The Distribution of Substituents in Vinyl Starch[1]

JAMES W. BERRY, ARCHIE J. DEUTSCHMAN, JR., AND JOHN P. EVANS

Department of Agricultural Biochemistry, University of Arizona, Tucson, Arizona

Received April 30, 1964

The distribution of vinyl groups in vinyl amylose and vinyl amylopectin has been determined by a sequence of catalytic hydrogenation, methanolysis, and chromatographic techniques. The 2-position has been found to be the predominant position of vinylation in vinyl starch prepared by reaction of starch with acetylene gas in the presence of potassium hydroxide.

A statistical study of starch vinylation which was previously reported from this laboratory showed that a response surface design is a good characterization of the relation between vinylation reaction variables and the degree of substitution (D. S.).[2] The present report describes a study of the distribution of vinyl groups in vinyl starch.

Previous work has shown that the position of vinyl ether groups on simple vinyl carbohydrates can be determined by two techniques.[3] First, catalytic hydrogenation gives the corresponding O-ethyl derivatives, which can be prepared by classical methods. Second, methylation, followed by dilute acid hydrolysis to remove the vinyl groups, results in partially methylated carbohydrates which can be separated and identified by gas–liquid partition chromatography.[4]

In this work, vinyl starch (D. S. approximately 1.0) was catalytically hydrogenated to yield the corresponding ethyl starch, which was then depolymerized by methanolysis to a mixture of methyl D-glucopyranoside and methyl O-ethyl-D-glucopyranosides. A small portion of the ethyl starch was hydrolyzed to a mixture of sugars; this mixture was shown by paper chromatography to consist predominantly of monosubstituted glucoses. Spots of approximately equal intensity were present for unsubstituted glucose and disubstituted glucoses, while that for trisubstituted glucose was very faint.

It was found that separation and identification of the ethyl ethers of methyl D-glucopyranoside were possible by gas–liquid partition chromatography. Also, by integration of peak areas the relative amounts of the various ethyl ethers could be determined, and these were assumed to represent the relative amounts of the corresponding O-vinyl ethers originally present in the vinyl starch. Several repetitions of vinylation, hydrogenation, methanolysis and gas–liquid partition chromatographic procedures for the same material gave negligible variation in the distribution profile.

In general the identity of the O-ethyl ethers was established by conversion of the known corresponding O-ethyl ethers of D-glucose to methyl O-ethyl-D-glucosides. The chromatographic behavior of these was compared with the appropriate mixture of methyl O-ethyl-D-glucosides derived from vinyl starch. In addition, samples of the latter were collected, hydrolyzed, and compared by paper chromatography with the authentic O-ethyl-D-glucoses.

The vinylation of amylose to a D. S. of 1.0 resulted in a ratio of mono- to disubstituents of 1.0:0.4. Considering only monosubstitution, the 2-position is somewhat more reactive than the 6-, as reflected in the ratio of 1.3:1.0. No appreciable substitution in the 3-position was observed. Only two disubstitution products were found in any appreciable amount, the 2,6- and 2,3-, in the ratio 8:1, respectively. A small peak was present which was assumed to be the trisubstitution product, 2,3,6-, but no characterization of the material was made.

The vinylation of amylopectin (D. S. 1.1) was found to be similar to amylose vinylation in that the ratio of 2–6 monosubstitution was 1.2:1.0. There was negligible substitution in the 3-position, and only two disubstitution products were found, the 2,6- and 2,3-, in the ratio of 3:1.

An interesting difference between amylose vinylation and amylopectin vinylation is apparent in the relative ratio of mono- to disubstitution in each case. For amylose the ratio was 1.0:0.4, but for amylopectin the ratio was 1.0:0.9. A possible explanation of this difference may be that the branched structure of amylopectin makes vinylation more difficult in the interior of the structure, while the relatively greater number of terminal and near-terminal anhydroglucose units undergo multiple vinylation more readily.

Our finding that the 2-position is the most reactive site for vinylation in the anhydroglucose unit is consistent with the isolation by Deutschman and Kircher[3] of methyl 2-O-vinyl-α-D-glucopyranoside as the main product of vinylation of methyl α-D-glucopyranoside to a D. S. of 1 with either vinyl chloride or acetylene. It is interesting to note that Croon and Flamm[5] determined that 2-O-ethyl-D-glucose is the predominant mono-O-ethyl-D-glucose in hydrolysates of ethyl cellulose, the latter having been prepared by reaction of ethyl chloride with alkali cellulose.

