[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

The Structure of a New Starch of High Amylose Content[2]

BY I. A. WOLFF, B. T. HOFREITER, P. R. WATSON, W. L. DEATHERAGE AND M. M. MACMASTERS

RECEIVED SEPTEMBER 13, 1954

Structural studies are reported on a starch containing about 50% amylose, derived from a corn variety having a high starch content. Like wrinkled-seeded peas, this corn contains a starch which is not only high in amylose but has an amylopectin fraction of unique structure and properties, intermediate between usual amylose and amylopectin fractions. Both inner and outer branches of the new amylopectin are longer than those of normal corn amylopectin. The new starch was fractionated after pretreatment in liquid ammonia. Its fractions were characterized by their extent of conversion by β-amylase, reducing power, iodine-complexing capacity, viscosity and periodate oxidation analysis. New modifications of the latter technique are reported which provide more suitable controls for the oxidation and furnish an estimate of the proportion of reducing end groups of a polysaccharide.

The potential utility of the linear starch component (amylose) or its chemical derivatives for the preparation of formed items such as films, fibers and molded articles[3] has motivated a continuing search for improved procedures for the fractionation of ordinary starches as well as for new plant sources with a starch of high amylose content.

While some progress has been made toward the development of better fractionation methods,[4] their commercial use has not been undertaken.

The starches from wrinkled-seed peas[5] and from certain varieties of sweet corn[6] have been reported to have high amylose contents varying from about 60 up to as much as 98%. Other workers have failed to confirm the latter value.[7] In any case, the total starch content of these materials is low. The most encouraging genetic development has been the discovery of a corn variety having a starch high in amylose content and which, unlike the previously studied "high-amylose corn" varieties, has a high starch content.[7b] A more detailed examination of the structure of starch from this new hybrid by study of its fractions appeared desirable and forms the basis of this report.

The high gelatinization temperature of this starch gave rise to difficulty in effecting its complete dispersion even when the usual conditions of starch fractionation[8] were modified to include drastic mechanical disintegration of the swollen granules. Solution of the starch in alkali, followed by neutralization, enabled satisfactory separation of the fractions by butanol precipitation. This procedure, however, necessitated repeated reprecipitation of the amylopectin fraction to obtain a product of low ash content.[9] The preferred procedure of fractionation was to pregelatinize the starch in liquid ammonia,[10] which converted it to a form more easily dissolved in hot water saturated with *n*-butyl alcohol. This is probably a phenomenon involving reduction in crystallinity similar to that effected by the action of various amines on cellulose.[11] Higher crystallinity in this native starch is apparently distributed through the entire granules since even when its granules were fractured by grinding they appeared microscopically to be disrupted by water to a lesser extent than damaged granules of the usual types of corn starch.

The starch fractions, after separation and (in case of the amylose) purification, were characterized by extent of conversion by β-amylase, reducing power, iodine sorption both potentiometrically and spectrophotometrically, viscosity and periodate oxidation analysis. The amylopectin fraction was further characterized by fractional precipitation with alcohol and by optical rotation of its tricarbanilate derivative.

Experimental

Raw Materials.—The starch was isolated from corn produced in the 1952 crop year by the Bear Hybrid Corn Company, Inc., and referred to by that company under the trade name of Amylomaize.[12] This starch is identical with that described by Deatherage, *et al.*[7b] The starch isolation was accomplished by conventional laboratory procedures[13] except that the sulfur dioxide content of the steep was progressively increased over a 48-hour period from 0.05 to 0.40% to simulate commercial countercurrent steeping procedures. The starch on a dry basis had 0.15% N, 0.035% P and 1.81% methanol-extractable material. This relatively high content of methanol extractables may be due to presence of some alcohol-soluble protein. Alternatively, there may be some relationship of the large amount of methanol-extractable material to the higher amylose content of the starch. Ability of amylose to complex with fatty material is well known. The starch was defatted by extraction with 85% methanol for fractionation by procedures B and C (see below).

The starch was incompletely dissolved by 0.1-0.2 *N* potassium hydroxide solution but was dispersed in 0.3–1.0 *N* potassium hydroxide. The rate and extent of solution

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

(2) Presented in two parts: Before the Division of Carbohydrate Chemistry at the 126th National Meeting of the American Chemical Society, New York, N. Y., September, 1954; and at the Midwest Regional Meeting of the American Chemical Society, Omaha, Nebr., November, 1954.

(3) I. A. Wolff, D. W. Olds and G. E. Hilbert, *Ind. Eng. Chem.*, **43**, 911 (1951); I. A. Wolff, H. A. Davis, J. E. Cluskey, L. J. Gundrum and C. E. Rist, *ibid.*, **43**, 915 (1951); R. L. Whistler and G. E. Hilbert, *ibid.*, **36**, 796 (1944); R. L. Whistler and I. A. Wolff, unpublished results on dry spinning of fibers from amylose triacetate.

(4) A. W. Bauer and E. Pacsu, *Textile Research J.*, **23**, 860 (1953); R. W. Kerr and W. J. Katzbeck, *Die Stärke*, **5**, 2 (1953); T. J. Schoch, U. S. Patents 2,515,095 and 2,515,096 (July, 1950).

