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

Acid Reversion in Relation to Isomaltose as a Starch Hydrolytic Product[1]

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It is demonstrated that the amount of crystalline β-isomaltose octaacetate isolable by column chromatographic techniques in the acid hydrolysis of amylopectin at low concentration (0.4%) is grossly (200 times) in excess of the small amount formed by the acid reversion process under like conditions. Therefore it is established that the 6-α-D-glucopyranosyl linkage is preformed in the amylpectin molecule.

The fact that oligosaccharides containing the 6-α-D-glucopyranosyl linkage can be isolated from the acid hydrolytic products of amylopectin has been offered by this Laboratory[3,4] as supporting evidence for the theory of the branched structure of this substance. It has long been known that D-glucose, when heated in acid solution undergoes condensation, a reaction specifically known as "reversion," to produce polymers of D-glucose units.[5] The question naturally arises whether such oligosaccharides, obtained from the acid hydrolysis of amylopectin, are fragments containing preformed linkages, or are products of such reversion. We wish herein to describe a series of experiments designed to answer this question. Conditions of acid concentration and temperature were chosen to bring about hydrolysis of a 0.4% suspension of amylopectin in ten hours to approximately 85% of completion. This degree of hydrolysis is safely under the value of 91% calculated[3,6] to produce a maximum yield of isomaltose; above this value the yield decreases rapidly. To determine the extent of isomaltose production by reversion during the hydrolysis, the procedure was repeated several times, using identical conditions of acid concentration, time and temperature of heating, but substituting varying initial concentrations of D-glucose for amylopectin. Since the quantities of isomaltose and gentiobiose formed are pertinent to the question, these sugars were isolated, identified and weighed as their crystalline β-octaacetates from the hydrolyzate of amylopectin and from each of the reversion reactions. This was accomplished by removing the D-glucose from the polymeric sugars by means of a carbon column,[7,8] followed by acetylation and chromatographic separation of the acetate mixture on a Magnesol–Celite[9] column. The yields are plotted in Fig. 1. No claim is made that these techniques are quantitative in the absolute sense but it is believed that the relative proportions recorded are essentially correct.

It should be noted that in the range of D-glucose concentrations studied, approximately equal quantities of gentiobiose and isomaltose are always formed by reversion, and that the amount of reversion increases with the concentration of the sugar. The yield of β-isomaltose octaacetate from the amylopectin hydrolyzate is of a grossly larger order of magnitude than that obtained from the corresponding reversion reaction. No β-gentiobiose octaacetate could be isolated from the amylopectin hydrolyzate. The methods employed would not have failed to detect a quantity of this substance comparable to the β-isomaltose octaacetate isolated.

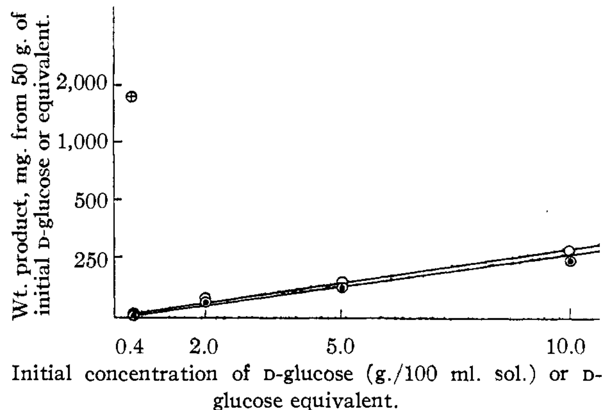

Fig. 1.—Yields of products obtained on hydrolysis for 10 hr. with 0.082 N HCl at 97° at varying initial reactant concentration; β-isomaltose octaacetate from amylopectin (⊕) and from D-glucose (O); β-gentiobiose octaacetate from D-glucose, ⊙.

These data indicate that the 6-α-D-glucopyranosyl linkage is actually preformed in the starch molecule and is only in a negligible part a product of reversion under the hydrolytic conditions employed.

