in water (*c* 0.6). Admixture with an authentic specimen (m.p. 111–112°)[25] gave m.p. 111–112°; lit.[26] m.p. 114–115°, $[\alpha]^{18}D$ +61.5° in water.

(h) **2-*O*-Methyl-D-glucose.**—Component 8 (Table III, 0.022 g.) which crystallized spontaneously was chromatographically identical with 2-*O*-methyl-D-glucose. After recrystallization from ethanol the 2-*O*-methyl-β-D-glucose had m.p. and mixed m.p. 156–158°, $[\alpha]^{23}D$ +63° (equilibrium value) in water (*c* 0.8); lit.[27] m.p. 157–159° and $[\alpha]_D$ +66° (equilibrium value) in water.

(i) **3-*O*-Methyl-D-glucose.**—Component 9 (Table III, 0.012 g.) which was chromatographically identical with 3-*O*-methyl-D-glucose, crystallized spontaneously as the β-anomer, m.p. and mixed m.p. 135°, $[\alpha]^{24}D$ +58° (equilibrium value) in water (*c* 0.4); lit.[28,29] m.p. 133.5–135° and $[\alpha]_D$ +55° (equilibrium value) in water.

(j) **6-*O*-Methyl-D-glucose.**—Component 10 (Table III, 0.007 g.), which showed the same R_f and color on paper chromatograms when sprayed with *p*-anisidine–trichloroacetic acid as 6-*O*-methyl-D-glucose, crystallized when nucleated with the authentic specimen. The crystalline 6-*O*-methyl-α-D-glucose had m.p. and mixed m.p. 143–144° and $[\alpha]^{26}D$ +54° (equilibrium value) in ethanol (*c* 1.2); lit.[30,31] m.p. 143–145° and $[\alpha]_D$ +60° (equilibrium value) in water. The osazone prepared in the usual way had m.p. and mixed m.p. 175–180°.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Degradation of Glycogen to Isomaltotriose[1] and Nigerose

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Isomaltotriose and nigerose were found among the oligosaccharides in the acid hydrolyzate of beef liver glycogen, indicating that some of the α-D-(1 → 6) linkages in this molecule lie in adjacent positions and that a small amount of α-D-(1 → 3) linkages are present. Crystalline panose is shown to be dimorphous.

There are five predictable trisaccharides bound by α-D-glucopyranosyl-(1 → 4) or (1 → 6) linkages or combinations thereof. Three of these entities, *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose (panose),[3-5] *O*-α-

(1) Preliminary communication: M. L. Wolfrom and A. Thompson, THIS JOURNAL, **78**, 4182 (1956).
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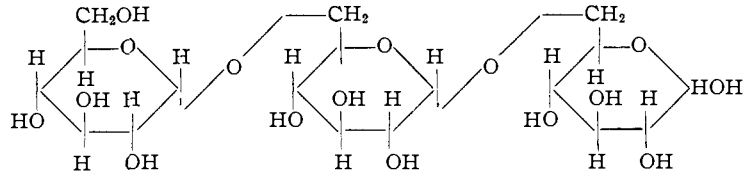
D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose (maltotriose)[6-8] and *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 6)-D-glucopyranose (isomaltotriose, I)[9,10] have been isolated from natural sources and characterized on a crystalline basis. The remaining two trisaccharides, *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 6)-D-glucopyranose and *O*-α-D-glucopyranosyl-(1 → 4)-*O*-[α-D-glucopyranosyl-(1 → 6)]-D-glucose have not been described. All of these substances are of value as reference materials in the identification of polysaccharide linkages by fragmentation analytical methods. Maltose, isomaltose,[11] maltotriose[11] and panose[12] have been described as hydrolytic products of glycogens. We report herein that isomaltotriose and nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) are likewise constituents of the glycogen molecule. Nigerose has been established as a constituent of amylopectin (waxy maize starch)[13] where it is encountered in larger amount than in glycogen. The hydrolytic conditions employed in our work were designed to reduce the formation of the isolated materials by reversion[14] to a negligible amount.