(5) J. P. Nielsen and Peggy C. Gleason, *Ind. Eng. Chem., Anal. Ed.*, **17**, 131 (1945); G. E. Hilbert and M. M. MacMaster, *J. Biol. Chem.*, **162**, 229 (1946); S. Peat, E. J. Bourne and M. J. Nicholls, *Nature*, **161**, 206 (1948).

(6) J. W. Cameron, *Genetics*, **32**, 459 (1947); G. M. Dunn, H. H. Kramer and R. L. Whistler, *Agron; J.*, **45**, 101 (1953).

(7) (a) R. M. McCready, J. Guggolz, V. Silviera and H. S. Owens, *Anal. Chem.*, **22**, 1156 (1950); (b) W. L. Deatherage, M. M. MacMasters, M. L. Vineyard and R. P. Bear, *Cereal Chem.*, **31**, 50 (1951).

(8) T. J. Schoch, THIS JOURNAL, **64**, 2957 (1942).

(9) A. L. Potter, V. Silveira, R. M. McCready and H. S. Owens, *ibid.*, **75**, 1335 (1953).

(10) J. E. Hodge, S. A. Karjala and G. E. Hilbert, *ibid.*, **73**, 3312 (1951).

(11) L. Segal, M. L. Nelson and C. M. Conrad, *J. Phys. Colloid Chem.*, **55**, 325 (1951).

(12) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

(13) Mary J. Cox, M. M. MacMasters and G. E. Hilbert, *Cereal Chem.*, **21**, 447 (1944).

were not visibly different at room temperature from at refrigerator temperature (approximately 4–5°).

Fractionation of Amylomaize Starch. Procedure A.—Complete dispersion of the Amylomaize starch granules was not effected when a 3% suspension in water saturated with *n*-butyl alcohol was subjected, alternately, to periods of autoclaving at 15 p.s.i.g. and agitation of the hot suspension in a Waring blendor. Conventional procedures of dispersion in water–butanol mixture without pretreatment of the starch were therefore abandoned.

Procedure B.—The procedure of Potter, *et al.*,[9] was followed, except that *n*-butyl alcohol instead of amyl alcohol was used as the complexing agent. The amylopectin solution was first separated from the amylose–butanol complex at room temperature and then refrigerated as recommended by those authors. Separation of a small quantity of precipitate occurred, which was indistinguishable by potentiometric iodine titration from the major portion of the amylopectin. Over-all total solids recovery in this procedure was 92%. The yield of amylopectin fraction was 41%. The yield of amylose was not calculated. Amylose samples taken at successive stages of purification had iodine affinities of 169, 190, 191 and 196 mg./g.

Procedure C.—The starch (100 g., dry basis), which had been pretreated in liquid ammonia,[10] was slurried in 300 ml. of *n*-butyl alcohol. This slurry was added to a stirred mixture of 2700 ml. of water and 300 ml. of butanol at 90°. After a heating period of 1 hour, an additional 200 ml. of butanol was added and the mixture (*p*H 6.7) was autoclaved for 1 hour at 15 p.s.i.g., then allowed to cool slowly. The amylose was recrystallized from hot water saturated with butanol. The solutions were autoclaved for 1 hour at each recrystallization stage. The thrice-recrystallized amylose (43% yield) sorbed 200 mg. of iodine/g. The amylopectin fraction showed no inflection in its potentiometric iodine titration curve (see below).

Normal Starch Fractions.—Starch was fractionated according to the procedure of Schoch,[8] and the amylose fraction was recrystallized twice from hot butanol-saturated water. The respective iodine sorptions of the fraction were 193 and 12 mg. I_2/g.

Characterization of the Fractions. Extent of Conversion by β-Amylase.—β-Amylase was prepared from wheat flour by the method of Ballou and Luck[14] with an acid treatment of the aqueous enzyme-containing extract (0.5 hour at *p*H 3 and 0°) included for inactivation of a major portion of any α-amylase contamination.[15] Potency was determined by the method of Kneen and Sandstedt[16] except that the Somogyi procedure[17] was used for determination of reducing sugar. Activity is expressed as the grams of starch converted by 1 ml. of the β-amylase solution in 1 hour at 30°.

Amylopectins were converted to limit dextrins by allowing 11 or 23 units enzyme/g. substrate to act on a 4% solution for 48 hours at 30° in a solution preserved with toluene and buffered by *p*H 4.6–4.8. Under these conditions an amylopectin fraction from ordinary corn starch was converted to the extent of 56%, the amylopectin from Amylomaize starch was converted to the extent of 58% of the reducing sugar, estimated as maltose,[17] theoretically obtainable by complete hydrolysis of the fraction to that sugar.

High extents of conversion of amylose to maltose occurred only when high ratios of enzyme to substrate were used. The procedure of Bernfeld and Gürtler[18] was used except that the alkaline amylose solution was added all at once to the buffered enzyme. Extents of conversion of 0.08% amylose solutions at 30° in the presence of toluene and sodium acetate–acetic acid buffer were

Amylose sample	β-Amylase units/g. amylose	Conversion to maltose, % 2.5 hr.	24 hr.
Normal corn	874	82	94
Amylomaize	874	76	90
Normal corn	262	79	83
Amylomaize	262	75	79

No significant differences in convertibility were found between Amylomaize fractions obtained by the various fractionation procedures.

