The Constitution of a Starch-Galactose Codextrin[1]

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ABSTRACT

The acid- and heat-catalyzed dextrinization of amylopectin in the presence of D-galactose afforded a codextrin containing 13.5% D-galactose. Periodate and methylation studies established that D-galactose had become incorporated into a polymer, whereas isolation of 6-*O*-α-D-glucopyranosyl-α-D-galactopyranose from a partial acid hydrolysate of a similar codextrin provided evidence, together with the methylation data, that the D-galactose was an integral part of a galactoglucan codextrin. The initial product of the codextrinization was heterogeneous, as shown by the fractionation studies. Structural studies on a major codextrin fraction showed that it had an average D.P. of 37 and was highly branched, as deduced from the high proportion of tetra-*O*-methyl-sugars recovered from the hydrolysate of the methylated codextrin. The methylation and periodate data indicate an average repeating unit of 5 for the codextrin.

The structural studies of corn (1,2,3) and wheat (4) starch dextrins established that transglycosylation occurred during the dextrinization process, in addition to molecular-weight (MW) reduction. The rearranged molecular structure produced by acidic transglycosylation required the cleavage of glycosidic linkages, with the resultant transient carbonium ion or levoglucosan residue reacting with hydroxyl groups of other glycosyl residues in the reaction mixture. It was suggested that new carbohydrate polymers, termed codextrins, might be obtained by introducing sugars, oligosaccharides, polyhydric alcohols, and other polysaccharides into the reaction (1). The reactive center produced by chain cleavage during dextrinization would react with the admixed carbohydrate to produce new glycosidic bonds.

Christensen confirmed this view; he prepared a series of codextrins by reacting a variety of polysaccharides with mono- and disaccharides (3). The purified products, free of low-MW reducing substances, were found to contain the added sugar when they were subjected to acid hydrolysis. Additional experiments with ^{14}C-labeled D-glucose and 2,3,6-tri-*O*-methyl-D-glucose (5) have confirmed that the foreign sugar is indeed incorporated into the dextrin molecule and is not present as a separate homogeneous polymer intermixed with the starch dextrin.

This process of codextrinization apparently offers a wide variety of heteropolysaccharides for industrial applications by the use of inexpensive and readily available reactants. The method is particularly attractive if definitive product characteristics are obtainable by controlling the percent incorporation of foreign sugar and the primary location it assumes in the rearranged molecular structure.

Starch-galactose codextrins have been prepared to gain insight into these questions and to lend additional support to the over-all concept of dextrinization.

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MATERIALS AND METHODS

Chromatographic separations were carried out by the descending method on Whatman No. 1 paper with the following solvents: (A) 1-butanol:ethanol:water (4:1:5, upper phase); (B) 1-butanol:ethanol:water (3:2:1); (C) 1-butanol:pyridine:water (6:4:3); (D) pyridine:ethyl acetate:water (2:5:7, upper phase); (E) pyridine:ethyl acetate:water (4:10:3); (F) butanone:water azeotrope; and (G) benzene:ethanol:water (200:47:15, upper phase). The sugars and their derivatives were detected by (H) ammoniacal silver nitrate (6) and (I) *p*-anisidine trichloroacetic acid (4).

Solutions were deionized with cation exchange resin, Amberlite IR-120, and anion exchange resin, Duolite A-4. All solutions were concentrated *in vacuo* at a bath temperature of 45°–50°.

Amylopectin was defatted by extraction with hot methanol.

Preparation of Starch-Galactose Codextrins

Defatted amylopectin (110 g., 0.69 mole; a mole of starch is intended to mean the gram formula weight of one anhydroglucose unit) and D-galactose (50 g., 0.28 mole) were ground together in a mortar and sprayed with 2.2*N* hydrochloric acid (5.2 ml.). The acid was applied in such manner as to distribute the catalyst uniformly throughout the reactants. After standing overnight the mixture was heated at 140° for 3 hr. in a nitrogen atmosphere with stirring. The product was treated with water (500 ml.), and a small amount of tarry insoluble material was removed by centrifugation. The codextrin was precipitated by adding the supernatant dropwise with stirring into acetone.

A second codextrin was prepared analogously from dry defatted waxy maize starch (55 g., 0.34 mole), D-galactose (32 g., 0.17 mole), and 2.4*N* hydrochloric acid (2.4 ml.). The isolated product was twice dissolved in water (300 ml.) and precipitated with methanol. The dry material (40 g.) was used without further purification for partial acid hydrolysis and is referred to hereafter as codextrin B.

