[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Constitution of a Wheat Starch Dextrin[1]

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An "acid converted" wheat starch dextrin of the "canary" type was investigated to determine the structural transformations brought about by the process of dextrinization. The dextrin was subjected to periodate and methylation studies. It is shown that dextrinization of starch involves transglycosidation and the development of a more highly branched structure.

Pyro-dextrins, the gum-like substances extensively used in the adhesives industry, are prepared by pyrolysis of native starches with or without acids under a wide variety of conditions. Although various theories have been advanced,[2-6] little precise information is available concerning the mechanism of the reaction and the structural changes that occur during the dextrinization of starch.

In a previous paper[7] periodate oxidation studies on an "acid-converted" corn starch dextrin showed that the amount (5%) of D-glucose stable to periodate oxidation is greater than in the parent native starch. The methylation results showed[7] (a) that while the hydrolyzate of methylated amylopectin contains 2,3,4,6-tetra-, 2,3,6-tri- and 2,3-di-*O*-methyl-D-glucose and (b) while the hydrolysate of methylated amylose contains 2,3,4,6-tetra- and 2,3,6-tri-*O*-methyl-D-glucose, the hydrolysate from the methylated corn starch dextrin contained ten different methyl derivatives of D-glucose, namely: 2,3,4,6-tetra-, 2,3,6-tri-, 2,3,4-tri-, 2,4,6-tri-, 2,3-di-, 2,6-di-, 3,6-di-, 2-, 3- and 6-*O*-methyl-D-glucose. Although the dextrin was prepared from the native starch, consisting of amylopectin and amylose, the nature and amounts of these ten cleavage fragments seemed to warrant the tentative conclusion that dextrinization not only involves a reduction in the molecular weight of the starch, a fact long recognized from physico-chemical studies, but that in addition, considerable transglycosidation occurs with the production of a highly branched structure.

This paper is concerned with the structural changes that take place when wheat starch is dextrinized in the presence of an acid catalyst, as revealed by methylation and periodate oxidation studies. Investigations into the dextrinization of amylose and of amylopectin are now being carried out, and they will form the subject of later communications.

The wheat starch dextrin was fractionated from aqueous solution with ethanol (Table I) and the major fraction (no. 10) purified by acetylation (Table II).

A sample of the purified dextrin, regenerated from the acetate, was subjected to periodate oxidation (Table III), sodium borohydride reduction and acid hydrolysis.[8] The periodate oxidation studies indicated that the molecule had an average repeating unit of 8 anhydroglucose residues. Since wheat starch, which is a mixture of the linear and branched chain components, has an average repeating unit of 20–25 glucose residues,[9a] it is apparent that a profound change in structure takes place during the dextrinization process. This value of 8 for the average repeating unit of the dextrin agrees reasonably well with the value of 9.5 from the analysis of the acid hydrolysis products of the dextrin polyalcohol which showed that for every molar proportion of glycerol there were 7.5 molar proportions of erythritol and 1 molar proportion of glucose.

In addition, since the wheat starch dextrin contains approximately 10% glucose that is immune to periodate cleavage, it is clear that new linkages must be produced during dextrinization (the original starch has not been investigated, but by analogy with other starches the amount of glucose stable to periodate is probably no more than 0.5%).[8,10]

Treatment of the dextrin first with methyl sulfate and then with silver oxide and methyl iodide furnished the methylated dextrin, which was found to be essentially homogeneous. After methanolysis of the methylated dextrin, followed by acid hydrolysis, the mixture of methylated sugars was separated by partition chromatography on a cellulose–hydrocellulose column[11] using butanone:water azeotrope[12] as the irrigating solvent and the components identified by preparing suitable crystalline compounds (Table IV).

