alkaline copper reduction and was stopped at 67% of completion by cooling to room temperature. Four such runs were made. The acid was removed from the cooled solution by passage through a column of Duolite A-4.[18] A preliminary separation was made on a column (900 × 70 mm.) of Unground Nuchar C[19] which had been pretreated by washing with 4 liters of 1% hydrochloric acid followed by 20 liters of water, 4 liters of 1% ammonium hydroxide solution and again with water until the effluent reached a *p*H of 7. The four amylopectin hydrolyzates were filtered successively through the carbon column, the D-glucose being removed by washing with water after the addition of each portion. The column was then washed with 5% ethanol (about 20 liters) until the effluent gave a negative Benedict test for reducing sugar. This effluent was concentrated to a sirup under reduced pressure, yield 24.5 g.

The dry sirup was acetylated by heating at the boiling point with 12 g. of sodium acetate and 200 ml. of acetic anhydride. After cooling, the reaction mixture was poured into 500 g. of ice and water, and after 3 hr. was extracted with chloroform. The chloroform solution was dried with anhydrous sodium sulfate and evaporated under reduced pressure to a sirup which was crystallized from ethanol (95%); yield 18.2 g. of β-maltose octaacetate which, after one recrystallization from ethanol (95%), showed m.p. 154–155°, $[\alpha]^{28}D$ +64° (*c* 4.5, chloroform). The mother liquor was evaporated under reduced pressure to a sirup, yield 22 g. This sirup was dissolved in benzene and chromatographed in 5-g. portions on Magnesol[20]–Celite[21] (5:1 by wt.) columns (275 × 75 mm., diam.) and developed with 4000 ml. of benzene–*t*-butyl alcohol (100:1 by vol.). The extruded and streaked (with 1% potassium permanganate in 10% sodium hydroxide) column showed a zone 190–220 mm. from the column top. A second zone occurred just above the first with only a slight interspace. The materials in these zones were eluted with acetone and evaporated to sirups under reduced pressure. The combined material from the bottom zone crystallized from ethanol (95%) as β-isomaltose octaacetate. The sirup (5 g.) from the combined upper zones was rechromatographed on Magnesol–Celite, as before, using 5000 ml. of benzene–*t*-butyl alcohol as developer. The bottom zone, 190–220 mm. from the top of the column, produced 200 mg. of β-isomaltose octaacetate; total yield from all columns 1.67 g., which, after one recrystallization from ethanol (95%), gave material of m.p. 144–146°, $[\alpha]^{28}D$ +98° (*c* 4.4, chloroform).

The material from the acetone eluate of the column section, located 110–190 mm. from the column top, was crystallized from ethanol (95%); yield 300 mg., m.p. 140–145°, $[\alpha]^{25}D$ +80° (*c* 3.2, chloroform). An additional yield of 50 mg. was obtained by rechromatography, performed in the manner described above, of the crystallization mother liquors. After further recrystallization from ethanol (95%), the melting point was 151–153°. The X-ray powder diffraction pattern was identical with that of known[14] β-nigerose octaacetate.

(18) A product of the Chemical Process Co., Redwood City, Calif.

(19) A product of the West Virginia Pulp and Paper Co., New York, N. Y.

(20) A product of the Westvaco Chemical Division of the Food Machinery and Chemical Corp., South Charleston, W. Va.

(21) A product of the Johns-Manville Co., New York, N. Y.

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[CONTRIBUTION FROM THE NATIONAL RESEARCH COUNCIL OF CANADA, PRAIRIE REGIONAL LABORATORY]

# A Chemical Synthesis of Sucrose. A Conformational Analysis of the Reactions of 1,2-Anhydro-α-D-glucopyranose Triacetate[1]

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RECEIVED FEBRUARY 13, 1956

Sucrose was synthesized by reaction of 1,2-anhydro-α-D-glucopyranose triacetate with sirupy 1,3,4,6-tetra-*O*-acetyl-D-fructose. The ability of the anhydride to form α-D-glucopyranosides is rationalized on the basis of its conformation and the stereochemical requirements for opening of the epoxide ring.

The reaction of 1,2-anhydro-α-D-glucopyranose triacetate (Brigl's anhydride[4]) with sirupy 1,3,4,6-tetra-*O*-acetyl-D-fructose[5] has afforded a chemical synthesis of sucrose.[6] The synthesis was anticipated[7] on the basis of a conformational analysis of the properties of the anhydride. It is therefore of some interest to consider this matter in detail.