Fractional Precipitation of Codextrin

The product (92.8 g.) was dissolved in water (1.0 liter) and fractionally precipitated by adding increasing amounts of ethanol. Each fraction was dissolved in water, reprecipitated in ethanol, washed with ethanol, ethyl ether, and petroleum ether, and dried *in vacuo*. The results are given in Table I.

Acetylation of Codextrin

A solution of the codextrin (31.85 g., fractions 9–13) in formamide (200 ml.) was added slowly with stirring to an ice-cold mixture of pyridine (160 ml.) and acetic anhydride (160 ml.) during 3.5 hr. The reaction mixture was kept at room temperature overnight and then warmed at 40° for 1 hr. The product was isolated by pouring the cooled mixture into ice-water (3.5 liters). The precipitate was washed with ethanol, ethyl ether, and petroleum ether and dried *in vacuo*. The acetylated codextrin showed $[\alpha]_D^{21}$ + 127.5° in chloroform (*c*, 1.0). After reacetylation with 4 parts of pyridine-acetic anhydride (1:1) for 6 hr. at 50°, the codextrin acetate (34.2

TABLE I

FRACTIONAL PRECIPITATION OF THE CODEXTRIN[a]

FRACTION	WEIGHT	ETHANOL ADDED	$[\alpha]_D^{22}$ IN WATER	FRACTION	WEIGHT	ETHANOL ADDED	$[\alpha]_D^{22}$ IN WATER
	g.	*liters*	*c, 1*		*g.*	*liters*	*c, 1*
1	6.60	1.0	+ 138	10	7.28	1.45	+ 134
2	1.98	1.05	+ 139	11	6.05	1.55	+ 133
3	3.96	1.075	+ 139	12	8.60	1.65	+ 136
4	4.11	1.1	+ 139	13	5.7	1.75	+ 133
5	1.30	1.125	+ 139	14	4.22	1.85	+ 134
6	1.67	1.15	+ 142	15	3.24	1.95	+ 134
7	7.07	1.2	+ 141	16	1.90	2.05	+ 133
8	4.31	1.25	+ 140	17	2.85	2.25	+ 132
9	7.07	1.35	+ 140	18	1.48	2.45	+ 132

[a]All fractions gave a reddish-brown color with iodine.

g.) showed $[\alpha]_D^{21}$ + 128.9° in chloroform (*c*, 1.0) with an acetyl value of 45.3% (calcd. OAc, 44.8%).

The acetylated codextrin (32.2 g.) was dissolved in chloroform (150 ml.) and fractionally precipitated by adding increasing amounts of petroleum ether. The results are summarized in Table II.

TABLE II

FRACTIONAL PRECIPITATION OF THE CODEXTRIN ACETATE

	FRACTION								
	1	2	3	4	5	6	7	8	9
Weight (g.)	0.26	4.11	6.79	3.21	2.20	5.30	3.49	2.42	3.10
Petroleum ether added (ml.)	180	190	200	210	220	240	260	280	310
$[\alpha]_D^{23}$ in $CHCl_3$ (*c*, 1)	+ 123	+ 124	+ 125	+ 123	+ 130	+ 132	+ 131	+ 131	+ 130

Deacetylation of Acetylated Codextrin

The acetate (11.21 g., fractions 6–8) was deacetylated by treatment with a mixture of acetone (100 ml.) and *N* sodium hydroxide (150 ml.) for 1 hr. at 50°. The acetone was removed under reduced pressure and the resulting alkaline solution was neutralized with glacial acetic acid. The product was isolated by pouring the solution into ethanol with stirring. The precipitate was washed with ethanol, ethyl ether, and petroleum ether, and dried to give a white amorphous powder (6.45 g.), $[\alpha]_D^{24}$ + 137.7° in water (*c*, 1.0).

Determination of Glucose and Galactose in Codextrin

The deacetylated codextrin (150 mg.) was dissolved in *N* sulfuric acid (3 ml.) and hydrolyzed in a sealed tube for 14 hr. in a boiling-water bath. The hydrolysate was neutralized with barium carbonate, filtered, and concentrated to a syrup.