Experimental

Hydrolysis of Amylopectin.—Amylopectin (waxy maize starch, 32.4 g., equivalent to 36 g. of D-glucose) was suspended in 9 l. of 0.082 N HCl and heated in a boiling water-bath for 10 hr. (approximately 85% hydrolysis on the basis of copper reducing value). The solution was cooled and passed through a column of Duolite A-4[10] to remove the acid.

Isolation of Disaccharides.—The above solution was then placed on a column (375 × 105 mm., diam.) of Darco G-60[11]; Celite[12] (1:1 by wt.) and washed with water until the effluent reacted negative to Benedict solution (*ca.* 25 l. after all sugar was on the column). The effluent, which up to this point is considered to contain only D-glucose, was discarded. The column was then washed with 25% ethanol and that portion of the effluent which reacted positive to Benedict solution (*ca.* 3 l.) was collected. The ethanol

(1) Reported in *Abstracts Papers Am. Chem. Soc.*, **123**, 14D (1953).

(2) Corn Industries Research Foundation Associate (A. T.) and Fellow (E. J. Q.).

(3) M. L. Wolfrom, J. T. Tyree, T. T. Galkowski and A. N. O'Neill, THIS JOURNAL, **73**, 4927 (1951).

(4) A. Thompson and M. L. Wolfrom, *ibid.*, **73**, 5849 (1951).

(5) W. R. Fetzer, E. K. Crosby, C. E. Engel and L. C. Kirst, *Abstracts Papers Am. Chem. Soc.*, **122**, 3R (1952).

(6) M. L. Wolfrom, E. N. Lassettre and A. N. O'Neill, THIS JOURNAL, **73**, 595 (1951).

(7) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

(8) M. L. Wolfrom, A. Thompson, A. N. O'Neill and T. T. Galkowski, *ibid.*, **74**, 1062 (1952).

(9) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

(10) A product of Chemical Process Co., Redwood City, California.

(11) Decolorizing carbon; a product of Darco Department, Atlas Powder Co., New York, N. Y.

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

solution was evaporated to a sirup under reduced pressure and dried by repeated distillation from methanol solution to an amorphous solid; yield 6.7 g. This material was acetylated by heating at the boiling point with 3.5 g. of fused sodium acetate and 50 ml. of acetic anhydride. The reaction mixture was then cooled and poured into 300 ml. of ice and water. After the destruction of the acetic anhydride, the mixture was extracted with chloroform, the chloroform solution was washed with water, dried with anhydrous sodium sulfate and evaporated to a sirup under reduced pressure. The five-gram portions of this sirup were chromatographed by placing each on a column (250 × 80 mm., diam.) of Magnesol[9]:Celite[9,12] (5:1 by wt.) and developing with 3500 ml. of benzene:*t*-butyl alcohol (100:1 by vol.). Zones near the middle of the columns produced β-isomaltose octaacetate; yield 1.03 g., m.p. 139–141°, $[\alpha]^{25}D$ +96.4° (*c* 4, chloroform); after one recrystallization from ethanol, m.p. 145–146°, mixed melting point with an authentic sample of β-isomaltose octaacetate unchanged, $[\alpha]^{25}D$ +98.4°; yield of crude product based on a quantity of starting material equivalent to 50 g. of D-glucose, 1890 mg. No trace of β-gentiobiose octaacetate could be found.