Reducing Power Determinations.—Reducing power determinations were carried out by the use of alkaline 3,5-dinitrosalicylic acid according to the procedure of Lansky, *et al.*,[19] and expressed in terms of degree of polymerization (DP). Values obtained were

	Normal corn starch	Amylomaize starch Procedure B	Procedure C
Amylose	320	320	285
Amylopectin	450	220	150

Potentiometric Iodine Sorption.—The fractions were analyzed for iodine affinity by the procedure of Bates, French and Rundle[20] as modified by Wilson, Schoch and Hudson.[21] The amylopectin fraction from Amylomaize starch showed no inflection in the titration curve. However, there is definite sorption of iodine, as indicated by the displacement of the curve from that of the blank (Fig. 1). Admixture of the amylopectin with a weighed amount of normal corn amylose of known sorption, to superimpose the inflection of the latter on the amylopectin curve, indicates the amylopectin fraction to sorb 50.5 mg. of iodine/g., which, if due to amylose, corresponds to an apparent amylose content of approximately 25%. The amylose fraction of Amylomaize starch, iodine affinities of which have already been cited, reacted in the usual fashion in the potentiometric iodine titration. The original, defatted Amylomaize starch sorbed 109 mg. of iodine/g. starch.

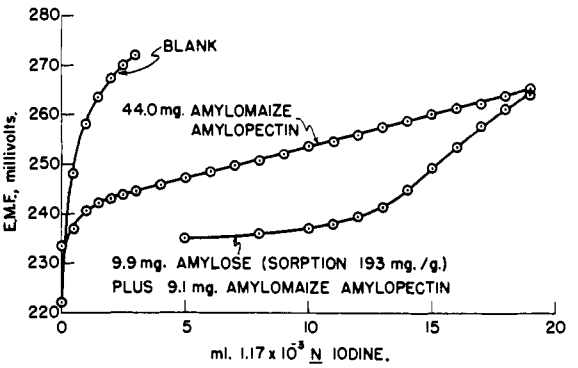

Fig. 1.—Potentiometric iodine titration curves of amylomaize amylopectin alone or in admixture with amylose.

Determination of Blue Values.—Blue values were determined by the procedure of McCready and Hassid[22] with the modifications that (a) samples were allowed to stand in 0.5 *N* sodium hydroxide overnight, then heated for 20 minutes at 60–63° under nitrogen for final dispersion; (b) final measurements were at 660 mμ and are reported as the optical density of a 1-cm. depth of solution; and (c) standards used for comparison were a recrystallized amylose fraction which sorbed 198 mg. of iodine/g., measured potentiometrically and the blank made with iodine–potassium iodide solution but containing no polysaccharide. For practical purpose of calculation, the blue value of the highly branched polysaccharide, corn glycogen, can be considered as zero (Fig. 2). The blue values (0.335–0.346) of the recrystallized amylose from Amylomaize starch were consistent with their purities as deduced from potentiometric iodine titration. The amylopectin fraction from Amylomaize starch had an apparent amylose content of 23–27% (blue values 0.078–0.092). A synthetic mixture of normal amylopectin (88%) and amylose (12%) having approximately the same blue value was used for comparative purposes. Spectral distribution curves of several of the solutions used for blue value determination are shown in Fig. 2. The Amylomaize starch had an apparent amylose content of 58%.

(14) G. A. Ballou and J. M. Luck, *J. Biol. Chem.*, **139**, 233 (1941).

(15) W. J. Olson, B. A. Burkhart and A. D. Dickson, *Cereal Chem.*, **20**, 126 (1943); E. Kneen, R. M. Sandstedt and C. M. Hollenbeck, *ibid.*, **20**, 399 (1943).

(16) E. Kneen and R. M. Sandstedt, *ibid.*, **18**, 237 (1941).

(17) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

(18) P. Bernfeld and P. Gürtler, *Helv. Chim. Acta*, **31**, 106 (1948).

(19) S. Lansky, M. Kooi and T. J. Schoch, THIS JOURNAL, **71**, 4066 (1949).

(20) F. L. Bates, D. French and R. E. Rundle, *ibid.*, **65**, 142 (1943).

(21) E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson, *ibid.*, **65**, 1380 (1943).

(22) R. M. McCready and W. Z. Hassid, *ibid.*, **65**, 1154 (1943).