The hydrolyzate from the methylated wheat starch dextrin was found to consist of 2,3,4,6-tetra- (12.2%), 2,3,6-tri- (74.8%), 2,3-di- (3.9%), 2,6-di- (7.9%), 2- (0.9%) and 3-*O*-methyl-D-glucose (0.3%). Since the dextrin was made from native starch, extensive structural deduction cannot be made, but it is apparent, however, from the percentage (12.2) of 2,3,4,6-tetra-*O*-methyl-D-glucose, that the dextrin must be much more highly branched than the parent wheat starch. It is noticeable that the amounts of tetra- and di-*O*-methylglucose are approximately the same, a result required by the general structural concept that the

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number of terminal, non-reducing ends should be the same as the number of branches in the molecular complex. Since the tetra-*O*-methyl-D-glucose arises from those glucose units which give rise to formic acid upon periodate oxidation, it is of interest to note that the average repeating unit (8) from periodate oxidation studies agrees with the value (8.3) from methylation studies.

The characterization of 2,6-di-*O*-methyl-D-glucose in relatively large amounts (7.9%) reveals the fundamental difference in structure between the dextrin and the parent starch since the methylated starch does not give rise to appreciable amounts of 2,6-di-*O*-methyl-D-glucose.[9a,c/9b,9c] This finding also shows that the dextrinization process not only causes degradation of the starch molecule but that it must also involve the generation of new 1,3-glycosidic linkages. The same phenomenon was observed in the previous studies on the "acid converted" corn starch dextrin.[7] The presence of these 1,3-linkages in the dextrin is also supported by the fact that the parent dextrin contains approximately 10% glucose that is immune to periodate oxidation.

Although the fraction of the wheat starch dextrin subjected to study comprised only 37% of the original dextrin, it seems likely that the same general transformations during dextrinization would be revealed by studies of the other dextrin fractions; the correctness of this view will form the subject of a later investigation.

From the above results, therefore, it seems reasonable to deduce that the physical properties of the dextrins, especially their adhesive, gum-like properties and solubility characteristics, must be attributed, at least in part, to the fact that the dextrins have a highly branched structure, the building units of which are mutually joined by a wide variety of glycosidic bonds.

Experimental

The partition chromatographic analyses were carried out with solvent A, butan-1-ol:ethanol:water (4:1:5),[13] solvent B, butanone:water azeotrope[12] or solvent C, propan-1-ol:water azeotrope.[14] The spray reagent used for the development of color with glucose methyl ethers was *p*-anisidine:trichloroacetic acid:water (1:5:250).[15] Unless stated otherwise all concentrations were carried out *in vacuo* at a bath temperature of 45–50°.

Preparation of the Wheat Starch Dextrin.—Commercial wheat starch was heated for 3 hr. at 120° and then for 5 hr. at 140° in the presence of 2 *N* hydrochloric acid added previously as a spray with mixing. The amount of 2 *N* acid used per gram of starch corresponded to that required to give a *p*H of 3 when added to a mixture of starch (1 part) and water (2 parts); this was predetermined in a separate experiment. The "canary" dextrin so obtained had a slight yellow color, and 95% of it was soluble in water.

Fractional Precipitation of the Wheat Starch Dextrin.—The dextrin (50 g. as prepared and without pre-drying) was dissolved in water (200 ml.) and fractionally precipitated by the addition of increasing amounts of ethanol. Each fraction was redissolved in water and the solution poured with stirring into an excess of ethanol. The white amorphous precipitate was removed by centrifugation and washed successively with ethanol, ethyl ether and light petroleum ether and then dried *in vacuo* at 50°. The results of this fractionation are recorded in Table I.

TABLE I

FRACTIONAL PRECIPITATION OF DEXTRIN FROM AQUEOUS SOLUTION WITH ETHANOL

Fraction no.	1	2	3	4	5	6	7	8	9	10	11	12
Wt., g.	1.5	2.4	2.2	2.6	0.6	1.0	0.4	1.7	7.1	18.5	2.1	5.8
Total ethanol added, ml.	7	17	37	52	72	92	112	137	157	207	257	307
Color with iodine[a]	P	P	P	P-L	P-L	P-L	P-L	L	L	L	L-R	R
$[\alpha]^{25}D$ (H_2O), *c* 1)	+175°	+153°	+153°	+154°	+155°	+158°	+162°	+160°	+156°	+130°	+122°	+121°

[a] P = Purple; L = Lavender; R = Red-brown.