Reversion Reaction of D-Glucose.—A series of four solutions of D-glucose (concentrations 0.4, 2.0, 5.0, and 10.0%) in 0.082 *N* HCl were heated for 10 hr. in a boiling water-bath, the solution temperature being maintained at 97°. In each case the quantity of D-glucose was 50 g., except the first, in which only 36 g. was used because of the large volume involved. After cooling, the acid was removed from the solutions by means of a Duolite A-4[10] column. Gentiobiose (zone 35–70 mm. from column top) and isomaltose (zone 90–150 mm. from column top) were then isolated as their β-octaacetates from the solutions by the procedure outlined above; all samples of β-gentiobiose octaacetate, m.p. 187 ± 2°, mixed m.p. with authentic sample unchanged, $[\alpha]^{25}D$ −4.5 ± 0.1° (*c* 2.5, chloroform) where yield was adequate for determination; all samples of β-isomaltose octaacetate, m.p. 144 ± 2°, mixed m.p. with authentic sample unchanged, $[\alpha]^{25}D$ +86.5 ± 0.5° (*c* 3.0, chloroform) where yield was adequate for determination. Upon further purification of β-isomaltose octaacetate the constants were: m.p. 145–146° $[\alpha]^{25}D$ +97.1° (*c* 3.0, chloroform). The quantities of eac! are recorded in Fig. 1.

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Synthesis of Phenanthrenes. V. A Mechanism for the Cyclization of β-Arylethylcyclohexanols[1]

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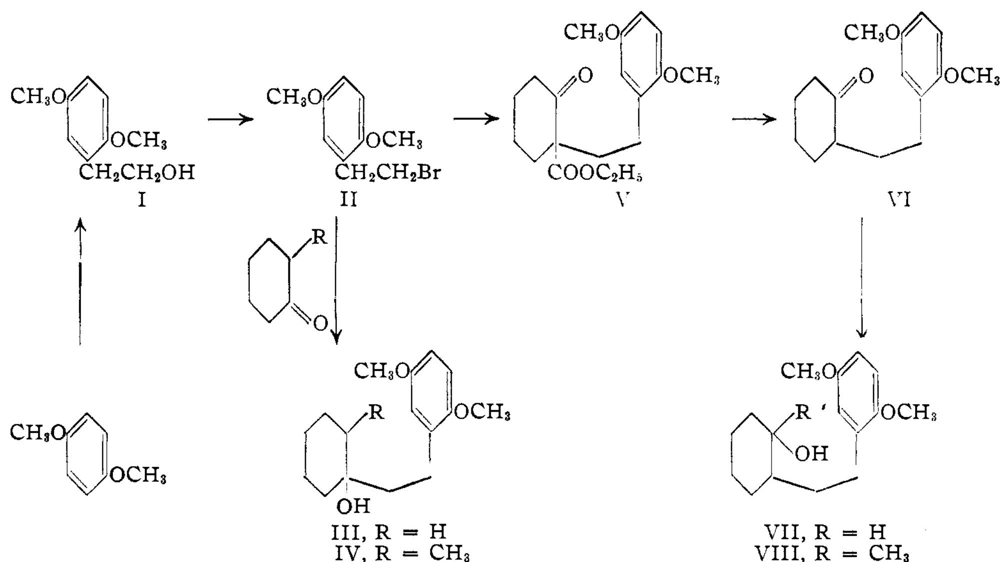
The cyclization of four new β-arylethylcyclohexanols has been carried out in order to obtain further information bearing on the mechanism of the reaction. The results obtained fron this study together with previous observations has made it desirable to formulate a reaction path in which a bridged ion is an important intermediate.

When a given β-arylethylcyclohexanol is to be cyclized it would be desirable if a prediction could be made concerning the amounts of spirane and phenanthrene to be expected. Some generalizations could be made from previous experimental results in our own and other laboratories; however, it was difficult to formulate a mechanism which explained all the facts.

The purpose of this work was to study the cyclization of four new alcohols which were chosen in an attempt to decide between the two mechanisms which were originally considered most likely. The four alcohols were synthesized from β-(2,5-dimethoxyphenyl)-ethyl bromide (II) which in turn was prepared from hydroquinone dimethyl ether.



Bromide II was rather unstable and hydrogen bromide was eliminated to some extent even during vacuum distillation; however, satisfactory yields of the desired alcohols could be obtained.

Each of the alcohols (III, IV, VII and VIII) was cyclized with a mixture of 85% phosphoric acid and phosphorus pentoxide. The yield of crystalline

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