glucose, anthraquinone and crotonyl chloride. Acetylacetone evidently suffered cleavage prior to or during the reduction; isopropyl alcohol was isolated in 63% yield.

Solutions of benzene diazonium chloride exhibited a rapid and strongly exothermic reaction with sodium borohydride. Benzene, aniline and phenylhydrazine were isolated in relatively small yields. The course of the reaction was qualitatively similar in neutral and in strongly alkaline solutions.

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Summary

Sodium borohydride, in water or methanol solution, is an effective reagent for the conversion of aldehydes and ketones to the corresponding alcohols. Its properties are compared with those of lithium aluminum hydride and it is shown to be superior in selective reductions. Acid chlorides are reduced to primary alcohols in non-aqueous media, but carboxylic acids, anhydrides, esters and nitriles are practically unaffected.

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

Crystalline Derivatives of Isomaltose[1,2]

BY M. L. WOLFROM, L. W. GEORGES[3] AND I. L. MILLER[4]

In the course of our studies on the structure of starch it became desirable to have a reference compound made up of two D-glucose residues combined by a 6-α-D-glucosyl linkage. Musculus,[5] Grimaux and Lefèvre[6] and Gautier[7] had prepared weakly reducing non-fermentable sirupy disaccharides by condensing D-glucose in the presence of acids. E. Fischer[8] in attempts to synthesize maltose, obtained a similar material which he called "isomaltose" and characterized as its phenylosazone (m. p. 158°). Georg and Pictet[9] prepared isomaltose according to the procedure of Fischer as modified by Friedrichs[10] and obtained a sirup which produced an amorphous acetate upon hot acetylation with acetic anhydride and sodium acetate. By means of a fractional precipitation procedure from ethanol the crude acetate was separated into three fractions: octaacetylgentiobiose, m. p. 190–195°; β-octaacetylisomaltose, amorphous, fusing at 72–77° and showing a rotation of $[\alpha]^{20}D$ +93.7° (c 4.8); a small amount of a third fraction, amorphous and fusing at 115–123°. The β-octaacetylisomaltose was converted into amorphous α-octaacetylisomaltose by heating with acetic anhydride and zinc chloride, $[\alpha]^{19}D$ +115.5° (c 4.2). The free sugar exhibited a rotation of $[\alpha]^{23}D$ +104.6° (ten minutes), +99.7° (twenty-four hours) (c 5.1, water), and produced a phenylosazone which melted at 160° and had a rotatory value of $[\alpha]^{23}D$ +23.1° (c 1.2, methanol). Upon methylation of the free sugar with subsequent hydrolysis, Georg[11] obtained 2,3,-4-trimethyl-D-glucose and 2,3,4,6-tetramethyl-D-glucose indicating a 6-D-glucopyranosyl structure. Myrbäck[12] prepared "isomaltose" as a reversion product of D-glucose by the action of hydrochloric acid and by methylation studies confirmed Georg's observation that it is 6-α-D-glucosyl-D-glucose. Berlin[13] has been able to isolate gentiobiose octaacetate from the "isomaltose" of Fischer. He believes that this preparation is a gross mixture containing gentiobiose. His observation is supported by Isaiev.[14] By acid hydrolysis and by Taka diastase treatment, Ahlborg and Myrbäck[15] obtained from corn starch a sirupy disaccharide which on methylation and hydrolysis yielded 2,3,4,6-tetramethyl-D-glucose and 2,3,4-trimethyl-D-glucose. It was thus established as an "isomaltose" derivative. Following the publication of our preliminary notice,[1] Montgomery, Weakley and Hilbert[16] hydrolyzed amylopectin (waxy corn starch) with a Taka diastase type of enzyme and isolated a disaccharide characterized by a crystalline octa-p-nitrobenzoate and by two isomeric crystalline octaacetates. One of the latter was shown to be identical with the product described herein by a direct comparison involving mixed melting point and comparative X-ray powder diffraction diagrams.

