The Constitution of an Amylopectin-Xylose Codextrin[1]

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ABSTRACT

Dextrinization of corn amylopectin in the presence of D-xylose gave a codextrin containing 13.4% D-xylose. Methylation analysis established that both transglycosylation of the amylopectin, to give a highly branched polymer, and polymerization of the xylose had occurred. Further evidence for these observations was provided by periodate oxidation studies which confirmed that a highly branched polymer was formed, and established that glucose and xylose were components of the same polymer.

Previous studies (1) have established that dextrinization of starch through the agency of acid and heat involves extensive chain scission and transglycosylation, resulting in considerable reduction in polymer molecular weight and formation of a new, highly branched polymer. Furthermore, when the dextrinization is performed in the presence of other monomers (2,3), they are incorporated into the dextrin molecule; and it was established that in a codextrin prepared from amylopectin and D-galactose, the incorporated monomer, D-galactose, was inserted into the molecule, principally as nonreducing terminal pyranose units and as (1 → 6)-linked alpha-D-galactopyranose units (4).

This reaction is not limited to sugars having a primary carbinol, for it has been shown that D-xylose (2) and 2,3,6-tri-*O*-methyl-D-glucose (3) are polymerized under these dextrinization conditions. The structural studies of the codextrins have now been extended to an amylopectin-D-xylose codextrin, and the results of these studies are reported herein.

MATERIALS AND METHODS

Paper-chromatographic analyses were carried out by the descending method on Whatman No. 1 paper with the following solvent systems: (A) pyridine-ethyl acetate-water, 2:5:7, (B) 1-butanol-ethanol-water, 3:2:1, and (C) butanone-water azeotrope; and mobilities are expressed relative to glucose (R_g). Paper electrophoresis was performed with 0.1*M* sodium borate buffer at 600 volts for 2 hr. Solutions were concentrated *in vacuo* below 45°.

Dextrinization of Amylopectin in the Presence of D-Xylose

Waxy maize starch (26 g.), defatted by extraction with hot methanol, was mixed with D-xylose (12.3 g.), and 2.4*N* hydrochloric acid (1.2 ml.) was sprayed into the mixture. After thorough mixing, the reaction mixture was kept at 25°C. for 12–13 hr. and then heated under nitrogen at 140°C. for 3 hr. with stirring. The product was dissolved in water (125 ml.) and the dextrin was precipitated by pouring the solution into 6 vol. of methanol. The precipitated dextrin was redissolved and precipitated three times in the same

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manner, and the product was then washed with ethanol and petroleum ether and dried *in vacuo* at 75°C. (yield 20 g.).

Paper chromatography of the product revealed no reducing sugars.

Composition of the D-Xylose-Amylopectin Codextrin

A portion of the codextrin was hydrolyzed with 1*N* sulfuric acid at 95°C. for 12 hr. The hydrolysate was chromatographed (solvent A) and the glucose and xylose were eluted and analyzed by the phenol-sulfuric acid method (5). The hydrolysate contained D-glucose and D-xylose in the molar ratio 5.50:1.06.

Acetylation of the Codextrin

The amylopectin-D-xylose codextrin (2 g.) was acetylated in formamide (15 ml.) with pyridine (15 ml.) and acetic anhydride (15 ml.) for 15 hr., and the acetate was isolated by pouring the reaction mixture into ice-water. After a second acetylation in pyridine and acetic anhydride at 50°C. for 6 hr., the product was isolated in the same manner and dissolved in chloroform. When the chloroform solution was poured into ethyl ether the acetate precipitated and was dried *in vacuo;* yield 2.3 g., $[\alpha]_D^{23}$ + 117.7° (*c* 0.4 in chloroform), OAc 44.02% (calcd. OAc 44.18%).

Methylation of the Codextrin

The codextrin (10 g.) was reduced with sodium borohydride (0.5 g.) and methylated by the Haworth procedure with dimethyl sulfate (150 ml.) and 30% sodium hydroxide (475 ml.). After three Haworth methylations the product had OCH_3 38.2% (yield 9.2 g.). A portion of this partially methylated product (4 g.) was remethylated three times by the Purdie method, giving the methylated product (yield 3.2 g.) with $[\alpha]_D^{24}$ + 131.7° (*c* 0.9 in chloroform) and OCH_3 41.92% (calcd. OCH_3 41.48%).

The methylated product was subjected to two methylations by the Muskat procedure with liquid ammonia, sodium (0.15 g.), and methyl iodide (3 ml.). After evaporation of the ammonia, the product was washed with hot water, dried, and dissolved in chloroform. The methylated product precipitated when the chloroform solution was poured into petroleum ether, $[\alpha]_D^{24}$ + 132.1° (*c* 1.0 in chloroform), OCH_3 42.15%.

Fractional precipitation of the methylated codextrin (3.02 g.) from acetone (30 ml.) with petroleum ether gave the fractions shown in the table below.