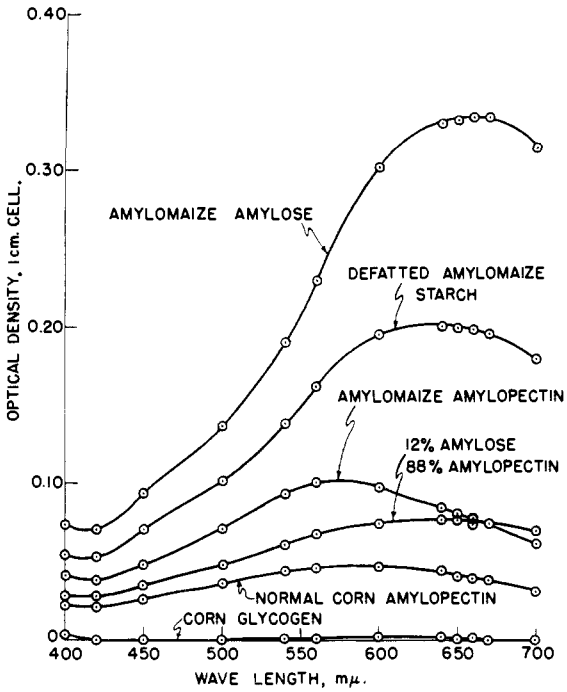

Fig. 2.—Spectral absorption of the polysaccharide–iodine complexes.

Viscosity Measurement.—Intrinsic viscosities were measured in 1 *N* potassium hydroxide by a procedure previously developed at this Laboratory.[23] The equations reported for calculation of intrinsic viscosity from measurements made at one concentration were found to be valid for amylose and amylopectin fractions of Amylomaize starch. Intrinsic viscosities of the fractions at 25° were: recrystallized amylose, 1.16–1.30; amylopectin, 1.12–1.29. There was as much variation in viscosity between different preparations following a given fractionation procedure as there was when different procedures were used. The fractions had viscosities of the same order of magnitude as fractions from ordinary corn starch.

Periodate Oxidations.—The average chain length (average number of anhydroglucose units per terminal non-reducing end group) of the various starch fractions was assessed by oxidation with sodium metaperiodate in the cold. Since amylaceous substrates have a tendency to over-oxidize, even at refrigerator temperatures, it has become customary to use a disaccharide as a reference standard. Although Potter, *et al.*,[9,24] found that maltose serves as a suitable standard and yields 3 moles of formic acid per mole sugar, other workers[25,26] have not had success with this procedure. The present authors, too, have found that maltose gives less than 3 moles of formic acid under the usual experimental conditions. Since the production of formic esters in periodate oxidation of appropriate sugars is now well substantiated,[27,28] it seemed reasonable to assume neither complete hydrolysis nor complete retention[29] of formic ester in oxidized maltose but to measure both the amount of free formic acid and that bound as formic ester. The procedure used for maltose was as follows: to 50 ml. of a precooled solution of 0.2220 g. of maltose hydrate in water was added 10.0 ml. of a solution of sodium metaperiodate containing 8 g./100 ml. Oxidations were carried out in the dark at 4–5°. At suitable time intervals, aliquots were withdrawn and 1 ml. of purified (distilled from KOH) ethylene glycol was added to react with the unused periodate. After 1 hour in the dark at room temperature, free formic acid was determined by titration with 0.01 *N* sodium hydroxide to the brom cresol purple end-point. After reaching the end-point, additional 0.01 *N* sodium hydroxide was added (approximately 1 meq. per meq. reducing end group), and this solution allowed to stand for 0.5 hour at room temperature to hydrolyze the formyl ester (up to 2 hours saponification time did not change the results). The solution was brought to the acid side with a known amount of 0.01 *N* sulfuric acid and again titrated to the brom cresol purple end-point with the alkali. The additional consumption of alkali, corrected for appropriate blanks and for the acid added, enabled calculation of the unhydrolyzed formyl ester. Results of the oxidation of maltose by this technique are shown in Fig. 3. The end-point of the reaction, when maltose is used as a reference standard, is taken at a total formic acid production of 3 moles/mole maltose. The authors have not verified that the acid titrated is exclusively formic. It is possible that acidic materials may be produced from the trialdehyde by the added alkali,[30] but this probably occurs to a negligible extent under the conditions used because of the rapid rate of hydrolysis of formate esters[28] and resultant consumption of the added alkali.

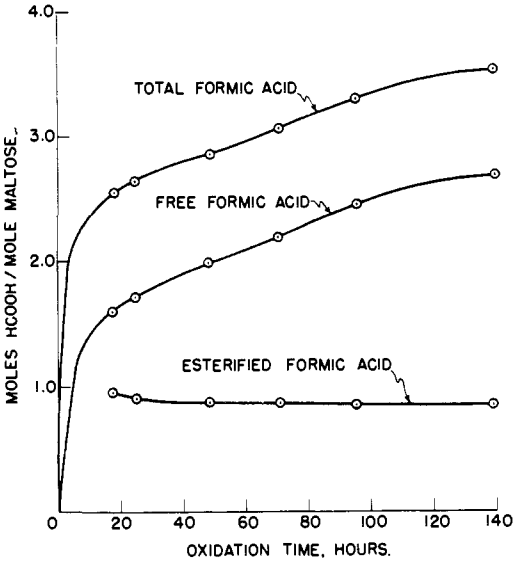

Fig. 3.—Production of formic acid by periodate oxidation of maltose.

