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

The Composition of Pyrodextrins

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Maltose, isomaltose, gentiobiose, sophorose and 1,6-anhydro-β-D-glucopyranose were isolated as their acetates from the partial acid hydrolyzate of a pyrodextrin made by roasting amylose at a temperature of 185–200° in the absence of an acid catalyst. This evidence indicates that (1 → 6), and to a lesser extent β-D-(1 → 2) linkages and 1,6-anhydro-β-D-glucopyranose moieties, are produced at the expense of α-D-(1 → 4) linkages during the roasting process. The 1,6-anhydro groups are necessarily chain terminal.

The pyrodextrins are those products made by roasting starch with or without the addition of acid catalysts or other modifying agents and have been produced commercially, in a wide variety, for many years. Slight variations in the reaction conditions will bring about striking differences in the product. Among factors which may be varied are the kind of starch, temperature, time of roasting, amount and kind of catalyst, and possibly the moisture content of the starch. Dextrins made at relatively low temperatures with an acid catalyst undoubtedly undergo a certain amount of hydrolysis and reversion (recombination) especially in the early part of the reaction when moisture is still present. The relatively high copper-reducing values[2] of this type of product indicate that some hydrolysis has taken place. When starch is heated in the presence of acid and a small amount of water, hydrolysis and reversion[3] occur resulting in the formation of relatively higher amounts of the more stable[4,5] (1 → 6) linkages at the expense of the predominant α-D-(1 → 4) linkage. The results of periodate and methylation techniques applied to this type of acid-formed dextrin[6,7] indicate that the molecule is reduced in size and has become considerably more branched.

If starch is roasted at temperatures as high as 200° without the addition of acid, it undergoes an exothermic reaction[8–10] to yield a product known commercially as British Gum. It acts like a gum when dampened, is almost completely soluble in water, does not separate from solution on standing and has a low reducing value. On the basis of end group assay by methylation procedures,[11] decrease in viscosity, decrease in hydrolysis by β-amylase, periodate oxidation and increase in water–butanol solubility, previous workers[2,12,13] have suggested that the starch loses its linear character and becomes more highly branched as the reaction proceeds.

In this communication we are concerned with pyrodextrins made by roasting a commercial amylose[14] at 185–200° without the addition of an acid catalyst. We wish to report the isolation of substantial quantities of maltose, isomaltose, gentiobiose, sophorose and 1,6-anhydro-β-D-glucopyranose as their crystalline β-D-acetates from partially acid-hydrolyzed samples of pyrodextrins made from this amylose. A sample which had been roasted at 200° until it softened to a brown semi-liquid, a treatment considerably more vigorous than usual, produced, after partial acid hydrolysis, D-glucose, isomaltose, gentiobiose, sophorose but no maltose. The hydrolyses were carried out under conditions which restricted the formation of disaccharides by reversion[15] to a negligible amount. Although there are many data in the literature concerning the nature of the linkages in dextrins, this is the first isolation and identification of these linkages by fragmentation analysis. Our data furnish direct evidence that α-D-(1 → 6), β-D-(1 → 6), β-D-(1 → 2) linkages and 1,6-anhydro-β-D-glucopyranose end groups exist in the pyrodextrin produced from amylose which was originally bound essentially by α-D-(1 → 4)-glycosyl linkages. Other linkages, especially α-D-(1 → 2), are probably also present in this dextrin, but were not established on an isolative basis.

The substantial absence of water in the reaction, and the fact that the resulting dextrin has a low reducing value, indicate that hydrolysis is not a significant factor in the modification at higher temperatures. The observation that the reaction is exothermic[8] indicates that the normal α-D-(1 → 4) linkages of amylose are converted to the more stable[4,5] (1 → 6) linkages.

Several courses for the reaction, all of which could possibly be operative, may be suggested. The first is analogous to that proposed[16] as the initial step in the thermal decomposition of cellulose. In this postulation a primary hydroxyl group on carbon 6 attacks the glycosyl linkage of the same D-glucose unit, resulting in a rupture of the chain with formation of an anhydro end group. Since the end product of the pyrolysis of starch is largely 1,6-anhydro-β-D-glucopyranose, it is possible that the

(1) Research Associate of the Corn Industries Research Foundation.

(2) Bernadine Brimhall, *Ind. Eng. Chem.*, **36**, 72 (1944).

(3) W. R. Fetzer, E. K. Crosby, C. E. Engel and L. C. Kirst, *ibid.*, **45**, 1075 (1953).

(4) E. A. Moelwyn Hughes, *Trans. Faraday Soc.*, **25**, 503 (1929).

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(6) C. M. Christensen and F. Smith, *ibid.*, **79**, 4492 (1957).