The sugars were isolated from the hydrolyzate of beef liver glycogen by a combination of carbon column chromatography, essentially as described by Whistler and Durso,[15] paper and silicate[16] column chromatography, and paper electrophoresis.[17,18]

The material (amorphous), which was identified as isomaltotriose, was chromatographically homogeneous, moving on paper at a rate slightly slower than panose but on paper electrophoresis moving at a rate much greater. A similar fast moving spot had been noted[12] in a yeast glycogen hydrolyzate but was not further investigated. The isomaltotriose displayed $[\alpha]_D$ +141°, which is in agreement with the reported value of +145°. The substance was further identified by molecular weight determination (on the dodecaacetate of its alditol) and by fragmentation analysis of the free sugar and its alditol. Paper chromatography and paper electrophoresis of a partial acid hydrolyzate of the alditol indicated the presence of only D-glucose, isomaltose, and unchanged material. Silicate chromatography of an acetylated partial hydrolyzate of the alditol (reduced trisaccharide) produced β-D-glucopyranose pentaacetate, D-glucitol (sorbitol) hexaacetate, β-isomaltose octaacetate and isomaltitol nonaacetate. These substances, which were fully identified by melting point and X-ray powder diffraction patterns, characterize the parent trisaccharide as isomaltotriose (I). The presence of this trisaccharide in a hydrolyzate of glycogen indicates that some of the α-D-(1 → 6) linkages occur in adjacent positions in the glycogen molecule.

In the course of this work, maltose, isomaltose, maltotriose and panose were again encountered and crystalline panose was shown to be dimorphous.



Isomaltotriose (I)

Experimental

Hydrolysis of Glycogen.—The glycogen used in this work was beef liver glycogen, $[\alpha]_D$ +198° (*c* 4, water). This material (92 g., dry basis) was hydrolyzed, in three portions, at a concentration of 0.4% in 0.1 *N* hydrochloric acid, by heating in a boiling water-bath. The hydrolysis was followed quantitatively by copper reduction and was stopped after 5.8 hr. at approximately 69% of completion. The hydrochloric acid was removed from the combined hydrolyzate by passage through a column of Duolite A4.[19]

Fractionation of the Glycogen Hydrolyzate on Carbon.—The sugars in the hydrolyzates were subjected to a preliminary separation on a carbon (Nuchar C unground[20]; no filter-aid added) column (800 × 65 mm., diam.) essentially according to the procedure of Whistler and Durso.[15] To prevent the accumulation of a large amount of D-glucose on the carbon, the neutralized hydrolyzate was placed on the same column in three portions, washing with water, after each addition, until the effluent exhibited a negative Benedict test. The water effluent, normally containing only D-glucose, was discarded. The developing solvent for the column was then changed to 5% ethanol and the addition continued (about 20 liters) until the effluent again showed a negative reaction toward Benedict solution. This effluent was evaporated to a sirup under reduced pressure; yield 15 g. (fraction I). The developer was changed to 10% ethanol and the washing continued until the effluent exhibited a negative reaction toward Benedict solution. Solvent was removed under reduced pressure; yield 7.2 g. of a sirup (fraction II).

Chromatography of Fraction I; Maltose, Isomaltose and Nigerose.—The sirupy fraction I (15 g.) was acetylated by heating to the boiling point with 100 ml. of acetic anhydride and 7 g. of anhydrous sodium acetate. The solution was then cooled and poured onto 300 ml. of ice and water. After the excess acetic anhydride was hydrolyzed, the solution was extracted with chloroform and the water-washed and dried extract was evaporated under reduced pressure; yield 25 g. of a sirup. The product was dissolved in ethanol and allowed to crystallize; yield 5.2 g.; after recrystallization from ethanol, m.p. 155–157° unchanged on admixture with known β-maltose octaacetate, $[\alpha]^{20}_D$ +62.7° (*c* 4, chloroform). The mother liquor was concentrated to a sirup; yield approx. 20 g.