The amylopectin samples (0.4750 g.) were oxidized and titrated in the same fashion as was the maltose, and were found to give appreciable amounts of titratable acid (Fig. 4) released by saponification. This is interpreted as being due to formyl ester at the reducing end group of the molecules. The reducing end group yields a total of 2 moles of formic acid. The average chain length may be considered as the reciprocal of the percentage of terminal non-reducing groups, or the reciprocal of the quantity [total HCOOH (moles/AGU) − 2 × ester formyl (moles/AGU)]. Calculated in this fashion (ester formyl corrected for percentage hydrolyzed, as indicated below), the average chain lengths of the normal corn starch amylopectin and the Amylomaize starch amylopectin were, respectively, 27 and 36. Furthermore, since 1 mole of formyl ester per molecule is produced, it should be possible to calculate from this figure an approximate number average molecular weight if no degradation occurred during the oxidation. Since the formyl ester of oxidized maltose is 13% hydrolyzed under our conditions at the end-point of the reaction, the assumption is made that a similar proportion of the ester at the amylopectin reducing end group is hydrolyzed. For normal corn amylopectin, for example, at 65 hours total formic acid (0.0413 mole/

(23) I. A. Wolff, L. J. Gundrum and C. E. Rist, THIS JOURNAL, **72**, 5188 (1950).

(24) A. L. Potter and W. Z. Hassid, *ibid.*, **70**, 3488 (1948).

(25) M. Morrison, A. C. Kuyper and J. M. Orten, *ibid.*, **75**, 1502 (1953).

(26) D. J. Manners, *Biochem. J.*, **55**, *xx* (1953).

(27) R. W. Lemieux and H. F. Bauer, *Can. J. Chem.*, **31**, 814 (1953).

(28) G. Neumüller and E. Vasseur, *Arkiv Kemi*, **5**, 235 (1953); F. S. H. Head and G. Hughes, *J. Chem. Soc.*, 603 (1954).

(29) K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **32**, 1102 (1949).

(30) F. S. H. Head, *J. Textile Inst.*, **38**, T389 (1947); E. M. Fry, E. J. Wilson, Jr., and C. S. Hudson, THIS JOURNAL, **64**, 872 (1942).

AGU) − free formic acid (0.0395 mole/AGU) = ester formyl (0.0018 mole/AGU) which corrected for 13% hydrolysis would be 0.0021 mole/AGU. This corresponds to a number average degree of polymerization of 476; the Amylomaize amylopectin, by similar calculation, had a DP_N of 386.

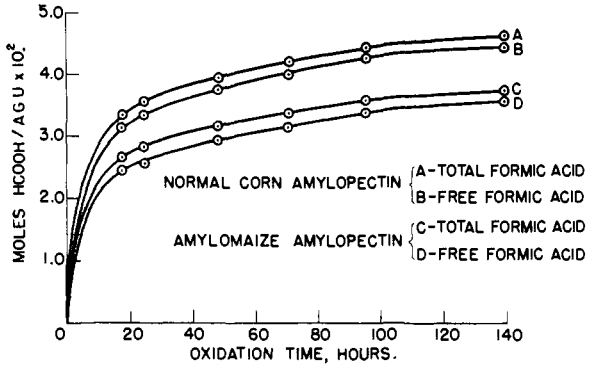

Fig. 4.—Production of formic acid by periodate oxidation of amylopectins.

Periodate oxidation of the amylose samples was carried out as before, but in the presence of 3% sodium chloride and a small amount of petroleum ether.[24] The tendency of the samples to clump and the smaller formic acid production from amylose make the results subject to somewhat greater error than in the case of amylopectins. The average chain lengths (calculated on the assumption that the molecules are linear)[24] for both normal corn amylose and Amylomaize amylose were 460 (total HCOOH acid production 0.0065 mole/AGU at 65 hours reaction time). The amount of ester formyl (corrected for 13% hydrolysis) at the end point was approximately one-fourth (0.0015 mole/AGU) of the total formic acid. This would indicate that on the average these amylose samples had about 2 (calcd. 2.3) chains per molecule. Kerr and Cleveland[31] have found corn amylose to be unbranched while Potter and Hassid[32] concluded that their corn amylose had on the average 2.9 chains per molecule.

Fractional Precipitation of Amylopectin with Methanol.—As a further comparison between Amylomaize amylopectin and normal corn amylopectin, 2% aqueous solutions of each were sub-fractionated with graded amounts of methanol at 25 ± 0.1°. The precipitated sub-fractions were sedimented by centrifugation and, after removal of the supernatant liquid, dissolved in water to a known volume and their amounts estimated by measurement of the optical rotation. The $[\alpha]^{25}D$ in water of both amylopectins was found to be +200 ± 5°. The original solutions were too turbid for more accurate measurement. Additional methanol was added to the supernatants and the process repeated. The data are shown graphically in Fig. 5.

Amylomaize amylopectin is more difficultly soluble in water than is normal corn amylopectin. It was necessary to autoclave the partial solution, prepared on a steam-bath, for 1 hour at 15 p.s.i.g. pressure before all of the swollen particles had dissolved. The autoclaved solution was more turbid than one similarly prepared from normal corn amylopectin. Furthermore, the Amylomaize amylopectin partially retrograded from solution on being refrigerated. A white flocculent precipitate, which was easily redissolved on warming, settled on standing. Certain of the sub-fractions also showed this type of retrogradation. Thus about two-thirds of the sub-fraction soluble in 50% methanol retrograded from aqueous solution in the cold.