It can be assumed that the pyranose ring of Brigl's anhydride possesses the half-chair conformation of cyclohexene oxide.[8] On this basis, the conformation of the anhydride is either II or III. Which of these two forms is the more stable, and the height of the energy barrier which separates the two forms, cannot be anticipated. Nevertheless, it has become clear that the substance possesses an inherent tendency to react in form II. The anhydride very likely is liberated in the latter conformation from the reaction of 3,4,6-tri-*O*-acetyl-β-D-glucosyl chloride (I) with ammonia since the reaction most probably involves replacement of axial chlorine through nucleophilic attack at $C_1$ by anionic $C_2$-oxygen in axial orientation in accordance with the steric requirements for neighboring group participation[9] and elimination reactions.[10]

The anhydride clearly shows a strong preference for reaction at the anomeric center rather than at $C_2$ since glucopyranosides are formed in high yield when the substance reacts with alcohols.[4,11–13] This tendency is not surprising in view of the attachment of $C_1$ to the ring-oxygen atom. It appears well established[14] that the preferred reaction route in the opening of an epoxide situated on a

(1) Presented in part at the 124th Meeting of the American Chemical Society, Chicago, Ill., September, 1953. Issued as Paper No. 224 on the Uses of Plant Products and as N.R.C. No. 4024.

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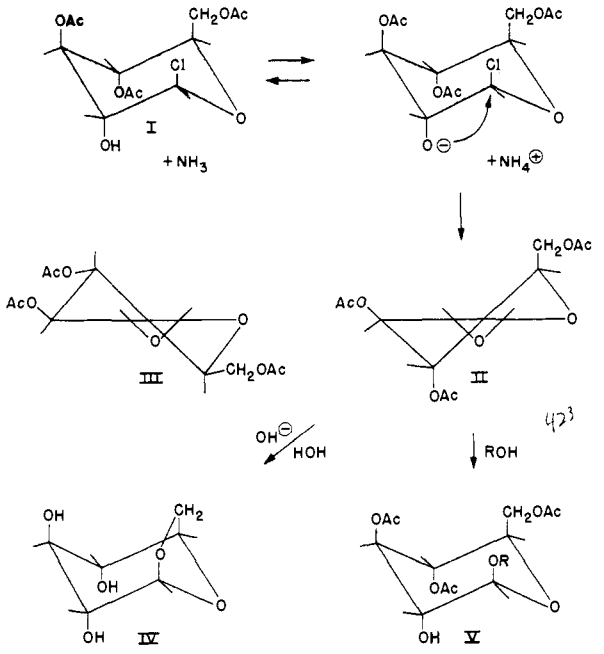
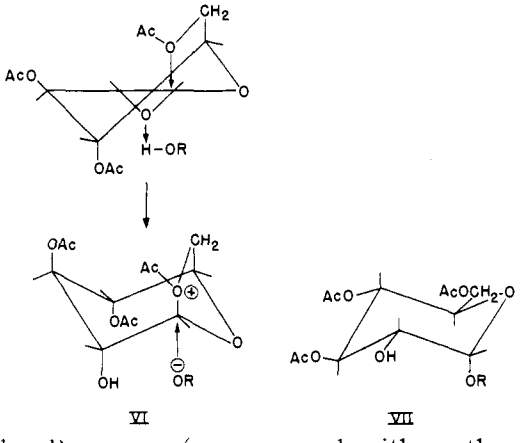


six-membered ring by a nucleophilic reagent is that which leads to the diaxial product. Conformation II is clearly required for the occurrence of this energetically favorable reaction route when the nucleophilic attack is at $C_1$. The fact that alkaline hydrolysis of the anhydride yields 1,2-diaxial 1,6-anhydro-β-D-glucopyranose (IV)[15-17] is definitive evidence that the conformation II is readily achieved. Therefore, it can be assumed that the reactive form of Brigl's anhydride is that (II) which possesses the $CH_2OAc$ group in axial orientation and the normal reaction of the anhydride with an alcohol to form a β-D-glucopyranoside (V) must proceed as shown.

Evidence has long existed that the anhydride reacts only sluggishly with alcohols (ROH) with



bulky R groups (as compared with methanol). Prolonged heating at elevated temperatures has been required[7,11,12,18] in many cases. Hardegger and De Pascual[12] have even resorted to catalysis by sulfuric acid. It has long been recognized[11,13] that the anhydride is converted to α-D-glucopyranoside as well as β-D-glucopyranoside in the sluggish reactions with the complex alcohols. These facts clearly suggest that there can develop strong steric hindrance to the normal *trans*-addition reaction. This steric effect must arise in part from increased shielding of the hydroxyl group by the R group. However, it seems probable that the main steric effect is the shielding of the anomeric center by the axial $CH_2OAc$ group in the reactive form (II) of the anhydride.