Acetylation of Starch Dextrin.—A solution of the dextrin (17.6 g., fraction 10, Table I) in formamide (100 ml.) was slowly added, with stirring during 3 hr., to a mixture of pyridine (100 ml.) and acetic anhydride (100 ml.) cooled in an ice-bath.[16] After standing for 10 hr. at room temperature the reaction mixture was poured, with stirring, into 8–10 volumes of ice-water to precipitate the acetate. The white amorphous precipitate was filtered, washed successively with absolute ethanol, ether and petroleum ether and dried *in vacuo*. The acetylated dextrin (yield 24 g.) showed $[\alpha]^{25}D$ +158° in chloroform (*c* 0.6). After reacetylation with 5 parts of pyridine–acetic anhydride (1:1) for 3 hr. at 55°, the dextrin acetate (23.5 g.) showed $[\alpha]^{25}D$ +155° in chloroform (*c* 0.6).

The acetyl value was determined by dissolving a sample of the acetylated material in a small volume of acetone, adding an excess of 0.1 *N* NaOH and warming for 2 hr. at 50°. After cooling, the excess alkali was back titrated with 0.1 *N* sulfuric acid; a blank was carried out at the same time (Found: Ac, 44.7%).

The acetylated dextrin (18.5 g.) was fractionally precipitated from chloroform solution by adding increasing quantities of petroleum ether in the usual manner. The results are given in Table II.

TABLE II

FRACTIONAL PRECIPITATION OF DEXTRIN ACETATE

Fraction no.	1	2	3	4	5	6
Total petr. ether added, ml.	10	35	55	75	115	145
Wt., g.	9.2	1.5	2.0	2.4	2.3	0.4
$[\alpha]^{25}D$ ($CHCl_3$, *c* 1)	+158°	+156°	+157°	+151°	+166°	...

Fractions 1, 2 and 3, being essentially the same, were combined and used for the experiments which follow.

Deacetylation of Dextrin Acetate.—A solution of the acetate (3 g.) in 10% (w./w.) aqueous potassium hydroxide (10 ml.) and acetone (15 ml.) was stirred for 1 hr. at room temperature and for 0.5 hr. at 50°. The solution was cooled, neutralized with acetic acid and poured into ethanol. The dextrin precipitate was collected (centrifuge), redissolved in water, reprecipitated with ethanol as before and finally dried *in vacuo* at 45°, giving a white amorphous powder which had $[\alpha]^{24}D$ +132° in water (*c* 0.6).

Periodate Oxidation of Wheat Starch Dextrin.—The dextrin fraction (0.9 g.) was oxidized with 0.1 *N* sodium periodate (100 ml.) at 5° in the dark and aliquots of the reaction solution periodically withdrawn in order to follow

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the rate of periodate consumption and formic acid production in the usual way.[17] Blank experiments were conducted at the same time. The results are summarized in Table III.

TABLE III

PERIODATE OXIDATION OF STARCH DEXTRIN

Reacn. time, hr.	$[\alpha]^{25}D$	Mols. of anhydrohexose per mol. of formic acid produced	Mols. of periodate consumed per mol. anhydrohexose unit
0.08	+77°	25.9	0.66
52	+19	12.7	1.01
138	+19	9.0	1.06
213	+18	7.9	1.06

Reduction of the Periodate Oxidized Dextrin with Sodium Borohydride.—The periodate oxidation reaction mixture was treated with barium chloride solution to precipitate the iodate and excess periodate and the solution centrifuged. Four times the theoretical amount of sodium borohydride was then added to the supernatant solution at room temperature to reduce the dextrin polyaldehyde. The solution was agitated periodically for 3 hr., after which time the evolution of hydrogen ceased and the reduced polysaccharide slowly precipitated from the solution. The precipitated dextrin polyalcohol was removed from the solution, washed with cold ethanol, redissolved in water and reprecipitated with alcohol. The material was then acidified with acetic acid and evaporated to dryness to decompose the residual borohydride. Methanolic hydrogen chloride (30 ml. of 1%) was added, and after shaking for 5 minutes the solution was evaporated *in vacuo* at 40° (bath temperature) to remove borate.[18] The residue was retreated with 1% methanolic hydrogen chloride to ensure complete removal of borates. The dextrin polyalcohol was hydrolyzed by treatment with 0.5 *N* HCl at room temperature for 2 hr. with constant stirring followed by warming at 50° for 1 hr.