A quantitative chromatographic separation of a portion of the syrup was carried out on paper with solvent B. The components were located by

spraying guide strips from the chromatogram with reagent H. The D-glucose, D-galactose, and appropriate blanks were eluted with water and analyzed colorimetrically by the phenol-sulfuric method (7). The codextrin and codextrin B contained 13.5 and 11.9% D-galactose, respectively.

Periodate Oxidation of Codextrin

A portion of the deacetylated codextrin (0.1910 g.) was oxidized with 0.09*N* sodium periodate (250 ml.) at 5° in the dark. Aliquots of the reaction solution were removed periodically and analyzed for periodate consumption and formic acid production in the usual manner (8). Blank experiments were conducted concurrently. The results are summarized in Table III.

TABLE III
PERIODATE OXIDATION OF THE CODEXTRIN

	Reaction Time (Hours)				
	1	24	68	140	201
Hexose, moles per mole formic acid	7.5	3.7	3.2	2.6	2.4
Periodate, moles per hexose unit	0.49	1.17	1.26	1.28	1.28

Reduction and Hydrolysis of Periodate-Oxidized Codextrin

The oxidation mixture was treated with a solution of barium chloride to remove iodate and excess periodate ions, and the polyaldehyde was reduced with sodium borohydride (0.2 g.) at room temperature for 18 hr. The solution, nonreducing to boiling Fehling's solution, was acidified (pH 5) and concentrated to dryness. The borates were removed by repeated treatment with 1% methanolic hydrogen chloride and concentration, and the codextrin polyalcohol was hydrolyzed with *N* hydrochloric acid in a boiling-water bath for 6 hr.

The hydrolysate was neutralized with ion-exchange resins and concentrated to a syrup. Chromatographic analysis, with solvent D and spray reagent H for identifying the components, indicated the presence of glycerol, erythritol, D-glucose, and D-galactose in the hydrolysate.

Determination of Glycerol, Erythritol, D-Glucose, and D-Galactose

Two aliquots of the hydrolysate (0.1795 ml.) were applied to separate chromatograms. The chromatogram for glycerol and erythritol analyses was developed with solvent A; the other chromatogram was developed with solvent D for assay of D-glucose and D-galactose. The components were located and quantitatively eluted from the paper.

D-Glucose and D-galactose were determined quantitatively with phenol-sulfuric acid (7), and chromotropic acid was used for quantitative determination of glycerol and erythritol (9). The molar ratio of D-galactose:D-glucose:glycerol:erythritol was 1:3:15:48.

Molecular-Weight Determination of Codextrin

The molecular weight was determined by the reducing end group analysis of Unrau and Smith (10). Two portions of the deacetylated codextrin (49.5 mg.) were dissolved in water (20 ml.) and one solution was treated with

sodium borohydride. Both solutions were acidified with glacial acetic acid and oxidized with 0.5*N* sodium periodate at 5° in the dark. The formaldehyde produced from the reduced sample was determined with chromotropic acid. Corrections were made for blank experiments.

Methylation of Codextrin

The deacetylated codextrin (2.5 g., 0.015 mole) was dissolved in liquid ammonia (300 ml.), and sodium (1.34 g., 0.06 mole) was added in small portions followed by methyl iodide (8.52 g., 0.06 mole). After 15 min., the process was repeated three times with sodium (0.23 g., 0.01 mole) and methyl iodide (1.42 g., 0.01 mole). The ammonia was evaporated and the product taken up in chloroform. The extract was washed with water, dried (magnesium sulfate), and concentrated to a glass (3.15 g.).

The partially methylated codextrin was dissolved in *N,N*-dimethylformamide (50 ml.), and silver oxide (15 g.) and methyl iodide (15 ml.) were added to the solution. The reaction mixture was shaken for 24 hr. at room temperature, filtered, and re-treated in the same way. The product was recovered from the salts by extraction with chloroform. Methylation was continued after removal of the solvent by twice refluxing the product with methyl iodide (25 ml.) and silver oxide (5 g.) for 15 hr. Excess methyl iodide was distilled, and the fully methylated codextrin (2.75 g.), OCH_3 45.1% (calcd. OCH_3, 45.5%), was extracted with acetone and recovered as a glass by concentration.

Fractional Precipitation of Methylated Codextrin

The methylated product was dissolved in acetone (25 ml.) and fractionally precipitated by adding increasing amounts of petroleum ether. Each fraction was washed with petroleum ether and dried *in vacuo*. The results are given in Table IV.