(7) J. D. Geerdes, Bertha A. Lewis and F. Smith, *ibid.*, **79**, 4209 (1957).

(8) J. R. Katz, *Rec. trav. chim.*, **53**, 555 (1934).

(9) J. R. Katz and A. Weidinger, *Z. physik. Chem.*, **A184**, 100 (1939).

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(11) W. N. Haworth, E. L. Hirst and (Miss) M. M. T. Plant, *J. Chem. Soc.*, 1214 (1935).

(12) R. W. Kerr and F. C. Cleveland, *Die Stärke*, **5**, 261 (1953); C. A., **48**, 1237 (1954).

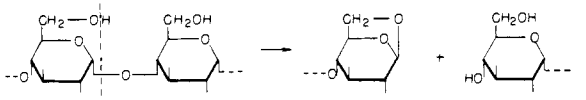
(13) R. W. Kerr and N. F. Sehink, *Paper Trade J.*, **120**, No. 8, 115 (1945).

(14) Superlose, obtained from Stein-Hall and Co., Inc., 285 Madison Ave., New York 17, N. Y.; *ca.* 95% amylose.

(15) A. Thompson, M. L. Wolfrom and E. J. Quinn, THIS JOURNAL, **75**, 3003 (1953).

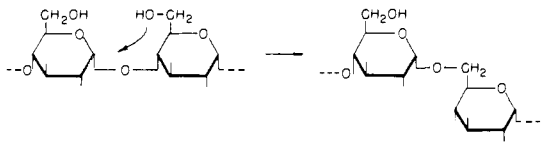
(16) W. G. Parks, R. M. Esteve, Jr., M. H. Gollis, R. Guercia and A. Petiarca, *Abstracts Papers Am. Chem. Soc.*, **127**, 6E (1955).

dextrinization process, especially at high temperatures, is an incipient pyrolysis in which α-D-(1 → 4) linkages are ruptured in the above manner without the formation of a reducing group. This mechanism is supported on the basis of alkali lability studies by Brimhall[2] but is discounted by

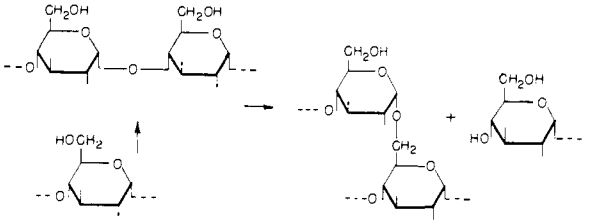


Kerr and Cleveland.[12] The presence of 1,6-anhydro-β-D-glucopyranose among the hydrolytic products of pyrodextrin would support this mechanism.

In the absence of added water and at the high temperature of the reaction, it is also possible that the glycosyl linkage may be attacked by other hydroxyl groups in the polymer molecule, preferably, but not exclusively, the primary hydroxyl of the adjacent D-glucose unit. This would produce (1 → 6) linkages for the most part, without branching. Brimhall[2] has suggested that other primary hy-



droxyl groups may attack the glycosyl linkage in the same way to produce branched structures.



Experimental

Preparation of Pyrodextrin.—Amylose (300 g., dried at 100° for 20 hr.) was placed in a 2-liter open, stainless steel beaker equipped with a mechanical stirrer and heated in an oil-bath. After about 90 min. the temperature of the oil-bath had risen to 225° and the contents of the beaker to 175°. The bath was maintained at 225° while the temperature of the starch increased to 200°. Dextrinization began at about 185° and continued fairly rapidly at 200°. The heating was continued for a total of 2.5 hr. at which time the material became almost completely soluble in water and was yellow in color. The bath was removed and the stirring continued until the material (dextrin A) had cooled.

A second sample was prepared in exactly the same way except that the heating was continued for about 30 min. longer when the material softened to a brown semi-liquid. After cooling, the glassy material (dextrin B) was powdered and stored for further use.