The hydrolysis solution was neutralized with $BaCO_3$, filtered and passed through Amberlite IR-120 cation and then through Duolite A_4 anion exchange resin to remove inorganic ions, and evaporated to a sirup. The hydrolyzate showed $[\alpha]^{25}D$ +8.5° in water (*c* 2.7).

Determination of Glucose, Erythritol and Glycerol in the Dextrin Polyalcohol.—A small portion of the sirupy hydrolysate of the dextrin polyalcohol was placed on Whatman No. 1 filter paper and developed with solvent C. Glucose, erythritol and glycerol were located in the boundaries of the paper and the three compounds were then quantitatively separated and eluted from the paper with a known volume of water.[19]

The quantitative determination of glucose was carried out colorimetrically with the phenol–sulfuric acid reagent,[20] and chromotropic acid was used for the quantitative determination of glycerol and erythritol.[21,22]

The molar ratios of glucose:erythritol:glycerol in the hydrolysate of the dextrin polyalcohol was found to be 1.0:7.5:1.0, respectively.

Methylation of Dextrin Acetate.—Wheat dextrin acetate (8.5 g.) was dissolved in acetone (190 ml.) and methylated with methyl sulfate (90 ml.) and sodium hydroxide (250 ml. of 30%) according to the method of Haworth.[23] The reaction was completed by heating for 0.5 hr. on the boiling water-bath when the methylated dextrin separated readily from the reaction mixture. This precipitate was washed by decantation with boiling water to remove salts and then dissolved in acetone in preparation for the next methylation. The mother liquor was extracted with chloroform and the combined extracts dried (Na_2SO_4) and concentrated. The residue was dissolved in acetone and added to the acetone solution of that portion of the dextrin that precipitated from the reaction mixture. Three additional methylations were applied as above, and the product was isolated each time by heating the reaction mixture (chloroform extraction was not necessary).

The methylated dextrin was subjected to three treatments with Purdie reagents using silver oxide (6 g.) and methyl iodide (30 ml.), the reaction mixture being refluxed for 18 hr. After recovering the excess of the methyl iodide, the product was isolated by extraction with acetone, traces of silver iodide being removed by centrifugation. Evaporation gave the methylated dextrin as a brown sirup (4.20 g.).

Fractional Precipitation of Methylated Dextrin.—The methylated dextrin (4.10 g.) was dissolved in acetone (40 ml.) and fractionally precipitated by adding petroleum ether in the usual way. Examination of the five fractions obtained in this manner showed that the product was essentially homogeneous; $[\alpha]D$ +182° in chloroform (*c* 1). Found: OMe, 43.5.

Methanolysis of Methylated Dextrin.—The methylated dextrin (3.30 g.) from a composite of fractions 2, 3 and 4 above was refluxed for 10 hr. with 2.5% methanolic hydrogen chloride. After 3 hr. the rotation of the solution became constant, $[\alpha]^{24}D$ +85° in methanol (*c* 3).

The solution was neutralized (Ag_2CO_3), filtered and the clear yellow solution evaporated with a current of air to avoid possible loss of the methyl 2,3,4,6-tetra-*O*-methyl-D-glucoside. Any residual solvent was removed *in vacuo* at 40°.

Separation of the Cleavage Products of the Methylated Dextrin.—The methyl glycosides (3.42 g.) were heated at 98° with *N* H_2SO_4 (40 ml.) for 19 hr. The solution became cloudy upon adding the acid, and the initial rotation could not be determined, but after 1 hr. it showed $[\alpha]^{25}D$ +49.5° and did not appear to change thereafter. The solution was neutralized ($BaCO_3$), filtered and the residue washed with water. The filtrate and washings were combined and extracted twice with chloroform. The sirup (1.75 g.) from the combined chloroform extracts and that (1.43 g.) from the aqueous layer were separately subjected to partition chromatography on a cellulose–hydrocellulose column using solvent B, the fractions being collected in test-tubes using an automatic fraction collector, during a period of 120 hr.[11]

The methylated sugars were isolated by concentrating the appropriate fractions and provisionally identified by paper chromatography using solvents A, B and C in the usual way. Results of the column chromatography are recorded in Table IV.