Amylomaize Amylopectin Tricarbanilate.—Amylomaize amylopectin was converted to its tricarbanilate derivative by a procedure previously described.[33] *Anal.* Calcd. for $C_{27}H_{25}N_3O_8$: N, 8.09. Found: N, 8.03. This derivative was $[\alpha]^{25}D$ −76° (pyridine, *c* 1), a value intermediate between that of the normal corn starch fraction tricarbanilates, but falling closer to that of the amylose derivative.

(31) R. W. Kerr and F. C. Cleveland, THIS JOURNAL, **74**, 4036 (1952).

(32) A. L. Potter and W. Z. Hassid, *ibid.*, **70**, 3774 (1948).

(33) I. A. Wolff and C. E. Rist, *ibid.*, **70**, 2779 (1948).

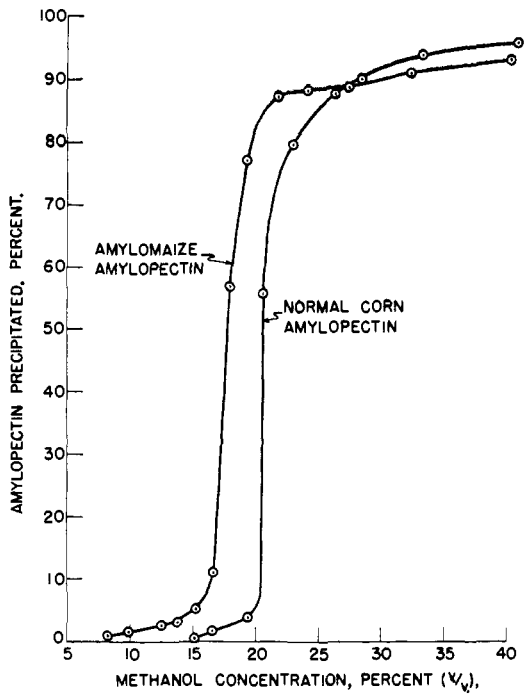

Fig. 5.—Fractional precipitation of amylopectins with methanol.

Discussion

The amylopectin fractions of starches having high amylose contents have been reported to be more branched[34] (for sweet corn) and less branched[9] (for wrinkled-seeded peas) than the corresponding fraction isolated from the usual types of starches. The amylose fractions were not found to differ from that of the normal starch from the plant source under study.

The starch from Amylomaize corn appears structurally to be very similar to if not identical with wrinkled-seeded pea starch.[9] Its amylose fraction is like that of normal corn starch in structure and molecular size. Thus the iodine sorption measured potentiometrically, blue value, intrinsic viscosity, formic acid production upon periodate oxidation and reducing power of Amylomaize amylose were identical, within limits of experimental error, with the values found for normal corn amylose. A new modification of the periodate oxidation technique showed a similarity in the number of chains per molecule. Since the conversion of an amylose fraction by β-amylase is dependent in such large measure on the completeness of its dispersion, no significance is attached at this time to the differences in rate of conversion (or extent at the end of 24 hours) found between the normal and Amylomaize amyloses.

In contrast, there is definite structural difference between amylopectin from normal corn and that from Amylomaize corn. The latter is more difficultly soluble in water and retrogrades rapidly from aqueous solutions in the cold. The viscosities of the amylopectin fractions are similar to each other and to those of the amylose fractions. Yet

(34) W. Dvonch, H. H. Kramer and R. L. Whistler, *Cereal Chem.*, **28**, 270 (1951).

the number average molecular weight of Amylomaize amylopectin as determined by alkaline dinitrosalicylic acid is less than that of amylose while that of normal corn amylopectin is greater. While these molecular weights may have no absolute significance,[19,35] taken as relative measures they enable the conclusion that Amylomaize amylopectin has a lower ratio of number average degree of polymerization to viscosity than does normal corn amylopectin. Two possible reasons for this might be (a) greater linearity of Amylomaize amylopectin, or (b) the existence of a type of molecular polydispersity for that fraction involving a greater proportion of quite high and quite low molecular weight components as compared with normal corn amylopectin. There is some evidence in favor of each of these possibilities, and it is quite probable that both factors are important. The iodine sorption, blue value, extent of conversion of β-amylase, periodate oxidation data, tendency toward retrogradation greater difficulty of solubility in water and rotation of the tricarbanilate derivative indicate that Amylomaize amylopectin has a greater degree of linearity than normal corn amylopectin. The precipitation curve (Fig. 5) of Amylomaize amylopectin has a slight discontinuity at a methanol concentration near 30% which may indicate some heterogeneity in that sample. Furthermore, there were portions which were precipitated at both lower and higher methanol concentration than corresponding normal corn amylopectin fractions. This would suggest the presence in the former of sub-fractions differing from one another by a greater amount in molecular structure, size or both. In a private communication Dr. T. J. Schoch has pointed out that methanol may be considered as a weak amylose-complexing agent and that the greater tendency of the Amylomaize amylopectin to precipitate with methanol may indicate greater linearity of structure.