Fragmentation Analysis of Amylose Pyrodextrin.—The yellow dextrin (dextrin A, 32.4 g., equivalent to 36 g. of D-glucose) was dissolved in 9 liters of 0.15 *N* hydrochloric acid and heated with stirring in a boiling water-bath. The course of the reaction was followed quantitatively by means of copper reducing values. The initial reducing value of the dextrin corresponded to a dextrose equivalent of 1.22%. After 4 hr. the hydrolysis had reached 65% completion. Three such runs were made and deacidified by passing through Duolite A-4[17] anion exchange resin. The solution was then placed on a carbon[18] (Nuchar C unground)[19] column which had been pretreated by washing successively with 2 liters of 5% hydrochloric acid, 10 liters of water, 2 liters of 5% aqueous ammonium hydroxide and then water until the effluent was neutral. The column containing the sugar was washed with water until the effluent was negative to Benedict solution. The effluent, normally containing only D-glucose, was discarded. The column then was washed with 3% ethanol, the portion (13 liters) of the effluent containing sugar was collected and was evaporated under reduced pressure to a sirup; yield 7.5 g. A second portion was removed with 5% ethanol; yield 8.0 g. The sirupy residues from the two zones were dried by repeated addition of methanol and evaporation of the solutions under reduced pressure. The material from each of the two zones was acetylated by heating to the boiling point with 10 parts of acetic anhydride and 0.5 part of sodium acetate. The cooled reaction mixtures were poured into 15 times their volume of water and ice. After standing, with occasional stirring, for 2 hr., the solutions were extracted with chloroform, the chloroform solutions dried with anhydrous sodium sulfate and evaporated to a sirup; yield from the 3% ethanol effluent, 15 g.; yield from the 5% ethanol effluent, 12 g.

The acetate of the material removed from the column by 3% ethanol was dissolved in benzene and chromatographed in 5-g. portions on a column (250 × 75 mm.) of Magnesol[20]–Celite[20] (5:1 by wt.) and developed with 2 liters of benzene–*t*-butyl alcohol (100:1 by vol.). The columns were extruded from the tube and streaked with indicator (1% potassium permanganate in 10% sodium hydroxide) to locate the zones. Five zones appeared and were sectioned and eluted with acetone. The acetone eluates were evaporated to sirups and crystallized from ethanol.

The eluate material from a zone 190–225 mm. from the column top crystallized; combined yield 570 mg. (I, see below), m.p. 126–128°. That from a second zone 160–185 mm. from the top crystallized; combined yield 510 mg., m.p. 106–108° cor. undepressed on admixture with known 1,6-anhydro-β-D-glucopyranose triacetate, $[\alpha]^{26}D$ −62.7° (*c* 2.7, chloroform) identifying it as the material named. The material in a third zone 120–140 mm. from the column top crystallized; combined yield 1.5 g. (II, see below), m.p. 151–153°. A fourth zone 65–110 mm. from the column top yielded crystalline material; combined yield 1.5 g. This substance was purified by recrystallization from ethanol; m.p. 144–145°, $[\alpha]^{26}D$ +97° (*c* 3.3, chloroform), identifying it as β-isomaltose octaacetate.

The material removed from the carbon column by 5% ethanol, after acetylation as above, crystallized directly from ethanol; yield 1.15 g., m.p. 187–189°. Pure material was obtained on further recrystallization from ethanol; m.p. and mixed m.p. 189–190° cor., $[\alpha]^{26}D$ −3.0° (*c* 3.5, chloroform), in agreement with the constants of β-gentiobiose octaacetate. The resulting mother liquor upon evaporation gave 10 g. of sirup which was chromatographed on Magnesol–Celite in 5-g. portions as described above. The material from a zone 210–225 mm. from the column top crystallized from ethanol; combined yield 240 mg. (III, see below), m.p. 124–127°. The material from a second zone 135–175 mm. from the column top crystallized from ethanol; combined yield 1.63 g. (IV, see below), m.p. 152–154°.

The fractions I and III were combined (810 mg.) and recrystallized from ethanol; m.p. and mixed m.p. 126-128° cor., $[\alpha]^{26}D$ +3.4° (*c* 2.8, chloroform). These constants are in agreement with those of β-D-glucopyranose pentaacetate. Fractions II and IV were combined (3.1 g.) and recrystallized from ethanol, m.p. and mixed m.p. 158.5-160° cor., $[\alpha]^{26}D$ +60.5°, identifying the substance as β-maltose octaacetate.

A partial hydrolysis of the dextrin B above was carried out as described for dextrin A and the products were examined by successive carbon and silicate chromatography as described. The zone material removed from carbon with 3% ethanol, after acetylation, yielded only β-D-glucopyranose pentaacetate; the zone material removed by 5% ethanol produced on acetylation only β-isomaltose octaacetate and a zone removed by 10% ethanol produced on acetylation β-gentiobiose octaacetate. The mother liquor (2 g.) from the latter material, upon chromatography using a column (250 × 55 mm.) of Magnesol–Celite (5:1 by wt.) and developed with 1500 ml. of benzene *t*-butyl alcohol (100:1 by vol.), produced a zone 145–190 mm. from the

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

(18) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

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

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

column top which upon removal and elution gave crystalline material; yield 100 mg., m.p. 184°, X-ray powder diffraction pattern[21] identical with that of β-sophorose (2-*O*-β-D-glucopyranosyl-β-D-glucose) octaacetate.

(21) A. Thompson, Kimiko Anno, M. L. Wolfrom and M. Inatome, THIS JOURNAL, **76**, 1309 (1954).