In summary, vinylation of starch, either amylose or amylopectin, to a D. S. of 1 results in predominant monosubstitution with the 2-position favored over the 6- and 3-positions as the most reactive site.

Experimental

Chromatography.—The gas–liquid partition apparatus was constructed in this laboratory. It employs a 10-ft. stainless steel column of 0.25-in. diameter packed with 20% DEGS on 40–60-mesh firebrick with helium carrier gas. An isothermal system, maintained by a Dynapac[6] proportional temperature controller, was used with a Gow-Mac[7] tungsten wire detection cell.

(1) Work was done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. Contract was supervised by the Northern Utilization Research and Development Division, Agricultural Research Service.

(2) J. W. Berry, H. Tucker, and A. J. Deutschman, Jr., *Ind. Eng. Chem., Process Design Develop.*, **2**, 318 (1963).

(3) A. J. Deutschman, Jr., and H. W. Kircher, *J. Am. Chem. Soc.*, **83**, 4070 (1961).

(4) H. W. Kircher, "Methods in Carbohydrate Chemistry," Vol. 1, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press, Inc., New York, N. Y., 1962, p. 13.

(5) I. Croon and E. Flamm, *Svensk Papperstid.*, **22**, 963 (1958).

(6) Lab-Line Instruments, Inc., Chicago, Ill.

(7) Gow-Mac Instrument Co., Madison, N. J.

The apparatus was constructed of Marinite with a 2-in. wall thickness throughout. An internal fan assured even heat distribution and markedly decreased stabilization time. A 1-mv. full-scale recorder with Disc Integrator was used. Vaporization of injected material was accomplished with a preheater with variable temperature control. Retention data are shown in Table I.

TABLE I

RETENTION TIMES FOR METHYL O-ETHYL-D-GLUCOSIDES[a]

Compound	Retention time, min.
Methyl 2,3-di-O-ethyl-D-glucoside, α form	28
β form	36
Methyl 2,6-di-O-ethyl-D-glucoside, α form	44
β form	48
Methyl 6-O-ethyl-D-glucoside	95
Methyl 3-O-ethyl-D-glucoside	106
Methyl 2-O-ethyl-D-glucoside	113

[a] Column temperature was 190°; helium pressure was 9 p.s.i.g.; 10-ft. diethylene glycol succinate (20%) column on 40–60-mesh firebrick.

Where required, paper chromatography was done in the descending manner, employing solvent systems and spray reagents described previously.[8,9]

Vinyl Starches.—The preparation and analysis of vinyl starch has been described.[2] The vinyl amylose used (D. S. 1.0) was prepared from corn amylose isolated by the Schoch procedure and having an amylose content of approximately 98% as indicated by iodine sorption.[10] The vinyl amylopectin used (D. S. 1.1) was prepared from Amioca powdered,[11] an unmodified waxy maize starch with an amylopectin content greater than 97%.

Hydrogenation of Vinyl Starch.—A solution of vinyl starch (1.0 g.) in absolute methanol (150 ml.) with 0.8 g. of 5% palladium-on-carbon catalyst[12] was hydrogenated for 70 hr. at 50° and 1800 p.s.i.g. The reaction mixture was centrifuged to remove the catalyst; evaporation of the solvent yielded 0.95 g. of product which showed no vinyl ether groups either by iodimetric or hydroxylamine hydrochloride analysis. The product, ethyl starch, was not further characterized.

Methanolysis of Ethyl Starch.—The material obtained by hydrogenation of vinyl starch was refluxed for 36 hr. in a mixture of absolute methanol (100 ml.) and concentrated hydrochloric acid (3 ml.). When cool, the reaction mixture was treated with Anion Resin CT-375[13] for 2 hr. at room temperature with stirring. The resin was removed by filtration, and the filtrate was reduced under vacuum to a clear pale yellow sirup.

Hydrolysis Procedure for Ethyl Starch or Methyl O-Ethyl-D-glucosides.—A 1–10-mg. sample of ethyl starch or methyl O-ethyl-D-glucoside was dissolved in 1.7 ml. of 72% aqueous sulfuric acid by adding the solid gradually to the acid while keeping the mixture ice-cold. After 30 min. the clear solution was diluted with water to 16-ml. total volume and refluxed for 6 hr. When cool, the reaction mixture was neutralized with Anion Resin CT-375, and the filtrate was concentrated to approximately 1 ml.