For a source of this disaccharide we turned to the synthetic polysaccharide dextran which is made from sucrose by the action of *Leuconstoc dextranicum*. Methylation studies[17,18] have shown that

(1) A preliminary communication by the present authors describing the crystalline octaacetate of 6-α-D-glucosyl-β-D-glucose appeared in THIS JOURNAL, **69**, 473 (1947).

(2) In the present communication we will employ provisionally the term isomaltose to designate 6-α-D-glucopyranosyl-D-glucose.

(3) Corn Industries Research Foundation Associate of The Ohio State University Research Foundation (Project 203).

(4) Corn Industries Research Foundation Fellow of The Ohio State University Research Foundation (Project 203).

(5) Musculus, *Bull. soc. chim. France*, [2] **18**, 66 (1872).

(6) E. Grimaux and L. Lefèvre, *Compt. rend.*, **103**, 146 (1886).

(7) A. Gautier, *Bull. soc. chim. France*, [2] **22**, 145 (1874).

(8) E. Fischer, *Ber.*, **23**, 3687 (1890); **28**, 3024 (1895).

(9) A. Georg and A. Pictet, *Helv. Chim. Acta*, **9**, 612 (1926).

(10) O. v. Friedrichs, *Arkiv Kemi Mineral. Geol.*, **5**, No. 4, 1 (1913); *Chem. Centr.*, **85**, I, 763 (1914).

(11) A. Georg, *Compt. rend. soc. phys. hist. nat. Genève*, **47**, 94 (1930).

(12) K. Myrbäck, *Svensk Kem. Tid.*, **53**, 67 (1941); **53**, 264 (1941).

(13) H. Berlin, THIS JOURNAL, **48**, 1107 (1926).

(14) B. J. Isaiev, *Chem. Listy*, **20**, 251 (1926); *C. A.*, **20**, 3159 (1926).

(15) K. Ahlborg and K. Myrbäck, *Biochem. Z.*, **308**, 187 (1941).

(16) Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, THIS JOURNAL, **69**, 2249 (1947).

(17) E. C. Fairhead, M. J. Hunter and H. Hibbert, *Can. J. Research*, **B16**, 151 (1938).

(18) S. Peat, E. Schlüchterer and M. Stacey, *J. Chem. Soc.*, 581 (1939).

this polysaccharide is bound at least to the extent of 90% by 6-α-D-glucopyranosyl linkages.

In this work we report the preparation of a disaccharide, isolated as its crystalline β-D-octaacetate (Fig. 1), by controlled acid hydrolysis of dextran under conditions which do not favor the production of reversion products. The isolation was effected by application of the acetate chromatographic techniques established in this Laboratory.[19] Molecular weight and acetyl determinations together with elementary analyses showed the acetate to be a disaccharide.

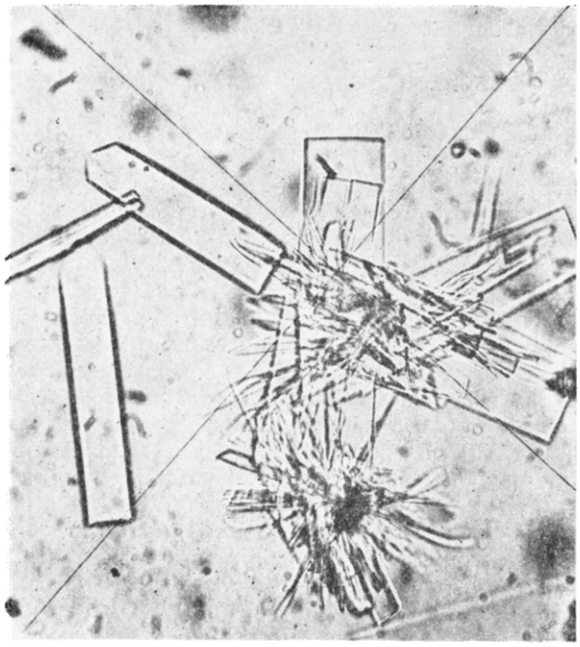

Fig. 1.—β-D-Isomaltose octaacetate.