TABLE IV
FRACTIONAL PRECIPITATION OF THE METHYLATED CODEXTRIN

	Fraction			
	1	2	3	4
Weight (g.)	0.43	0.77	1.15	0.30
Petroleum ether added (ml.)	60	64	70	excess
OCH_3, %	44.9	45.1	45.0	45.0
$[\alpha]_D$ in $CHCl_3$ (*c*, 1)	+ 157	+ 158	+ 158	+ 160

Hydrolysis of Methylated Codextrin

The methylated codextrin (1.88 g., fractions 2 and 3, Table IV) was dissolved in methanolic hydrogen chloride (50 ml., 2.15%) and the solution was refluxed for 15 hr. until the rotation became constant. The solution was neutralized with silver carbonate and the filtrate was concentrated to a syrup (2.2 g.).

The mixture of glycosides was heated in *N* sulfuric acid (45 ml.) in a boiling-water bath for 15 hr. The rotation of the solution became constant

in 8 hr. The hydrolysate was neutralized with barium carbonate, filtered, deionized, and concentrated to a colorless syrup (1.724 g.).

Separation of Components of the Hydrolysate

The methylated sugar mixture (1.706 g.) was separated into its components by column chromatography with cellulose-hydrocellulose and solvent F. Fractions from the column which contained more than one component were separated by paper chromatography with solvents F and G and the compounds eluted from the paper. The results are given in Table V.

TABLE V

COLUMN CHROMATOGRAPHY OF THE HYDROLYSATE OF METHYLATED CODEXTRIN

Component	Methyl Derivative	Weight	Mole Percent
		g.	%
1	2,3,4,6-tetra-*O*-methyl-D-glucose	0.278	16.24
2	2,3,4,6-tetra-*O*-methyl-D-galactose	0.064	3.72
3	2,3,4-tri-*O*-methyl-D-glucose	0.039	2.41
4	2,3,6-tri-*O*-methyl-D-glucose	0.904	56.16
5	2,4,6-tri-*O*-methyl-D-galactose	0.014	0.86
6	2,4,6-tri-*O*-methyl-D-glucose	0.019	1.17
7	2,3,4-tri-*O*-methyl-D-galactose	0.049	3.04
8	2,3-di-*O*-methyl-D-glucose	0.134	8.87
9	2,4-di-*O*-methyl-D-glucose	0.024	1.58
10	3,6-di-*O*-methyl-D-glucose	0.015	0.99
11	2,6-di-*O*-methyl-D-glucose	0.041	2.71
12	2,4-di-*O*-methyl-D-galactose	0.021	1.38
13	3-*O*-methyl-D-glucose	0.012	0.86
14	2-*O*-methyl-D-glucose		
15	6-*O*-methyl-D-glucose		
16	6-*O*-methyl-D-galactose		
		1.614 g. = 94% recovery	

Identification of Components from Methylated Codextrin

(A) 2,3,4,6-Tetra-*O*-methyl-D-glucose, component 1, crystallized spontaneously and after recrystallization from petroleum ether gave 2,3,4,6-tetra-*O*-methyl-α-D-glucose, m.p. and mixed m.p. 97°–98°, $[\alpha]_D^{23}$ + 81.7° in water (*c*, 0.9); lit. (11) m.p. 96°, $[\alpha]_D$ + 84° in water.

(B) 2,3,4,6-Tetra-*O*-methyl-D-galactose, component 2, was isolated as a syrup, $[\alpha]_D^{24}$ + 130° (eq.) in water (*c*, 1.0); lit. (11) $[\alpha]_D^{24}$ + 162° → + 138° (42 hr., eq.) in water. Treatment with aniline yielded the crystalline anilide of 2,3,4,6-tetra-*O*-methyl-D-galactose, m.p. and mixed m.p. 195°–197°, $[\alpha]_D^{24}$ – 143° in pyridine (*c*, 0.7); lit. (11) m.p. 197°, $[\alpha]_D^{22}$ – 141° in pyridine (*c*, 0.8).

(C) 2,3,4-Tri-*O*-methyl-D-glucose: The syrupy component 3 showed $[\alpha]_D^{22}$ + 58° in methanol (*c*, 1.3); lit. (11) $[\alpha]_D^{24}$ + 61° in methanol.