TABLE IV

COLUMN CHROMATOGRAPHY OF THE HYDROLYSATE OF METHYLATED STARCH DEXTRIN

Component	Identity of *o*-methyl-D-glucose	*Rf*[a]	Wt., g.	Ratio (approx.) Mole	%
1	2,3,4,6-Tetra-	0.82	0.373	31.5	12.2
2	2,3,6-Tri-	.51	2.290	208	74.8
3	2,3-Di-	.18	0.119	11.5	3.9
4	2,6-Di-	.15	.240	23	7.9
5	2-	.042	.029	3	0.9
6	3-	.062	.010	1	0.3
		Total	3.061[b]		

[a] By paper chromatography with solvent B. [b] Recovery = 96%.

Identification of Cleavage Products of Methylated Dextrin. 1. 2,3,4,6-Tetra-*O*-methyl-D-glucose.—Component 1 (Table IV, 0.373 g.) crystallized spontaneously upon removal of the solvent and after recrystallization from ethyl ether gave 2,3,4,6-tetra-*O*-methyl-α-D-glucose, m.p. 89°, mixed m.p. 88–89°, $[\alpha]^{24}D$ +81.5° equilibrium value in water (*c* 5); lit.[24] m.p. 88–89°, $[\alpha]D$ +83.3° in water. To a solution of the tetra-*O*-methyl-D-glucose (20.7 mg.) in dry pyridine (2 ml.), was added *p*-nitrobenzoyl chloride (48.3 mg.). The mixture was heated at 80–85° (bath temperature) for 40 min. After cooling, a few drops of water was added to the reaction mixture which was then poured into a cold, saturated, sodium bicarbonate solution (10 ml.). Extraction of the product with chloroform and crystallization from aqueous methanol gave the mono-*p*-nitrobenzoate of

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2,3,4,6-tetra-*O*-methyl-D-glucose, m.p. and mixed m.p. 101–102°; lit.[25] m.p. 102°.

2. **2,3,6-Tri-*O*-methyl-D-glucose.**—Component 2 (Table IV, 2.29 g.) crystallized spontaneously and after recrystallization from ethyl ether, gave 2,3,6-tri-*O*-methyl-D-glucose, m.p. and mixed m.p. 112–115°, $[\alpha]^{24}D$ +62° equilibrium value in water (*c* 1); lit.[26] m.p. 121–123°, $[\alpha]D$ +70.5° equilibrium value in water. Treatment of the trimethyl sugar with *p*-nitrobenzoyl chloride in the usual way[27] gave the 1,4-di-*p*-nitrobenzoate of 2,3,6-tri-*O*-methyl-D-glucose, m.p. and mixed m.p. 190–191°, $[\alpha]^{24}D$ −32° in chloroform (*c* 2); lit.[27] m.p. 189–190°, $[\alpha]D$ −33° in chloroform.

3. **2,3-Di-*O*-methyl-D-glucose.**—Component 3 (Table IV, 0.119 g.) crystallized spontaneously from ethyl ether and methanol, giving 2,3-di-*O*-methyl-D-glucose, m.p. and mixed m.p. 85–86°, $[\alpha]^{24}D$ +49° in methanol (*c* 4); lit.[28] m.p. 85–87°, $[\alpha]D$ +48.3° in acetone. Treatment with aniline gave the characteristic N-phenyl-D-glucopyranosylamine 2,3-dimethyl ether, m.p. and mixed m.p. 134°, $[\alpha]^{22}D$ −83° in chloroform (*c* 4) (after recrystallization from ethyl acetate); lit.[29] m.p. 134°.