Fraction	*Weight*	*Pet. Ether Added*	$[\alpha]_D$ *in Chloroform*	*Methoxyl*
	g.	*ml.*	*degrees*	*%*
1	0.12	39	....	....
2	2.42	85	+132	42.05
3	0.25	excess	+132	42.60

Hydrolysis of Methylated Codextrin and Identification of Components

The methylated codextrin (1.75 g. of fraction 2) was refluxed with 2% methanolic hydrogen chloride (50 ml.) for 20 hr. The methanolysate was neutralized with silver carbonate, filtered, and concentrated, and the glycosides were hydrolyzed with *N* sulfuric acid (50 ml.) at 100°C. for 14 hr. The hydrolysate was neutralized with barium carbonate and concentrated to a syrup (1.57 g.), and a portion of this hydrolysate (1.36 g.) was fractionated

TABLE I

COLUMN FRACTIONATION OF THE HYDROLYSATE OF THE METHYLATED CODEXTRIN[a]

FRACTION	METHYL DERIVATIVE	WEIGHT	
		g.	*mole %*
1	2,3,4,6-tetra-*O*-Methyl-D-glucose		16.2
		0.268[b]	
	2,3,4-tri-*O*-Methyl-D-xylose		4.2
2	2,3,6-tri-*O*-Methyl-D-glucose	0.625	47.7
3	2,4,6-tri-*O*-Methyl-D-glucose	0.036	2.7
4	2,4-di-*O*-Methyl-D-xylose	0.014	1.4
5	2,6-di-*O*-Methyl-D-glucose	0.096	7.8
6	2,3-di-*O*-Methyl-D-glucose	0.049	4.1
7	2,3-di-*O*-Methyl-D-xylose	0.069	6.5
8	3-*O*-Methyl-D-xylose	0.059	6.0
9	2-*O*-Methyl-D-glucose	0.023	2.0
10	3-*O*-Methyl-D-glucose	0.007	0.5
11	D-Xylose	0.010	1.2
	Recovery	1.246	

[a] Components isolated from 1.363 g. of hydrolysate.

[b] The amounts of tetra-*O*-methyl-D-glucose and tri-*O*-methyl-D-xylose calculated from the specific optical rotation of the mixture are 0.219 and 0.048 g. respectively.

on a hydrocellulose column (6) (solvent C). Fractions were purified further when necessary by paper chromatography with solvent B or C, or by paper electrophoresis. The methylated sugars were detected with *p*-anisidine trichloroacetate. The methyl sugars shown in Table I were identified as follows:

(A) 2,3,4,6-tetra-*O*-Methyl-D-glucose, fraction 1, $[\alpha]_D^{26}$ + 71.2° (*c* 0.9 in water), was separated by paper chromatography (solvent B), giving pure 2,3,4,6-tetra-*O*-methyl-D-glucose which was recrystallized from petroleum ether, m.p. 93°–95°, and $[\alpha]_D^{25}$ + 82° (*c* 0.6 in water); lit. (7), m.p. 96° and $[\alpha]_D$ + 84° in water.

(B) 2,3,4-tri-*O*-Methyl-D-xylose was identified in fraction 1 by paper chromatography.

(C) 2,3,6-tri-*O*-Methyl-D-glucose: recrystallization from ethyl ether gave the pure component with m.p. 121°–122° and $[\alpha]_D^{24}$ + 68.8° (*c* 1.2 in water); lit. (7), m.p. 121°–123° and $[\alpha]_D$ + 70.5° in water.

(D) 2,4,6-tri-*O*-Methyl-D-glucose: the crystalline compound gave m.p. 127°–129° and $[\alpha]_D^{26}$ + 66° (*c* 0.7 in methanol); lit. (7), m.p. 123°–126° and $[\alpha]_D$ + 70° in methanol.

(E) 2,4-di-*O*-Methyl-D-xylose: after recrystallization from ethyl acetate, this component had m.p. 110°–112° and $[\alpha]_D^{25}$ + 22° (*c* 1.4 in water); lit. (7), m.p. 111° and $[\alpha]_D^{24}$ + 23° in water.

(F) 2,6-di-*O*-Methyl-D-glucose: the syrup, $[\alpha]_D^{25}$ + 61.2° (*c* 1.0 in water), was transformed into the 1,3,4-triphenylazobenzoate, m.p. 207°–209°; lit. (7), m.p. 206°–208°.

(G) 2,3-di-*O*-Methyl-beta-D-glucose: the compound was recrystallized from ethyl acetate, m.p. 105°–107° and $[\alpha]_D^{25}$ + 48° (*c* 0.5 in acetone); lit. (7), m.p. 110° and $[\alpha]_D$ + 51° in acetone.

(H) 2,3-di-*O*-Methyl-D-xylose: the compound was isolated as a syrup,

$[\alpha]_D^{25}$ + 24° (*c* 0.7 in water), and transformed into N-phenyl-2,3-di-*O*-methyl-D-xylosylamine, which was recrystallized from ethyl acetate, m.p. 124°–126°; lit. (7), m.p. 123°–125°.

(I) 3-*O*-Methyl-D-xylose had $[\alpha]_D^{25}$ + 16.8° (*c* 0.6 in water) and was identified by paper chromatography; lit. (7), $[\alpha]_D$ + 17° in water.