The syrup yielded a crystalline anilide on treatment with aniline. The recrystallized product showed $[\alpha]_D^{23}$ – 100° in ethanol (*c*, 0.5), m.p. 147°–149°, mixed m.p. 148°; lit. (11) m.p. 150° and $[\alpha]_D$ – 103° in ethanol.

(D) 2,3,6-Tri-*O*-methyl-D-glucose, component 4, crystallized immediately upon concentration, and after recrystallization from ethyl ether the 2,3,6-tri-*O*-methyl-D-glucose had m.p. 119°–121° and mixed m.p. 118°–

120°, $[\alpha]_D^{23}$ + 69.4° in water (*c*, 0.9); lit. (11) 121°–123°, $[\alpha]_D$ + 70.5° in water.

(E) 2,4,6-Tri-*O*-methyl-D-glucose, component 5, crystallized partially when seeded with an authentic specimen. The isolated compound had m.p. and mixed m.p. 124°–127°, $[\alpha]_D^{24}$ + 64° in methanol (*c*, 0.5) (on syrup); lit. (11) m.p. 123°–126°, $[\alpha]_D$ + 70° in methanol.

The syrup on treatment with aniline gave a crystalline anilide. The anilide melted 159°–161°, mixed m.p. 160°–162°; lit. (11) m.p. 162°–166°.

(F) 2,4,6-Tri-*O*-methyl-D-galactose, component 6, was a syrup having a rotation of $[\alpha]_D^{23}$ + 89.4° in water (*c*, 0.9); lit. (11) $[\alpha]_D$ + 90° in water.

The anilide of 2,4,6-tri-*O*-methyl-D-galactose was prepared and melted alone and in admixture with an authentic specimen at 176°–178°; lit. (11) m.p. 179°.

(G) 2,3,4-Tri-*O*-methyl-D-galactose, component 7, was a syrup showing $[\alpha]_D^{26}$ + 112° in water (*c*, 1.0); lit. (11) $[\alpha]_D$ + 114° in water.

Treatment of component 7 with aniline gave *N*-phenyl-D-galactosylamine-2,3,4-tri-*O*-methyl ether, m.p. 164°–166°, mixed m.p. 165°–167°; lit. (11) m.p. 167°.

(H) 2,3-Di-*O*-methyl-D-glucose, component 8, crystallized when it was seeded with an authentic specimen. After recrystallization it had m.p. and mixed m.p. 110°–112°, $[\alpha]_D^{26}$ + 52.4° in acetone (*c*, 0.6); lit. (11) m.p. 110°, $[\alpha]_D$ + 51° in acetone.

The crystalline anilide melted 132°–135° and had mixed m.p. 133°–135°; lit. (11) m.p. 134°.

(I) 3,6-Di-*O*-methyl-D-glucose: This fraction had $[\alpha]_D^{23}$ + 68.6° in water (*c*, 1.3) and was a mixture of 3,6-di-*O*-methyl-D-glucose ($[\alpha]_D$ + 76.5° in water) (11), since oxidation of a portion of the syrup (1 mg.) with periodic acid under conditions known to oxidize 3,6-di-*O*-methyl-D-glucose completely to 2,5-di-*O*-methyl-D-arabinose without oxidizing 2,4-di-*O*-methyl-D-glucose (12) gave two components. These two components were identical by paper chromatography (solvent F) with authentic 2,5-di-*O*-methyl-D-arabinose and 2,4-di-*O*-methyl-D-glucose.

The syrupy mixture was treated with methanolic hydrogen chloride (1%) at room temperature to convert the 3,6-di-*O*-methyl-D-glucose into the methyl furanoside, leaving 2,4-di-*O*-methyl-D-glucose unchanged. The two components were separated by preparative paper chromatography. The glycofuranoside was eluted from the paper and hydrolyzed by heating with Amberlite IR-120 cation-exchange resin. Concentration of the hydrolysate gave crystalline 3,6-di-*O*-methyl-D-glucose, m.p. and mixed m.p. 115°–117°; lit. (13) m.p. 114°–115°.

(J) 2,4-Di-*O*-methyl-D-glucose: The slower-moving component from (I) was eluted from the paper and concentrated to a syrup. It was heated with *p*-nitroaniline and glacial acetic acid in ethanol in a sealed tube at 85° for 1 hr. Evaporation of the solvent afforded *N*-*p*-nitrophenyl-2,4-di-*O*-methyl-D-glucosylamine, m.p. 248° (dec.); lit. (11) m.p. 250° (dec.).