Identical partial hydrolysis and analysis[5] of a sample of the unheated amylose (Superlose[14]) yielded only β-D-glucopyranose pentaacetate, β-maltose octaacetate, β-maltotriose octaacetate and no β-isomaltose octaacetate or β-gentiobiose octaacetate.

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[CONTRIBUTION FROM THE FERMENTATION SECTION, NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION[1]]

A Kinetic Study of Dextransucrase[2a]

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Dextran synthesis by cell-free enzyme preparations derived from *Leuconostoc mesenteroides* NRRL B-512F requires both sucrose and a suitable "acceptor" cosubstrate. Molecules of the latter serve as initiators for the formation of new chains. At sucrose concentrations greater than about 0.01 *M*, the initial rate of reaction depends upon the availability of the acceptor, as well as upon sucrose concentration. The effect of a model acceptor substrate, α-methyl glucoside, upon some of the kinetic properties of dextransucrase is reported. The initial rate of reaction has a first order dependence upon α-methyl glucoside concentration in the range 0.15–0.8 *M*; below 0.15 *M* the measured rate is in part due to an unidentified acceptor substrate. Data indicate that this substance is not a contaminant of the enzyme preparation. First-order dependence of the rate upon sucrose concentration is confirmed in the range 0.01–0.11 *M*; the dependence changes through zero order to an inverse dependence (inhibition) at higher sucrose concentrations, as Hehre[3] earlier reported, in the absence of α-methyl glucoside. α-Methyl glucoside relieves the inhibition. Estimates of the Michaelis constant and maximum velocity for either sucrose or α-methyl glucoside depend upon the concentration of the other reactant. An equation is derived which describes the dependence of rate upon the concentrations of sucrose and α-methyl glucoside over the ranges 0.01–0.11 and 0.15–0.80 *M*, respectively. The data are compared with the behavior predicted by certain reaction mechanisms.

Dextransucrase catalyzes a transglucosylation which may be represented empirically as

sucrose + acceptor ⟶ fructose + glucosyl-acceptor

The second product may react repeatedly with sucrose, giving rise to polymeric products. Although the enzyme is, for practical purposes, specific for sucrose as the donor,[3] any of several carbohydrates will serve as acceptor.[4–9] Since the latter serve as chain initiators, some degree of control of the molecular weight distribution has been achieved by making a suitable selection of the acceptor substrate and by the adjustment of the ratio of its concentration to that of sucrose. (Other factors, particularly temperature and enzyme concentration, also affect the molecular weight distribution of the product.[7])

This communication presents a study of the effect of a model acceptor substrate upon some of the kinetic properties of dextransucrase. The acceptor concentration was varied over a range sufficient to shift the average degree of polymerization of the product from the order of several million[10] to the oligosaccharide range.[4,9]

(1) One of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) (a) Presented in part at the 55th General Meeting, Society of American Bacteriologists, New York, N. Y., May 8–12, 1955. Abstracted, Bacteriological Proceedings, 1955, p. 126. (b) Department of Chemical Engineering, Universty of Minnesota, Minneapolis 14, Minn.

(3) E. J. Hehre, *J. Biol. Chem.*, **163**, 221 (1946).

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(9) R. W. Jones, A. Jeanes, C. S. Stringer and H. M. Tsuchiya, *ibid.*, **78**, 2499 (1956).

(10) F. R. Senti and N. N. Hellman, Abstracts of Papers, Am. Chem. Soc., 121st Meeting 8 O (1952).

The high molecular weight dextran synthesized by *Leuconostoc mesenteroides* NRRL B-512F is believed to consist of a number of α-1,6-linked main chains, to which short branches (predominately single glucosyl units) are attached by α-1,3-linkages.[11–13] The main chains may be several hundred units in length and are thought to be associated in a "macro-branched" structure.[12] Although it has been shown[14] that the two types of glucosidic linkages are synthesized by separate enzymes, the ratio of α-1,6;α-1,3 linkages in B-512F dextran (95:5)[11] indicates that the predominant activity of the dextransucrase preparations used in this work is the formation of α-1,6 linkages. The oligosaccharides synthesized in the presence of α-methyl glucoside have been shown to be α-methyl isomaltoside and its α-1,6-linked homologs.[9]

Kinetic constants with respect to sucrose have been reported by Hehre,[3] Carlson,[15] Goodman[16] and their co-workers. Earlier studies[3,4] indicated no effect upon the rate of reaction by an added cosubstrate (dextran), possibly because only a very low molar concentration could be attained experimentally. Some saccharides known to be effective acceptors have been shown to have an effect upon the rate of reaction, as well as upon the molecular weight of the product.[4] Bovey has

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