The above sirup was dissolved in 200 ml. of benzene and chromatographed in four portions on Magnesol[16]–Celite[18] (5:1) columns (270 × 75 mm., diam.) using 4000 ml. of

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(20) A product of the West Virginia Pulp and Paper Co., Chicago, Ill.

benzene–*t*-butyl alcohol (100:1 by vol.) as developer. The column was extruded and streaked with indicator (1% potassium permanganate in 10% aqueous sodium hydroxide). A zone appearing 145 to 215 mm. from the column top was excised and eluted with acetone. Upon evaporation of the combined acetone solutions from the four columns, a sirup was obtained which was crystallized from ethanol; yield 4.6 g., m.p. 145–146° unchanged upon admixture with an authentic sample of β-isomaltose octaacetate, $[\alpha]^{20}D$ +96° (*c* 4, chloroform). These constants are in agreement with those of β-isomaltose octaacetate.[21]

The material in a zone appearing at 90 to 125 mm. from the column top was eluted with acetone. After evaporation, the resulting sirup (2.95 g.) was rechromatographed, in the same manner, on a Magnesol[16]–Celite[16] (5:1) column (270 × 75 mm., diam.) with 4000 ml. of benzene–*t*-butyl alcohol (100:1 by vol.) as developer. The material obtained on solvent removal from the acetone eluate of a zone appearing 90 to 125 mm. from the column top yielded a small amount of a gel from ethanol. The gel was isolated by decantation and from its ethanol solution a small amount of crystalline material separated; yield 2 mg., X-ray diffraction identical with that recorded[13,14] for β-nigerose (3-*O*-α-D-glucopyranosyl-β-D-glucopyranose) octaacetate.

Chromatography of Fraction II.—The material in fraction II (7.2 g.) was dissolved in 100-mg. portions, in a few drops of water and applied evenly to a Whatman 3MM filter paper (47 × 56.5 cm.) on a line 12.5 cm. from one end. The chromatogram was developed with 1-butanol–pyridine–water (3:2:1.5 by vol.) for 40 hr. A pilot strip, cut from the edge of the paper, was sprayed with aniline phthalate (1.66 g. of phthalic acid, 0.93 g. of aniline in 100 ml. of 1-butanol saturated with water) and heated for a few minutes in an oven at 105° to indicate the positions of the zones on the paper. Two main zones, A and B, appeared with several smaller zones. These two main zones, which moved at rates approximating those of maltotriose and panose as determined by comparison with the known sugars, were cut out of the papers. A total of 72 papers were prepared in this manner and the respective zones were combined. The sugars were recovered by pulping the paper in water, filtering and washing with water. The combined filtrates and washings were evaporated under reduced pressure to sirups; yield 2.95 g. from zone A and 1.58 g. from zone B.

Panose and its Dimorphism.—The slower moving zone A material above (2.95 g.) crystallized from methanol on being maintained at 60° overnight; yield 1.2 g. Pure material (form 2) was obtained on recrystallization from methanol; yield 1.05 g., m.p. 220–221° dec., unchanged on admixture with an authentic specimen of panose (form 1) received from Dr. S. C. Pan (m.p. 220°), $[\alpha]^{25}D$ +160.4 → 150.6° (140 min., *c* 3.8, water); X-ray powder diffraction data of our isolated crystals (form 2): 6.88[22] vw,[23] 5.96w 5.38m, 5.11w, 4.84vs, 4.25m, 4.03vw, 3.90vw, 3.81w, 3.58w, 3.29w, 3.19w, 3.01m, 2.76vw, 2.71vw, 2.60 vw. These data are identical with those obtained on a sample of panose isolated from potato starch and submitted to us by Dr W. J. Whelan.

Anal. Calcd. for $C_{18}H_{32}O_{16}$: C, 42.85; H, 6.39. Found: C, 42.76; H, 6.44; sample dried before analysis 2 hr. at 78° and 1 mm. pressure over phosphoric anhydride, 0.55% loss, no loss on further heating for 4 hr. at 110°, X-ray powder diffraction pattern unchanged.