The determined rotation of the disaccharide octaacetate is in close agreement with that of the amorphous acetate of Georg and Pictet[9] and with that predictable for a 6-α-D-glucopyranosyl disaccharide linkage by application of Hudson's rules of isorotation. The value may be calculated from the rotations of three known octaacetates containing 6-D-glycosyl linkages as follows

Gl + L + Gal	= 69,560	(6-α-D-galactosyl-β-D-glucose octaacetate) (β-melibiose octaacetate)
−(Gl − L + Gal)	= 0	(6-β-D-galactosyl-β-D-glucose octaacetate)
2L	= 69,560	
L	= 34,780	
Gl − L + Gl′	= −3,590	(6-β-D-glucosyl-β-D-glucose octaacetate) (β-gentiobiose octaacetate)
+ L	= 34,780	
Gl + Gl′	= 31,190	

(19) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

$$Gl + L + Gl' = 31,190 + 34,780 = 65,970$$

= (6-α-D-glucosyl-β-D-glucose octaacetate) (β-isomaltose octaacetate)

$$[\alpha]_D = \frac{65,970}{678.6} = +97°$$

in which L, Gl, Gl′ and Gal represent portions of the molecular rotation due to the glycosyl carbon atom binding the disaccharide, the two acetylated pyranoid D-glucose entities and the pyranoid D-galactose entity, respectively.

Deacetylation of this crystalline acetate yielded the free reducing disaccharide in amorphous form with a specific rotation of $[\alpha]^{24}D$ +103.2° (water) in good agreement with the value of 104.6° cited by Georg and Pictet.[9] The disaccharide was not fermentable by yeast.

The crystalline disaccharide octaacetate was converted to the crystalline 6-α-D-glucopyranosyl-α-D-glucopyranosyl bromide heptaacetate and this in turn was transformed into the crystalline methyl 6-α-D-glucopyranosyl-β-D-glucopyranoside heptaacetate. Deacetylation of the latter compound yielded an amorphous glycoside which on periodate oxidation produced the data shown in Table I, which table also cites comparative oxidation data with the corresponding glycosides of the β-1,4-linked cellobiose and the β-1,6-linked gentiobiose. These data definitely establish the new glycoside as a methyl 6-α-D-glucopyranosyl-β-D-glucopyranoside. The ring structure in the nonreducing portion of the disaccharide and in both the D-glucose entities in the glycoside, octaacetate, and acetohalogen compound can be considered as established.

TABLE I

PERIODATE OXIDATION AT 25° OF THE METHYL GLYCOSIDES OF β-D-FORMS OF CELLOBIOSE, GENTIOBIOSE AND ISOMALTOSE

Substance	Time, hr.	Oxidant consumed[a]	Formic acid formed
Methyl β-D-cellobiopyranoside	8	3.01	
	10	3.02	1.05
Methyl β-D-gentiobiopyranoside	3	4.06	
	4.5	4.08	1.95
Methyl 6-α-D-glucopyranosyl-β-D-glucopyranoside (Methyl β-D-isomaltoside)[b]	3	4.02	
	4	4.04	1.91

[a] Eighteen:one (moles periodate:glycoside). [b] Prepared through its crystalline acetate and chromatographically homogeneous.

Experimental

6-α-D-Glucopyranosyl-β-D-glucopyranose Octaacetate.—An amount of dextran (100 g.), prepared by the action of *Leuconostoc dextranicum* on sucrose[19] (rotation $[\alpha]_D$ +180° (*c* 1, water)), which is in agreement with the value reported by Peat[18]) was dissolved by shaking in 3 liters of 30% hydrochloric acid. The mixture was held at 24–26° and polarimetric readings were taken at intervals. When the rotation had dropped to $[\alpha]_D$ +105°, which required about twelve hours, the solution was poured into 15 liters of water, and the acid neutralized with basic lead carbonate. The precipitate was filtered and washed with

500 ml. of water. The lead ion was precipitated from the combined filtrate and washings with hydrogen sulfide and after filtration the solution was neutralized to a *p*H of 7.0 by the addition of sodium bicarbonate. The solution was concentrated to about 700 ml. under reduced pressure at 50°. The remainder of the ionic material was removed by passing the solution successively over Amberlite[20] resins IR-100 and IR-4. The solution and washings were concentrated to a sirup at 50° and this was dried to an amorphous powder by azeotropic distillation with absolute ethanol under reduced pressure.