Preparation of Authentic Substances for Chromatographic Comparison. Methyl 2-O-Ethyl-α-D-glucopyranoside.—The synthesis and characterization of this compound which was in our possession has been described previously.[3]

Methyl 3-O-Ethyl-D-glucopyranoside.—The method of Glen, *et al.*,[14] was used to prepare 3-O-ethyl-D-glucose. The product, m.p. 136.5–137.5°, $[\alpha]^{24}D$ +26.0° (5 min.) (water, *c* 2), was refluxed for 20 hr. with a solution of 3% anhydrous hydrogen chloride in absolute methanol. After treatment of the reaction mixture with Anion Resin CT-375 and evaporation of the solvent, a very pale yellow sirup was obtained which was not further purified or characterized.

Methyl 6-O-Ethyl-D-glucopyranoside.—The method of Ohle and Tessmar[15] was used to prepare 6-O-ethyl-D-glucose. The product, m.p. 155.5–156.5°, $[\alpha]^{26}D$ +57° (water, *c* 2), was converted to the glucoside as described above and was not further purified or characterized.

Methyl 2,3-Di-*O*-ethyl-D-glucopyranoside.—The method of Croon and Flamm[5] was used to prepare 2,3-di-O-ethyl-D-glucose. The product did not crystallize, but was homogeneous by paper chromatography; it had $[\alpha]^{24}D$ +51.7° (water, *c* 2). The product was converted to the glucoside as described above and was not further characterized.

Methyl 2,6-Di-O-ethyl-D-glucopyranoside.—The method of Reeves[16] was utilized in the preparation of 2,6-di-O-ethyl-D-glucose. The product,[17] m.p. 112.5–114.5°, $[\alpha]^{24}D$ +54.9° (water, *c* 2), was converted to the glucoside as described above and was not further characterized.

(8) E. L. Hirst, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).
(9) R. W. Lenz and C. V. Holmberg, *Anal. Chem.*, **28**, 7 (1956).
(10) We are indebted to Dr. R. J. Dimler, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Ill., for the preparation of the corn amylose.
(11) American-Maize Products Co., Roby, Ind.
(12) Englehard Industries, Inc., Newark, N. J.
(13) Eli Lilly and Co., Indianapolis, Ind.
(14) W. Glen, G. Myers, and G. Grant, *J. Chem. Soc.*, 2568 (1951).
(15) H. Ohle and K. Tessmar, *Ber.*, **71B**, 1843 (1938).
(16) R. E. Reeves, *J. Am. Chem. Soc.*, **70**, 259 (1948).
(17) Croon and Flamm[5] report the following data for this sugar: m.p. 90–92°, 114–115°; $[\alpha]^{24}D$ +56° (water, *c* 2).

Reactions of α-Substituted Polynitrotoluenes. I. Synthesis of 2,2′,4,4′,6,6′-Hexanitrostilbene

K. G. SHIPP

The Organic Chemistry Division, U. S. Naval Ordnance Laboratory, Silver Spring, Maryland

Received March 4, 1964

2,2′,4,4′,6,6′-Hexanitrostilbene (**1**) has been prepared from 2,4,6-trinitrobenzyl chloride by reaction with alcoholic potassium hydroxide. The product melts at 316°, 105° higher than the melting point for a product reported to be hexanitrostilbene, obtained from the reaction of 2,4,6-trinitrobenzyl bromide with alcoholic potassium hydroxide. That the product reported here is indeed **1** has been confirmed by molecular weight determination, elemental analyses, and by an unambiguous independent synthesis. The lower melting product obtained previously was probably 2,2′,4,4′,6,6′-hexanitrobibenzyl.

Reich, Wetter, and Widmer[1] reported a 40% yield of a crystalline product from the reaction of 2,4,6-trinitrobenzyl bromide with alcohol potassium hydroxide. Upon recrystallization from nitrobenzene this product formed pale yellow needles melting at 211°, and was assumed to be 2,2′,4,4′,6,6′-hexanitrostilbene. When this procedure was repeated in these laboratories in an effort to obtain a sample of **1** for study in a series of polynitroaromatic compounds, the results obtained were at variance with those reported by Reich. No product similar to that reported was obtained.

With 2,4,6-trinitrobenzyl chloride as the substrate in the reaction with alcoholic potassium hydroxide, however, a scintillating crystalline precipitate formed. This product crystallized from nitrobenzene in beauti-

(1) S. Reich, O. Wetter, and M. Widmer, *Ber.*, **45**, 3055 (1912).