The above data on crystalline form 2 are in agreement with those for the sample of panose prepared by Dr. Pan with the exception of the X-ray powder diffraction data[22,23] which, for this modification (form 1) are: 9.51vw, 8.29vw, 6.09vw, 5.81vw, 5.48m, 5.18w, 4.87vs, 4.69w, 4.43vw, 4.27vw, 4.16vs, 4.01vw, 3.76w, 3.64vw, 3.49vw, 3.23m, 3.05w, 2.89vw, 2.73w. A sample of form 1 was heated over phosphoric anhydride for 2 hr. at 78° and 1 mm. and lost 0.26% with no further loss after 2 hr. at 110°.

Our infrared absorption spectrum for form 1 (Nujol mull) agrees with that published by Whiffen[24] in the range of 700 to 900 cm.$^{-1}$, but is slightly different from the spectrum (Nujol mull) of the new modification (form 2) in this range as well as in the range 1100 to 1400 cm.$^{-1}$.

Sirupy panitol (50 mg.), which had been prepared by reducing form 2 of panose with Raney nickel catalyst and 2000 p.s.i. of hydrogen at 80°, was acetylated with 5 ml. of acetic anhydride and 50 mg. of sodium acetate at the boiling point and the product was isolated as described above and crystallized from ethanol; yield 70 mg., m.p. 145–147° with no depression on admixture with a sample of known panitol dodecaacetate prepared from form 1; X-ray powder diffraction data[22,23]: 12.11vs, 10.43vw, 9.36vw, 8.63vs, 7.80w, 7.03w, 6.41vw, 6.07vw, 5.72vw, 5.29vw, 4.96vw, 4.62s, 4.44s, 4.14vw, 4.03vw 3.89vw, 3.76w, 3.62w, 3.50w, 3.39m, identical with those of a sample of panitol dodecaacetate[4,25] prepared from a sample of panose (form 1) submitted to us by Dr. Pan.

An amount of 50 mg. of panose (form 1) was acetylated with hot acetic anhydride and sodium acetate in the manner described above for the disaccharide portion of the hydrolyzate. There resulted a sirupy acetate which was deacetylated by dissolving in 0.5 ml. of methanol and adding a drop of a solution of sodium methoxide (0.5 g. of sodium dissolved in 100 ml. of methanol). The sodium was removed by shaking with a small amount of Amberlite 120.[26] The sirup resulting on solvent removal was crystallized from boiling methanol; yield 25 mg., X-ray powder diffraction data identical with those of form 2.

The crystalline modification of panose isolated in this work (form 2) was recrystallized five times by dissolving in a small amount of water, filtering with carbon, removing the water by evaporation under reduced pressure and refluxing with methanol. The resulting material then gave an X-ray powder diffraction pattern identical with that of form 1.

These data indicate that the two crystalline forms are dimorphous and have indistinguishable melting points (decomposition points).