Twenty grams of the amorphous hydrolyzate, 20 g. of freshly fused sodium acetate and 200 ml. of acetic anhydride were placed in a liter flask fitted with a reflux condenser and heated gradually by means of an oil-bath to 110–120° and held there until completion of the vigorous reaction. The reaction mixture was then cooled and poured into 2 liters of ice and water. When the oily layer had hardened to an amorphous solid, it was filtered and washed free of acetic acid with water; yield *ca.* 25 g.

Five grams of the above-described amorphous acetylated hydrolyzate of dextran was dissolved in 100 ml. of benzene and placed on a column of Magnesol[21]–Celite[22] (5:1 by wt.) (240 × 80 mm. in diam.[23]) and developed with 3000 ml. of benzene–ethanol (100:1 by vol.). The first zone near the bottom of the extruded column, as located by means of streak indicator (1 part of potassium permanganate, 10 parts of sodium hydroxide and 100 parts of water) contained β-D-glucose pentaacetate. The second zone located about one-third of a column length from the bottom contained the disaccharide octaacetate. It was cut out, eluted with 750 ml. of acetone and evaporated to a sirup; yield 0.5 g. Material (1.6 g.) obtained in this manner was dissolved in 50 ml. of benzene and placed on a column of Magnesol[21]–Celite[22] (5:1 by wt.) (240 × 43 mm.[23]) and developed with 1200 ml. of benzene–ethanol (100:1). The first significant zone from the bottom of the column was sectioned and eluted with 750 ml. of acetone to produce a sirup on solvent removal; yield 0.9 g. This material was crystallized from ethanol and was recrystallized from the same solvent; yield 0.7 g., m. p. 143–144°, $[\alpha]^{25}D$ +96.9° (*c* 2.7, chloroform).

Anal. Calcd. for $C_{12}H_{14}O_{11}(CH_3CO)_8$: C, 49.56; H, 5.63; CH_3CO, 11.78 ml. of 0.1 *N* NaOH per 100 mg.; mol. wt., 679. Found: C, 49.74; H, 5.67; CH_3CO, 11.86 ml.; mol. wt. (Rast), 680.

6-α-D-Glucopyranosyl-D-glucose.—Two grams of 6-α-D-glucopyranosyl-β-D-glucopyranose octaacetate was deacetylated according to the method of Isbell.[24] The acetate was dissolved in 40 ml. of absolute methanol and cooled to 0°. Barium methoxide (2.4 ml. of 0.4 *N*) was added to the solution and the whole kept at 0° for twenty-four hours. It was then diluted with 200 ml. of cold water and the ionic material removed by passing over Amberlite resins IR-100 and IR-4.[20] The effluent from the ion exchange columns was concentrated to a sirup under reduced pressure. The residual water was removed by repeatedly stirring with ethanol and evaporating to dryness in a vacuum desiccator. All attempts to crystallize the substance failed. It is a very hygroscopic solid; $[\alpha]^{24}D$ +103.2° (*c* 3.85, water). This material displayed only one zone when placed on Silene[25]–Celite[22] (5:1 by wt.) (240 mm. × 43 mm.[23]) and developed with 120 ml. of 90% dioxane. The free sugar is not fermented by bakers' yeast.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.48. Found: C, 42.73; H, 7.10.

(20) A product of The Resinous Products and Chemical Co., Philadelphia, Pennsylvania.

(21) A product of Westvaco Chlorine Products Corp., South Charleston, West Virginia.

(22) No. 535, a product of Johns-Manville Co., New York, N. Y.

(23) Dimensions of the adsorbent.

(24) H. S. Isbell, *Bur. Standards J. Research*, **5**, 1185 (1930).