(J) 2-*O*-Methyl-D-glucose and 3-*O*-methyl-D-glucose were separated by paper electrophoresis in 0.1*M* sodium borate buffer and identified by paper chromatography and electrophoresis.

Periodate Oxidation of the Codextrin

The codextrin (0.208 g.) was oxidized with 0.1*N* sodium metaperiodate (100 ml.) at 5°C. Aliquots were titrated periodically for formic acid and periodate by the Fleury-Lange method (8). The consumption of periodate and the formic acid released are shown below.

	Time			
	48 hr.	*96 hr.*	*168 hr.*	*240 hr.*
Formic acid released (moles hexose/mole formic acid)	3.3	3.2	3.2	3.2
Periodic acid consumed (moles IO_4^-/mole hexose)	1.14	1.15	1.20	1.20

An aliquot of the oxidation solution (20 ml.) was treated with barium acetate to precipitate the iodate and periodate ions, and the filtrate was treated with sodium borohydride (0.20 g.) for 24 hr. The excess borohydride was decomposed with acetic acid and the solution was passed through a column of Amberlite IR 120 (H^+) cation-exchange resin and concentrated to dryness. The boric acid was removed from the residue by distillation with methanol. A portion of the syrupy product was hydrolyzed with 1*N* sulfuric acid at 95°C. for 14 hr. The deionized hydrolysate was examined by paper chromatography (solvent B) and shown to contain D-glucose, D-xylose, erythritol, and glycerol. Each component was separated by paper chromatography, and the sugars were analyzed quantitatively by the phenol-sulfuric acid method (5); the alditols were analyzed by the chromotropic acid method (9). The molar ratio of D-glucose:D-xylose:erythritol:glycerol was 1.16:1.05:7.9:4.5.

The codextrin (10 g.) was subjected to periodate oxidation for 15 days at 5°C. and reduction with sodium borohydride as described previously. The polyalcohol obtained thus was partially hydrolyzed with 0.1*N* sulfuric acid (250 ml.) at 24°C. for 6 hr. and the hydrolysate was deionized with Amberlite IR-120 (H^+) and Duolite A-4 (OH^-) resins. On concentration of the hydrolysate and paper chromatographic analysis (solvent B), glycerol, erythritol, and three other components with R_g 1.2, 1.0, and 0.85 were detected.

The components were separated by preparative paper chromatography with Whatman No. 3 paper. The compound with R_g 1.2 was purified further by paper chromatography with solvents A and B. Hydrolysis of the purified compound with 0.5*N* sulfuric acid for 3 hr. gave D-glucose, D-xylose, and erythritol in the molar ratio 1:1:1.

DISCUSSION

A mixture of waxy maize starch and D-xylose heated at 140°C. in the presence of hydrochloric acid gave a dextrinized product which was freed

from low-molecular-weight sugars and degradation products by repeated precipitation from aqueous solutions with alcohol. Acid hydrolysis of this dextrin, which was readily soluble in cold water and gave a red color with iodine, gave D-glucose and D-xylose in the molar ratio of 5.50:1.06. From the yield of the dextrin it is apparent that 20% of the original xylose had been incorporated into the polymeric product.

That extensive transglycosylation of the amylopectin and the xylose had occurred during the dextrinization reaction was evident from the periodate oxidation data. Oxidation of the dextrin by periodate was complete in 7 days, resulting in the liberation of 1 mole of formic acid for every 3.2 sugar residues.[5] This is indicative of a highly branched structure with one branching residue for every 3 to 4 monomer units. Furthermore, approximately 15% of the sugar residues, consisting of equal proportions of glucose and xylose, were resistant to attack by periodate, compared with less than 1% periodate-resistant glucose residues in corn amylopectin.

Reduction of the periodate-oxidized codextrin with sodium borohydride and subsequent hydrolysis with 0.1*N* sulfuric acid at room temperature afforded three glycosides in addition to glycerol and erythritol. One of these glycosides was composed of equal proportions of D-glucose, D-xylose, and erythritol. The isolation of this fragment of the polymer constitutes proof that D-xylose is in part at least an integral structural unit of the starch dextrin and that the theoretical concept of a codextrin has been realized.

Evidence for the highly branched structure of the codextrin was also forthcoming from the methylation data, which indicated a branched residue in every 4 to 5 monomer units. The predominant new linkages introduced into the amylopectin molecule were the 1 → 3-glycosidic linkages which resulted in a new branched glucose residue substituted at C-3 and at C-4. This structural feature of dextrinization was detected in previous structural studies of corn (10) and wheat (11) starch dextrins. The xylose residues were incorporated into the polymer primarily as nonreducing terminal groups, as linear residues substituted at C-4, and as branching residues substituted at C-2 and C-4.

Although extensive fractionation of this dextrin was not carried out, previous studies of corn and wheat dextrins (10,11) and codextrins (2,4) revealed extensive heterogeneity of the products. It is reasonable to assume that in this case also, the structural features detected represent the average of a series of dextrin molecules containing the different linkages in various proportions.

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