The mechanism of the abnormal reaction of the anhydride to form α-D-glucopyranosides (VII) cannot be predicted with certainty. Of the possible reaction routes, that which would involve participation of the $CH_2OAc$ group in the first stage of the reaction[7] to yield the 1,2-diaxial carboxonium ion (VI) appears most plausible in view of the facts that the $C_6$-oxygen atom is certainly suitably positioned for such a participation, that such participations which can provide anchimeric assistance have a wide occurrence in replacements which proceed with retention of configuration and that the route affords a diaxial opening of the epoxide ring.

In view of the above considerations, it seemed reasonable to expect that Brigl's anhydride would serve as the long-sought reagent[5,19] for the synthesis of the α-D-glucosidic linkage of sucrose. This opinion was strengthened by the synthesis of maltose[7] and provided the necessary confidence to face the rather formidable isolation problem presented by the reaction product. Fortunately, a combination of the techniques of preparative paper chromatography[20,21] and of extrusion chromatography of sugar acetates on Magnesol-Celite developed by McNeely, Binkley and Wolfrom[22] allowed a solution to this problem. Replacement chromatography on a Darco G60—Celite column[23] can be substituted for the preparative paper chromatogram.

We report herein the preparation of sucrose in which the first crystalline product, sucrose octaacetate, was isolated in highest yield, 5.5%. In this preparation the anhydride was heated with the 1,3,4,6-tetra-*O*-acetyl-D-fructose for 104 hours at 100°. A reaction at 80° for 168 hours gave an 8.8% yield as shown by isotopic dilution analysis. In view of the fact that the synthesis is only of academic interest no serious attempt was made to establish the optimum reaction conditions. At the beginning of this work, the reaction mixture underwent extensive charring. It was decided that this result was due to impurities in the tetraacetylfructose (most likely traces of acetobromofructose) since the use of more carefully purified material led to strong discoloration but no ap-

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preciable charring. Since the completion of this work, Lemieux and Bauer[24] have found that Brigl's anhydride can be the source of trouble. Reaction of the anhydride at 100° with 1,2,3,4-tetra-*O*-acetylglucoses (to form isomaltose, gentiobiose and maltose) led to extensive discoloration when the anhydride was purified merely by recrystallization. However, when the anhydride was purified by distillation,[25] the reaction products were essentially colorless.

In summary, 1,2-anhydro-α-D-glucopyranose triacetate, first prepared by Brigl[4] in 1922, has served as the crucial reagent for the first purely chemical synthesis of the important naturally occurring disaccharides sucrose, maltose[7] and trehalose[26] and a new synthesis of isomaltose.[24] The fact that the latter three disaccharides are definitely α-D-glucopyranosides together with our rationalization of the properties of Brigl's anhydride are offered as synthetic evidence for α-D-glucosidic linkage in sucrose. The previous evidence for this configuration rests on the fact that sucrose is hydrolyzed by the enzyme maltase which is specific for α-D-glucopyranosides[27] and on the facts that invertase being a β-D-fructofuranosidase[28] must attack the anomeric center of the fructosyl moiety and α-D-glucopyranose is a first product of the reaction.[19,29,30] Wolfrom and Shafizadeh[31] have very recently confirmed these conclusions by application of Hudson's isorotation rules.