(K) 2,6-Di-*O*-methyl-D-glucose, component 11, was a syrup with a rota-

tion of $[\alpha]_D^{24}$ + 63.9° in water (*c*, 0.6); lit. (11) $[\alpha]_D$ + 63.6° in water. The syrup was converted into 2,6-di-*O*-methyl-D-glucose 1,3,5-tri-*p*-phenylazobenzoate, m.p. 203°–206°, mixed m.p. 203°–206°, $[\alpha]_D^{25}$ – 323° in chloroform (*c*, 0.3); lit. (11) m.p. 206°–208°, $[\alpha]_D^{25}$ – 341° in chloroform.

(L) 2,4-Di-*O*-methyl-D-galactose: The syrupy component 12 showed $[\alpha]_D^{24}$ + 85.9° in water (*c*, 0.6); lit. (11) $[\alpha]_D$ + 85.6° in water. The crystalline anilide was prepared and had m.p. and mixed m.p. 215°–217°; lit. (11) m.p. 216°.

The mono-*O*-methyl sugars were identified only by chromatography.

Partial Acid Hydrolysis of Codextrin B

Codextrin B (10 g.) was hydrolyzed with 0.1*N* sulfuric acid (1.0 liter) for 10 hr. at 90°. The reducing value based on glucose was 50% of the maximum value possible by complete hydrolysis. The hydrolysate was neutralized with barium carbonate, deionized and concentrated *in vacuo* to a syrup was converted into 2,6-di-*O*-methyl-D-glucose 1,3,5-tri-*p*-phenylazobenand oligosaccharides.

Fractionation of Codextrin Partial Hydrolysate

The syrup (7.6 g.) was dissolved in water (50 ml.) and added to a column (3 × 50 cm.) of activated coconut charcoal (14). The monosaccharides were eluted with water and the disaccharides with 5% ethanol. The latter eluate (8 liters) was concentrated *in vacuo* to a small volume (25 ml.) and examined by paper chromatography with the use of solvent D. Isomaltose and three other oligosaccharides, $R_{melibiose}$ 0.96, 0.85, and 0.72, were detected.

A portion of the oligosaccharide mixture was separated quantitatively on Whatman No. 3MM paper with the use of solvent D. The oligosaccharides with $R_{melibiose}$ 0.96 (A) and 0.85 (B) were eluted with water (yield 30 mg. and 150 mg. respectively). Each component was purified further by two paper-chromatographic separations in solvent E. At this point each oligosaccharide gave a single spot on a chromatogram with solvents B, C, D, and E. Oligosaccharide A had $[\alpha]_D^{22}$ + 102° in water (*c*, 0.84). All attempts to crystallize disaccharide A or its acetate were unsuccessful.

Identification of Oligosaccharides

Hydrolysis. Paper chromatography of the acid hydrolysate of oligosaccharide A revealed D-glucose and D-galactose present in equimolar quantities. Similarly, oligosaccharide B contained D-galactose only and was not investigated further.

Reduction. Disaccharide A was reduced with sodium borohydride and the borates were removed by treatment with methanolic hydrogen chloride and concentration. Acid hydrolysis of the reduced compound and paper-chromatographic analysis of the neutralized hydrolysate with solvent B revealed D-glucose as the only reducing sugar detectable with aniline phosphate and aniline oxalate spray reagents (15).

Methylation. The disaccharide (6 mg.) was dissolved in *N,N*-dimethylformamide (5 ml.) and shaken with methyl iodide (5 ml.) and silver oxide

(0.5 g.) for 18 hr. at room temperature in the dark. The reaction mixture was filtered and concentrated, and the entire process was repeated twice. The final concentrate was hydrolyzed with *N* sulfuric acid in a boiling-water bath for 12 hr. The hydrolysate was neutralized with barium carbonate, filtered, and chromatographed on paper with solvents B and F. Two methylated sugars, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-galactose, were detected and identified by their comparable mobility with authentic samples.