A continuous variability in the lengths of linear portions of Amylomaize amylopectin would account[20] for the non-appearance of an inflection point in the iodine potentiometric titration curve, a possibility also considered by Potter, *et al.*[9]

Although polydisperse in size, and perhaps not homogeneous in chemical structure, Amylomaize amylopectin is best considered as a polysaccharide entity. Three lines of evidence indicate that Amylomaize amylopectin is not a mechanical mixture of amylose and amylopectin, but that it is a representative of a new polysaccharide type, intermediate in structure between amylose and amylopectin: (A) A 1% aqueous solution of Amylomaize amylopectin retrogrades to the extent of 94% on refrigeration, more than would be expected from admixture of a branched polysaccharide with 23–27% amylose even if the latter on retrogradation mechanically occluded some amylopectin. (B) In mechanical mixtures of amylose and amylopectin the former can be identified by an inflection in the potentiometric iodine titration curve. (C) The spectral distribution curve (Fig. 3) of the polysaccharide–iodine complex was quite different for Amylomaize amylopectin from that for a synthetic mixture of normal corn starch fractions having substantially the same blue value. The curves fall close to one another at 660 mμ, the wave length taken for calculation of apparent amylose, but the Amylomaize amylopectin–iodine complex showed an absorption maximum displaced toward the shorter wave lengths.

The existence of such intermediate fractions has been anticipated.[19,36,37]

Extension of this line of thought has important implications on our method of expression of the amylose content of starches such as Amylomaize. Rigorous assay of the true amylose content necessitates fractionation to establish the iodine-complexing capacity of the constituent fractions of a starch for use as standards. In the case of the 1952 crop year Amylomaize starch, use of the fractions originating from that starch as standards would (on the basis of blue value) indicate its amylose content as 47% instead of an apparent 53% if ordinary corn amylopectin was used as a standard or apparent 58% with an iodine blank as standard. If one wishes to report apparent amylose on the basis of blue values for surveying a series of starch samples, the use of a glycogen or an iodine blank as one standard (Fig. 2) is recommended. The potentiometric titration as conventionally used is, of course, a measure of apparent amylose content. Total iodine-sorptive capacity of a starch, even though not a measure of separable amylose fraction, may be found to correlate well with other starch properties such as film-forming ability, which are dependent on the total linear material present

If it is accepted that Amylomaize amylopectin is an intermediate type of amylaceous polysaccharide with an average chain length of 36 anhydroglucose units, comparisons are in order between the lengths of the inner and outer branches of this substance and those of normal corn amylopectin. Average chain length multiplied by extent of conversion by β-amylase plus 2[38] gives a figure approximating the outer-branch length. Inner-branch length may be taken as the difference between the average chain length and the outer-branch length, using the terminology and structural conceptions of other recent workers in the field.[38] The results of such calculations are

	Branch length	
	Outer	Inner
Normal corn amylopectin	17	10
Amylomaize amylopectin	23	13

It is seen that the difference in the amylopectins is reflected in both the inner- and outer-branch lengths.

Further structural studies on Amylomaize amylopectin through its sub-fractionation and by examination of its β-amylase limit dextrin are contemplated. Study of the fractions of other samples of starch of high amylose content derived from corn

(35) It is interesting to note that the DPn obtained by the new modification of the periodate oxidation procedure is of the same order of magnitude as that from the dinitrosalicylic acid procedure.

(36) R. W. Kerr and O. R. Trubell, *Paper Trade J.*, **117**, No. 15, 25 (1943).

(37) J. E. Hodge, E. M. Montgomery and G. E. Hilbert, *Cereal Chem.*, **25**, 19 (1948).

(38) B. Illingworth, J. Larner and G. T. Cori, *J. Biol. Chem.*, **199**, 631 (1952); J. Larner, B. Illingworth, G. T. Cori and C. F. Cori, *ibid.*, **199**, 641 (1952).

hybrids similar to the one presently considered should clarify the relationship, if any, between genetic composition of the corn and the type of amylopectin component present in the starch.

Acknowledgment.—The authors gratefully acknowledge the coöperation of R. P. Bear in furnishing the corn from which the starch was isolated. Thanks are also due to Mrs. Margaret M. Holzapfel for extracting the starch from the corn; R. L. Lohmar, Jr., for furnishing the corn glycogen; T. A. McGuire for determination of methanol extractables in the starch; Mrs. Phyllis L. Patrick for viscosity analyses and β-amylase convertibility determinations; J. W. Sloan for microscopic observations on the fractured starch granules; C. H. Van Etten for nitrogen and phosphorus analyses; B. H. Alexander for assistance in several periodate oxidation analyses; and C. E. Rist for advice and encouragement in the course of this work.