**Acknowledgment.**—The infrared absorption spectra were determined by Miss Agnes Epp.

## Experimental

**Sucrose Octaaacetate.**—1,3,4,6-Tetra-*O*-D-fructofuranose[5] (1.4 g.) was dissolved in 40 ml. of dry benzene. About 30 ml. of the benzene was removed by distillation to free the system of water. 1,2-Anhydro-α-D-glucopyranose triacetate[4] (1.16 g.) was then added, and the total volume was reduced to about 3 to 4 ml. by further distillation of benzene. The remaining solution was heated on a steam-bath for 104 hours in a sealed tube. The reaction mixture was acetylated by heating with 20 ml. of acetic anhydride and 1 g. of sodium acetate for 1 hr. on the steam-bath. The acetylated mixture was dissolved in 50 ml. of benzene, and the solution was washed 3 times with 50 ml. of water. The dried benzene solution was evaporated *in vacuo* and the residue was deacetylated by treatment with 30 ml. of 0.02 *N* methanolic sodium methoxide solution for 6 hours at room temperature and 16 hours at 0°. After neutralization with carbon dioxide, the solvent was evaporated *in vacuo*. The residue was dissolved in 4.5 ml. of water and the brownish sugar solution was applied as uniform streaks to six 50 × 50 cm. sheets of Whatman 3 MM filter paper for chromatographic separation[20,21] with butanol:ethanol:water (5:1:4). The bands on the resulting chromatogram were detected on strips cut from the sheets by the periodate-permanganate spray reagent.[32] The disaccharide fraction was extracted from the paper with water and the solvent was removed *in vacuo*. The residue was acetylated by heating with 15 ml. of acetic anhydride and 0.75 g. of sodium acetate for two hours on the steam-bath, to yield 0.633 g. of product. The sirupy material was dissolved in about 5 ml. of benzene and added to the top of a 50 × 205 mm. column of Magnesol–Celite (5:1)[22] wetted with benzene. The chromatogram was developed with 2 liters of 100:1 benzene–ethanol, the column was extruded, and a streak was applied to the length of the column by spraying with a freshly prepared aqueous solution of 1% potassium permanganate in 2.5 *N* aqueous sodium hydroxide through a 2 mm. wide slit cut from a sheet of Teflon. Four zones could be detected. Elution of the zone 6 mm. to 47 mm. with acetone gave 0.042 g. of sirupy material with $[\alpha]_D$ + 74° (in chloroform), the zone 47 mm. to 73 mm. gave 0.118 g. of sirupy material with $[\alpha]_D$ + 74° (in chloroform) and the zone 73 mm. to 96 mm. gave 0.223 g. of sirupy material which, after being dissolved in about 1 ml. of ethanol and standing at room temperature for several hours, deposited 0.142 g. of crystalline material, m.p. 81–86°, $[\alpha]_D$ +62.5° (*c* 0.8 in chloroform). After three recrystallizations from ethanol, the melting point was that reported for sucrose octaacetate, 88–90°,[33] with $[\alpha]_D$ +60° (*c* 1.0 in chloroform). The melting point was unchanged by admixture of an authentic sample of sucrose octaacetate. The substance pressed with potassium bromide[34] possessed an infrared absorption spectrum identical to that determined for sucrose octaacetate under the same conditions. The mother liquor was combined with the material from zone 47 mm. to 73 mm. and a second chromatogram was made. Crystalline material, m.p. 78–80° (5 mg.), was isolated which after purification had the physical properties of sucrose octaacetate. The total yield of crude crystalline material was therefore 0.147 g., 5.5% yield. This is a minimum yield since the paper chromatography was attended by considerable loss of material.

**Sucrose.**—The pure sucrose octaacetate (74 mg.) was dissolved in 5 ml. of 0.02 *N* methanolic sodium methoxide and kept at room temperature for six hours. The mixture was neutralized with carbon dioxide and the solvent evaporated *in vacuo*. The residue was dissolved in about 3 ml. of water and the aqueous solution percolated through a mixed bed of ion exchange resins containing 0.5 g. of Amberlite IR 120H and 0.5 g. of Amberlite IR 4B. The solvent was removed *in vacuo* and the residue was crystallized from 0.5 ml. of water and 10 ml. of ethanol. The material (34 mg.) melted at 187°, $[\alpha]_D$ +66.7° (*c* 0.7 in water). The mixed m.p. with authentic sucrose, m.p. 187°, $[\alpha]_D$ +66.5° (in water), was 187°. A positive Raybin test[35] was obtained on dissolving 1 mg. of the synthetic sucrose in 2 ml. of 0.05 *N* aqueous sodium hydroxide solution, and adding 1 mg. of diazouracil. After shaking and standing for one minute a green color developed. A precipitate was formed on addition of magnesium sulfate.

**Yields by Isotopic Dilution Analysis.**—The products from two preparations were analyzed by isotopic dilution. Radioactive sucrose octaacetate (prepared from carbon-14 labeled sucrose) was added to the mixture obtained on treating Brigl's anhydride, 2 mmolar, with a twofold excess of the fructose tetraacetate. The product was then worked up in the manner described above except that chromatography on Darco G60–Celite[23] was substituted for the preparative paper chromatograms. The radioactivity of the isolated sucrose octaacetate was determined by combustion to barium carbonate and counting at infinite thickness with a gas-flow counter. The percentage yield was calculated from the expression, $100x(C_i - C_f)/yC_f$, where $x$ is the weight of radioactive sucrose octaacetate added of radioactivity $C_i$ = 364,000 c.p.m., $y$ is the theoretical yield of sucrose octaacetate (2 mmolar), and $C_f$ is the radioactivity of the sucrose octaacetate finally isolated.

A reaction at 80° for 168 hours gave a product which was diluted with 10.07 mg. of the radioactive sucrose octaacetate. The product yielded 120 mg. of crude crystalline sucrose octaacetate which after purification possessed a radioactivity of 26,600 c.p.m. which corresponds to a 8.8% yield.

A similar experiment where the reaction mixture was heated at 120° for 72 hours indicated the formation of sucrose in only 2.2% yield.

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