Isomaltotriose.—The mother liquors from the crystallization of panose were combined and evaporated under reduced pressure to an amorphous product; yield 1.79 g. This material was separated by means of paper zone electrophoresis with an apparatus similar to that described by Foster.[17] Whatman 3MM paper (14 × 57 cm.) was used and the material was applied in 75-mg. portions uniformly on lines 70 mm. and 225 mm. from the ground end of the plates. Three such papers were wet with borate buffer (*p*H 10) and clamped, one above the other, between the glass plates. In this way a total of 150 mg. of material could be separated in each run. A current of 400 volts and 45 milliamp. was applied for 4 hr. After the papers were removed from the apparatus, a pilot strip was removed from one edge and the zones were located therein by means of aniline phthalate indicator[17] and excised from the paper. Only two zones appeared. The faster moving zone had a M_G[17] value of 0.71 as compared with those of isomaltose 0.67,[17,27] maltose 0.37,[17,27] panose 0.33 and maltotriose 0.33. After 1.79 g. of amorphous material had all been subjected to this treatment, the papers holding the fast moving zone were pulped by stirring in water and the resultant suspension was filtered and washed with water. The filtrate and washings were combined and the sodium ion was removed by stirring the solution with Amberlite 120[26] until neutral. The solution was then evaporated to dryness under reduced pressure. The boric acid residue was removed as the volatile methyl ester by repeated distillation with methanol. The residue was dissolved in methanol, filtered with carbon and evaporated under reduced pressure to dryness; yield 0.67 g., $[\alpha]^{20}D$ +141°, a value comparable with that (+145°) found by Jeanes and associates[9] for isomaltotriose. A sample (50 mg.) of this material was dissolved in 2.5 ml. of 0.05 *N* hydrochloric acid and heated on a boiling water-bath for 5 hr. The acid was removed by shaking with a small amount of Duolite A4[19] ion exchange resin and the solution evaporated to dryness under reduced pressure. Paper chromatography, using 1-butanol, pyridine, water (3:2:1.5 by vol.) as developer, and electrophoresis, using a

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(22) Interplanar spacing, Å., CuK_α radiation.

(23) Intensity of lines, estimated visually: vs, very strong; s, strong; m, medium; w, weak; vw, very weak.

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borate buffer (*p*H 10), indicated the presence of only D-glucose, isomaltose and unhydrolyzed material.

The amorphous unhydrolyzed product (250 mg.) was dissolved in 30 ml. of water and reduced with hydrogen at 2000 p.s.i. and 80° with Raney nickel catalyst. The solution was filtered and evaporated to a sirup under reduced pressure; yield 200 mg., $[\alpha]^{20}D$ +114° (*c* 2.5, water). A 50-mg. sample of this material was acetylated with 3 ml. of hot acetic anhydride and 25 mg. of sodium acetate as described above; yield 60 mg. of sirup.

Anal. Calcd. for $C_{18}H_{22}O_6(CH_3CO)_{12}$: mol. wt., 1010.9. Found: mol. wt., 1018 (Rast).

Fragmentation Analysis of the Reduced Trisaccharide (Isomaltotriitol).—An amount of 134 mg. of the reduced and unacetylated product described above was dissolved in 10 ml. of 0.05 *N* sulfuric acid and heated on a boiling water bath for 7 hr. The excess acid was removed by shaking with a slight excess of Duolite A4[19] and filtering. The solution was evaporated under reduced pressure to a sirup which was acetylated with hot acetic anhydride (5 ml.) and sodium acetate (75 mg.) as described above. The resulting sirup was chromatographed on a column (235 × 35 mm., diam.) of Magnesol–Celite (5:1 by wt.) using 1000 ml. of benzene–*t*-butyl alcohol (100:1 by vol.) as developer. Three zones were located on the extruded column by streaking with the alkaline permanganate indicator. These were excised and eluted with acetone. The sirups obtained on solvent removal from the acetone eluates and from the column effluent were crystallized from ethanol. That from a zone located 25–40 mm. from the column top was identified as isomaltitol nonaacetate[28]; yield 3.8 mg. The material was crystallized from ethanol; m.p. 111–113°; X-ray powder diffraction pattern,[22,23] identical with that of authentic material: 10.98vs, 9.31s, 8.35w, 7.22vw, 6.86m, 6.39vw, 5.75vw, 5.14m, 4.72m, 4.43vw, 4.09s, 3.81w, 3.60vw. The product obtained from the zone 55–85 mm. from the column top was identified as β-isomaltose octaacetate[21]; yield 0.5 mg., m.p. 142–144°, X-ray powder diffraction data[14] identical with that of the authentic substance. The crystalline material found in the third zone located 240–290 mm. from the column top was identified as D-glucitol (sorbitol) hexaacetate; yield 0.8 mg., m.p. 95–96°; X-ray powder diffraction pattern,[22,23] identical with that of an authentic sample: 10.34w,, 8.43w, 7.58w, 7.02s, 6.56vw, 6.35vw, 5.96vw, 5.62vw, 5.05vs, 4.69w, 4.37w, 4.20vw, 4.01w, 3.89vw, 3.74m, 3.43s, 3.27vw, 3.18vw, 2.95m, 2.80vw, 2.72vw, 2.65w. The crystalline material from the column effluent was identified as β-D-glucopyranose pentaacetate; yield 25 mg., m.p. (on recrystallization from ethanol) 126–128° unchanged on admixture with an authentic specimen.