4. **2,6-Di-*O*-methyl-D-glucose.**—Component 4 (Table IV, 0.240 g.), a sirup, showed $[\alpha]^{24}D$ +63° equilibrium value in water (*c* 5); lit.[30,31] $[\alpha]D$ +63.3° in water. Chromatographic analysis using solvents A and B indicated that it was 2,6-di-*O*-methyl-D-glucose. Treatment with *p*-phenylazobenzoyl chloride in pyridine gave the 1,3,4-triazobenzoate of 2,6-di-*O*-methyl-D-glucose, m.p. and mixed m.p. 202–206° (after recrystallization from ethyl acetate–petroleum ether), $[\alpha]^{23}D$ −260° in chloroform (*c* 0.1); lit.[31] m.p. 205–207°, $[\alpha]_{6252}$ −275° in chloroform.

5. **2-*O*-Methyl-D-glucose.**—Component 5 (Table IV, 0.029 g.) crystallized spontaneously and afforded 2-*O*-methyl-D-glucose, m.p. and mixed m.p. 157–158°, $[\alpha]^{24}D$ +65° equilibrium value in water (*c* 1) (after recrystallization from ethanol); lit.[32–34] m.p. 157–158°, $[\alpha]$ +66° equilibrium value in water.

6. **3-*O*-Methyl-D-glucose.**—Component 6 (Table IV, 0.01 g.) crystallized spontaneously from ethanol and appeared to be essentially pure 3-*O*-methyl-α-D-glucose, m.p. and mixed m.p. 157–158°, $[\alpha]^{24}D$ +56° equilibrium value in water (*c* 1); lit.[28,35] m.p. 161°, $[\alpha]D$ +55.5° equilibrium value in water.

In addition to the six components identified above, column chromatography of the hydrolysate of the methylated dextrin yielded 10 mg. of material whose R_f value, 0.062, corresponded to D-glucose. However, the substance failed to crystallize, and since its rotation, $[\alpha]^{24}D$ +17° in water (*c* 0.2), showed that only one-third of it could be D-glucose, it was not examined further.

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[CONTRIBUTION FROM PULP MILLS RESEARCH AND THE DEPARTMENTS OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF WASHINGTON]

Lignin. VIII. Molecular Weights of Lignin Sulfonates during Delignification by Bisulfite–Sulfurous Acid Solutions

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The average molecular weight has been estimated of the sodium lignin sulfonates dissolved from hemlock, spruce and maple woods into bisulfite–sulfurous acid solutions after several periods of treatment at elevated temperatures. With the gymnosperm woods, it is found that lower molecular weight lignin sulfonates are obtained first, and then as the time of treatment is extended, the average molecular weight of the dissolved lignin sulfonates increases to a maximum, decreases to a minimum and finally begins to rise again. These changes are attributed to the effects of the following three processes which are thought to be proceeding simultaneously but at different rates: hydrolysis of hydrolyzable bonds in the lignin polymer, polycondensation of some lignin molecules with others and diffusion of soluble lignin sulfonates from the wood tissue into solution. With maple wood, the molecular weights of the dissolved lignin sulfonates obtained are about constant and strikingly smaller than those observed for the gymnosperm lignins].

Introduction

In prior reports from this Laboratory, diffusion[2a] and light scattering[2b] methods have been described for the estimation of the average molecular weights of lignin sulfonate preparations. Fractionation of such preparations followed by estimation of the molecular weights of the lignin sulfonates in the resultant fractions has provided information concerning the distribution in lignin sulfonate molecular weights.[2c] Since these distributions were found to be somewhat different for several preparations examined, the present research was undertaken to obtain more knowledge concerning the average molecular weights and the distribution in molecular weights of lignin sulfonates which exist at various stages of delignification of wood by use of bisulfite–sulfurous acid solutions.

Experimental

A description previously has been given[2b] of the preparation of the Western Hemlock wood meal (*Tsuga heterophylla;* 10 to 30 mesh; ethanol–benzene and hot water extracted; 6.14% H_2O and 5.15% OCH_3) used for the experiments. Delignifications were carried out by sealing into a Pyrex tube 1.00 or 4.50 g. of wood meal with ten times its weight of an aqueous solution which contained 50 g. of SO_2/liter and 9.67 g. of Na_2O/liter for series I, and 90 g. of SO_2/liter and 9.67 g. of Na_2O/liter for series II. Each bomb was placed in a steel tube which was closed, fastened to a "Ferris" wheel in an oil-bath at 135° (±0.1°) and rotated

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