PEORIA, ILLINOIS

[CONTRIBUTION FROM THE STARCH AND DEXTROSE SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Kinetics of Hydrolysis of Isomaltotriose and Isomaltotriitol[2]

BY R. W. JONES, R. J. DIMLER AND C. E. RIST

RECEIVED OCTOBER 21, 1953

Conflicting evidence appears in the literature regarding the relation between rate of acid hydrolysis of glucosidic bonds and their position in polysaccharide molecules. The present studies were undertaken to provide information on such a relationship in the α-1,6′-glucosidically linked homologous series of oligo- and polysaccharides. Attention has been centered on the first two members of this series, isomaltose and isomaltotriose, and the alcohols obtained by reduction of these sugars. Procedures employing quantitative paper chromatography were developed for the study of the kinetics of hydrolysis of these carbohydrates. In the series isomaltose, isomaltotriose and dextran, the over-all rate constant for hydrolysis decreases with increase of chain length, the rate constant for dextran B-512 being about one-third that for isomaltose. For the individual bonds in the reduced oligosaccharide, isomaltotriitol, the bond farthest removed from the sorbitol end is cleaved twice as fast as the other linkage. It is postulated, on the basis of these data and certain assumptions concerning the effects of reduction of isomaltotriose, that the non-reducing end bond in isomaltotriose is hydrolyzed about 1.7 times as fast as the reducing end bond.

Considerable success has been attained in recent calculations of length of external branches in dextran molecules on the basis of measurements of the amounts of glucose and low molecular weight oligosaccharides formed during partial acid hydrolysis of the dextrans.[3] The interpretation of the data has been dependent in part upon the knowledge of whether the position of an α-1,6′-glucosidic linkage in the polymer chain influences its rate of hydrolysis. Since there is a lack of agreement in the literature concerning the effect of the position of a bond on its hydrolysis rate constant, K, the present studies were undertaken to provide evidence of such effects in the α-1,6′-linked glucose polymers.

A greater rate of hydrolysis of terminal compared with internal bonds in polysaccharides has been postulated by Freudenberg, Carlqvist, and others on the basis of studies of acid hydrolysis of cellulose,[4-7] starch,[4a,5,8-10] glycogen,[11] Schardinger dextrins[12] and low-molecular weight oligosaccharides.[4b,7] This hypothesis was based on the increase in K during hydrolysis of polysaccharides and the extent to which the velocity of hydrolysis increased in the order cellulose, cellohexaose, cellopentaose, cellotetraose, cellotriose, cellobiose. Swanson and Cori,[13] however, failed to detect an increase in K during hydrolysis of polysaccharides from starch. Likewise they found no difference in hydrolysis rate constant for amylose and maltose. The results obtained by Swanson and Cori may reflect the effects of differences in hydrolysis conditions[11] or in analytical methods used.

In the present studies attention was centered on the first two members of the α-1,6′-glucosidically linked, homologous series, isomaltose and isomaltotriose, which had been made available as a result of their preparation by carbon column chromatography of enzymic hydrolyzates of dextran from *Leuconostoc mesenteroides* NRRL B-512.[14] A procedure employing quantitative paper chromatography was developed for determining the effect of chain length on the over-all rates of hydrolysis (total bond cleavage) of these oligosaccharides. In addition, chromatographic techniques facilitated measurement of the rate constants for hydrolysis of the individual bonds in the reduced trisaccharide, isomaltotriitol. These data provided a basis for estimating the rates of cleavage of the individual bonds in the parent trisaccharide, isomaltotriose, although certain assumptions were necessary to allow for the difference between the over-all hy-

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

(2) Presented before the Division of Carbohydrate Chemistry at the 124th National Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

(3) R. W. Jones, R. J. Dimler, Allene Jeanes, C. A. Wilham and C. E. Rist, *Abstr. Papers, Amer. Chem. Soc.*, **126**, 13 D (1954); a somewhat similar approach has been initiated independently in a study of the peripheral structure of limit dextrins from starch by D. French, J. Calamari and G. M. Wild, *ibid.* **122**, 5R (1952).

(4) (a) K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz and G. Steinbrunn, *Ber.*, **63**, 1510 (1930); (b) K. Freudenberg and G. Blomqvist, *ibid.*, **68**, 2070 (1935).

(5) K. Freudenberg, *Trans. Faraday Soc.*, **32**, 74 (1936).

(6) L. G. Sillén, *Svensk Kem. Tidskr.*, **55**, 221 and 266 (1943).

(7) M. L. Wolfrom and J. C. Dacons, THIS JOURNAL, **74**, 5331 (1952).

(8) K. H. Meyer, H. Hopff and H. Mark, *Ber.*, **62**, 1103 (1929).

(9) K. Myrbäck and B. Magnusson, *Arkiv Kemi, Mineral. Geol.*, **A20**, No. 14, 22 pp. (1945).

(10) K. Myrbäck, B. Örtenblad and K. Ahlborg, *Biochem. Z.*, **307**, 53 (1940).

(11) B. Carlqvist, *Acta Chem. Scand.*, **2**, 759 (1948).

(12) K. Myrbäck, *Arkiv Kemi*, **1**, 161 (1949).

(13) Marjorie A. Swanson and C. F. Cori, *J. Biol. Chem.*, **172**, 797 (1948).

(14) Allene Jeanes, C. A. Wilham, R. W. Jones, H. M. Tsuchiya and C. E. Rist, THIS JOURNAL, **75**, 5911 (1953).