β-Maltotriose Hendecaacetate.—The material (1.58 g.) of the faster moving zone B from the paper chromatography of fraction II was placed upon paper and separated by electrophoresis, using a sodium borate buffer at *p*H 10, by the procedure described above for the separation of isomaltotriose. There resulted two zones, the slower of which had an M_G value of approximately 0.33 or that of maltotriose. The material in this zone was isolated; yield 950 mg. Acetylation of this product with hot acetic anhydride and sodium acetate was effected as described above for the acetylation of fraction I. This material failed to crystallize from ethanol and was chromatographed on a column (210 × 42, diam.) of Magnesol–Celite (5:1 by wt.) by development with 1000 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). Two zones were located on the extruded column by streaking with the alkaline permanganate indicator. The amorphous material (180 mg.), obtained from the acetone eluate of a zone located 25–50 mm. from the column top, was not further investigated. The material obtained on solvent removal from the acetone eluate of a zone located 25–50 mm. from the column top, was crystallized from ethanol; yield 120 mg., m.p. 134–136° unchanged on admixture with authentic β-maltotriose hendecaacetate.[6,7]

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[CONTRIBUTION FROM THE ROBERT W. LOVETT MEMORIAL LABORATORIES FOR THE STUDY OF CRIPPLING DISEASES, MASSACHUSETTS GENERAL HOSPITAL, AND THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

The Synthesis of D-Gulosamine Hydrochloride[1a,b]

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D-Gulosamine hydrochloride has been prepared in crystalline form from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside and has been characterized through the following crystalline derivatives: *N*-(2′-hydroxynaphthylidene), methyl *N*-acetyl-α-D-glycoside and methyl *N*-acetyl-3,4,6-tri-*O*-acetyl-α-D-glycoside.

The isolation of a new aminosugar from streptothricin and streptolin B has been reported recently, and the structure of a 2-amino hexose, D-gulosamine (VII), has been proposed for it.[2] This appears to be the first isolation of a 2-amino hexose from natural sources since D-galactosamine was extracted from cartilage 40 years ago and the well known D-glucosamine from chitin nearly a century ago. It seems also to be the first isolation of a natural sugar with the D-gulose configuration.

Confirmation of the structure proposed by van Tamelen, *et al.*, by an unequivocal synthetic procedure seemed of interest. The preparation of D-allosamine from D-glucosamine has been accomplished in our laboratory.[3] In the present study a similar reaction sequence was applied to D-galactosamine and methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (I)[4] was transformed into the 3-*O*-methylsulfonyl derivative II. Reaction with sodium acetate in methyl cellosolve[5] failed, however, to proceed in a similar way as described for the glucosamine derivative[3] and the starting material was recovered unchanged. This failure was unexpected, as the benzylidene derivative of galactosamine II possesses the general conformation of a *cis*-decalin, much more flexible than the *trans*-decalin confor-

(1) (a) Studies on hyaluronic acid and related substances XVII. This is publication No. 211 of the Robert W. Lovett Memorial Laboratories for the Study of Crippling Diseases, Department of Medicine, Harvard Medical School, Boston, and the Massachusetts General Hospital. This investigation has been supported by a research grant from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service (Grant A-148-C3). Presented before the Division of Carbohydrate Chemistry at the 131st Meeting of the American Chemical Society, Miami, Florida, April, 1957. (b) A preliminary note has been published, THIS JOURNAL, **79**, 2660 (1957).

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(5) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, *ibid.*, **76**, 4044 (1954).