DISCUSSION

The results of the structural analysis of this codextrin are consistent with analogous studies on corn and wheat starch dextrins (1,4); that is, there is a drastic reduction in molecular weight and development of a highly branched structure. The crude codextrin was heterogeneous as shown by fractionation studies, and structural studies were carried out on one fraction. The average degree of polymerization of the codextrin was 37 as determined by the end group analysis method of Unrau and Smith (10). This value is far removed from the D.P. 2250 obtained for amylopectin by the same method (16). The complexity of structure is shown by the broad spectrum of methylated D-glucose and D-galactose derivatives produced on hydrolysis of the methylated codextrin (Table V). Of the 11 D-glucose derivatives listed in Table V only components 1, 4, and 8 are obtained in significant quantities from methylated amylopectin. The methylated D-galactose derivatives strongly support the concept of codextrinization—that is, the incorporation of a foreign sugar into a polysaccharide under conditions of acid and heat. The mole percents of tetra- (3.72) and di-*O*-methyl-D-galactose (1.38) are significantly different. This affords evidence that the D-galactose is not present as a homogeneous polymer contaminating a D-glucose dextrin, for the general structural concept demands that in a polysaccharide the number of terminal, nonreducing ends must be equal to the number of branches in the molecule. This requirement is satisfied in the total analysis, however, since the mole percent of tetra-*O*-methyl sugars, 19.96%, agrees favorably with the mole percent of di- (15.53%) and mono-*O*-methyl sugars (0.86%).

The total amount (19.96 mole %) of 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methyl-D-galactose suggests an average repeating unit of 5. This value is in good agreement with periodate oxidation data.

Analysis of the acid hydrolysis products of the codextrin polyalcohol produced by periodate oxidation and sodium borohydride reduction showed that for every molar proportion of D-galactose there were 3 molar proportions of D-glucose, 15 of glycerol, and 48 of erythritol. The molar ratio of erythritol, D-galactose, and D-glucose to glycerol, therefore, is 3.5 to 1. Glycerol is derived from nonreducing terminal D-glucopyranosyl and D-galactopyranosyl residues, which would give rise to tetra-*O*-methyl sugars in the methylated dextrin, and the average repeating unit on this basis would be 3.5. Since this value is lower than that indicated by the methylation data, it suggests that some of the glycerol is derived from nonterminal units; this is supported by the methylation data, which predict that 20% of the glycerol is derived from

1→6 linkages. With this taken into account, the average repeating unit would be about 5.5.

The formic acid production, 1 mole of formic acid per 2.4 moles of anhydrohexose, is lower than expected from the glycerol derived from the polyalcohol (1 glycerol per 4.5 hexose residues) or that predicted by the methylation data (1 mole formic per 4 hexose residues). This discrepancy may result from overoxidation.

Further experimental evidence for structural rearrangement and monomer incorporation follows from (a) the fact that 6% of D-galactose and D-glucose (in a mole ratio of 1 to 3) was immune to periodate oxidation; (b) the isolation of 2,4,6-tri-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-galactose, 2,4-di-*O*-methyl-D-glucose, 3,6-di-*O*-methyl-D-glucose, and 2,4-di-*O*-methyl-D-galactose from the methylated dextrin; and (c) the isolation of 6-*O*-α-D-glucopyranosyl-D-galactose by partial hydrolysis of the dextrin.

The disaccharide was isolated from the partial acid hydrolysate of codextrin B under conditions that give rise to a minimum of reversion (17). That it was a disaccharide is apparent from the chromatographic mobility and from the observation that further hydrolysis of the compound produced equimolar quantities of D-glucose and D-galactose. Sodium borohydride reduction and subsequent acid hydrolysis gave D-glucose as the only reducing sugar, thereby establishing D-galactose at the reducing end of the disaccharide. This was confirmed by identification of 2,3,4-tri-*O*-methyl-D-galactose from the hydrolysate of the methylated compound, along with 2,3,4,6-tetra-*O*-methyl-D-glucose arising from the nonreducing portion of the molecule. The structure of the disaccharide is established, therefore, as 6-*O*-α-D-glucopyranosyl-D-galactose (18).

From the variety of tri- and di-*O*-methyl sugars that have been characterized in the methylated codextrin, it is apparent that the branching is highly diversified, as evidenced by single linkages at C-3, C-4, and C-6 and branch points involving carbons 4 and 6, 2 and 4, 3 and 6, and 3 and 4 in the D-glucose and D-galactose residues.

It is encouraging to find relatively the same galactose incorporation into two different codextrin preparations. Even so, it is apparent that extensive investigations of the various reaction parameters is needed before predictable products will become available.

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