(25) A product of Columbia Chemical Division, Pittsburgh Plate Glass Co., Barberton, Ohio.

6-α-D-Glucopyranosyl-α-D-glucopyranosyl Bromide Heptaacetate.—Two grams of 6-α-D-glucopyranosyl-β-D-glucopyranose octaacetate was dissolved in 20 ml. of dry, freshly prepared, and ethanol-free chloroform. The solution was cooled in an ice–salt-bath and to it was added 5.2 ml. of dry glacial acetic acid containing 30–32% of hydrogen bromide. The mixture was allowed to stand at 0° for two hours after which it was poured into a separatory funnel containing cracked ice. The chloroform solution was washed four times with cold water and dried over anhydrous sodium sulfate. A sirup was obtained on solvent removal that was crystallized from absolute ethanol; m. p. 131–133°, $[\alpha]^{24}D$ +202° (*c* 1.75, chloroform).

Anal. Calcd. for $C_{26}H_{35}O_{17}Br$: C, 44.64; H, 5.04; Br, 11.41. Found: C, 44.74; H, 5.01; Br, 10.98.

Methyl 6-α-D-Glucopyranosyl-β-D-glucopyranoside Heptaacetate.—An amount of 0.19 g. of crystalline 6-α-D-glucopyranosyl-α-D-glucopyranosyl bromide heptaacetate was dissolved in 3 ml. of absolute methanol containing 0.2 g. of powdered Drierite, 0.2 g. of silver carbonate, 2 ml. of alcohol-free chloroform and a trace of iodine. The mixture was shaken overnight, filtered and evaporated to a sirup. The sirup was crystallized from ethanol–water and was recrystallized in the same manner; yield 0.06 g., m. p. 114–116°, $[\alpha]^{25}D$ +88.5° (*c* 2.0, chloroform).

Anal. Calcd. for $C_{26}H_{35}O_{17}(OCH_3)$: C, 49.84; H, 5.89; OCH_3, 4.77. Found: C, 49.97; H, 6.17; OCH_3, 4.96.

Methyl 6-α-D-Glucopyranosyl-β-D-glucopyranoside.—Methyl 6-α-D-glucopyranosyl-β-D-glucopyranoside heptaacetate (0.45 g.) was dissolved in 2.5 ml. of absolute methanol and heated under reflux for fifteen minutes with 0.2 ml. of 0.1 *N* sodium methoxide according to the method of Zemplén and Pacsu.[26] The reaction mixture was cooled and the methanol was removed under reduced pressure at 50–55°. The resulting sirup was dissolved in 5 ml. of water and the ionic material removed by passing the solution over Amberlite resins IR-100 and IR-4.[20] The effluent from the ion exchange columns was concentrated to a viscous sirup and the product was obtained as an amorphous solid by stirring in dioxane and concentrating under reduced pressure in a vacuum desiccator. This operation was repeated several times; yield 0.2 g., $[\alpha]^{24}D$ +50° (*c* 5.8, water).

Anal. Calcd. for $C_{12}H_{21}O_{16}(OCH_3)$: C, 43.82; H, 6.79; OCH_3, 8.71. Found: C, 44.11; H, 6.83; OCH_3, 8.68.

Acknowledgment.—We are pleased to acknowledge the advice of Professor Grant L. Stahly of the Department of Bacteriology of this University, in the preparation of the dextran.

Summary

1. 6-α-D-Glucopyranosyl-β-D-glucopyranose octaacetate has been separated in crystalline condition by chromatographic methods from the acetylated hydrolyzate of dextran (from *Leuconostoc dextranicum*). This acetate was converted to the crystalline 6-α-D-glucopyranosyl-α-D-glucopyranosyl bromide heptaacetate.

2. 6-α-D-Glucopyranosyl-D-glucose and methyl 6-α-D-glucopyranosyl-β-D-glucopyranoside have been prepared in amorphous but chromatographically pure condition through their crystalline acetates and the 1,6-linkage established by periodate oxidation of the latter compound.

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(26) G. Zemplén and E. Pacsu, *Ber.*, **62**, 1613 (1929).