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Dispersion of Starch Granules and the Validity of Light Scattering Results on Amylopectin[1,2]

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Immature and mature dent, sweet and waxy corn starch granules were dispersed by refluxing at 113° in 6 *M* LiBr and at 125° in 8 *M* LiBr for various time lengths or by refluxing at 93° in a buffered or unbuffered aqueous solution saturated with amyl alcohol. The dispersions were examined by light scattering. Two sedimentation constants were obtained for immature waxy corn amylopectin, one sample being dispersed in 6 *M* LiBr and the other in 8 *M* LiBr. A sedimentation constant also was obtained on an immature dent corn amylopectin which had been dispersed in a buffered aqueous amyl alcohol solution. Light scattering molecular weights were obtained on various amylopectin samples using *N* KOH, 6 *M* LiBr, and water as solvents. Acid hydrolysis studies (random depolymerizations) show that immature corn amylopectin consistently behaves as an A–R–B_2 type statistical polymer.[3] Two components were found in immature waxy corn starch. It is thought that both polymers are branched polysaccharides. From the schlieren ultracentrifuge patterns the relative amount of the slow component appeared to increase in concentration with dilution of the sample. This is thought to be due to a concentration anomaly of the schlieren pattern.

Introduction

For the branched polymers amylopectin and glycogen, the weight-average molecular weight (obtained primarily from light scattering data) is over a hundred times as large as the number-average molecular weight. In a previous paper[3] it was shown that this large difference can be explained if one assumes that the branched polysaccharide is synthesized by a completely random condensation of glucose units. Results cited in the literature (see below) indicate that the high molecular weight obtained from light scattering data is not due to physical aggregation but is due to a broad size distribution of molecules as found in A–R–B_2 type polymers.[3,4] The purpose of this paper is to examine further the validity of the high light scattering molecular weights and to examine whether amylopectin consistently behaves as a statistically formed polymer.

Two major factors that may give large, erroneous light scattering molecular weights are: (a) physical aggregation due to intermolecular hydrogen bonding or entanglement of exterior branches and (b) the presence of a small amount of extremely large particles. In the extreme case a single molecular species could give aggregates which would possess a broad size distribution such as found in the statistical model. The dispersion may also exist as a mixture of high molecular weight aggregates and low molecular weight molecules. Secondly, dust, undissolved granules, or the like, even though present in minute amounts would give broad size distributions and high apparent molecular weights.

Experiments in this laboratory and others[5–7] have shown that the light scattering molecular weight for a given amylopectin solution can be reproduced within the accuracy of the instrument. Also the light scattering results on potato amylopectin measured at 75 and 25° gave the same results.[6] The consistency of these results indicates that undissolved granular segments or foreign particles such as dust are not present since it would be extremely improbable that the same size impurities would be present in each molecular weight determination. Fractionation of amylopectin and glycogen by centrifugation[5,8] shows that both glycogen and amylopectin have broad size distributions. This would also outrule the presence of a small amount of extremely large particles. In addition, if hydrogen bound aggregates are present, they are not dispersed by heating in Pentasol or 0.5 *N* KOH.[6] Stacy and Foster[7] found that formamide, water, *N* KOH, anhydrous ethylenediamine and ethylenediamine hydrate give essentially the same large $\bar{M}_w$. Also Witnauer, Senti and Stern[6] found that the amylopectin or amylopectin acetate gave reproducible weight-average molecular weights in acetone, chloroform, dioxane, nitromethane and acetonitrile. In addition, a high $\bar{M}_w$ of 70.8 × 10^6 for glycogen was obtained using 15% $MgCl_2$ as a solvent.[5] These results indicate that physical aggregation—if present—cannot be destroyed by hydrogen bond-breaking solvents. A comparison of the weight-average molecular weight of amylopectin or glycogen with the $\bar{M}_w$ of their respective β-amylase limit dextrins[5,7] indicates that if aggregation of the glycogen or amylopectin molecules is present, it is not due to the intermolecular hydrogen bonding or entanglements of their exterior branches.

The results cited above seem to completely outrule the presence of a small amount of extremely large particles. While the evidence is not so overwhelming against the first factor discussed above, all evidence seems to indicate the absence of any type of physical aggregation. However, the authors believed that further experimentation was needed before physical aggregation could be completely ruled out.

So far no study has been made to establish when the dispersion of the starch granules is complete or if degradation occurs during the dispersion. As reported in this paper this type of study was made

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[1] Journal Paper No. J-3244 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1116. This work was taken in part from a thesis submitted by S. R. Erlander to the faculty of Iowa State College in partial fulfillment of the requirements for the degree of Ph. D.

[2] This research was supported in part by a grant from the Corn Industries Research Foundation.

[3] S. Erlander and D. French, *J. Polymer Sci.*, **20**, 7 (1956).

[4] S. Erlander and D. French, *ibid.*, to be published.

[5] M. R. Stetten, H. M. Katzen and D. Stetten, Jr., *J. Biol. Chem.*, **222**, 587 (1956).

[6] L. P. Witnauer, F. R. Senti and M. D. Stern, *J. Polymer Sci.*, **16**, 1 (1955).

[7] C. J. Stacy and J. F. Foster, *ibid.*, **20**, 57 (1956).

[8] C. J. Stacy, J. F. Foster and S. R. Erlander, *Makromol. Chem.*, **17**, 181 (1955).

on the dispersion of starch granules in heated 6 or 8 *M* LiBr by observing the change in turbidity and dissymmetry of the starch solution during dispersion. Following dispersion of the starch granules under a variety of conditions, the resulting solutions were examined by ultracentrifugation, light scattering and acid hydrolysis. If amylopectin behaves as an A–R–B_2 type polymer formed by the random condensation of glucose units, then the acid hydrolysis of amylopectin (a random depolymerization) should be the reverse of its random synthesis.[4]

Experimental

Isolation of Samples.—The variety of corn plants used were Seneca Chief sweet corn, Iowa 4297 dent corn and Iowax 5 hybrid waxy corn. The isolation of the immature and mature waxy, dent and sweet corn starch granules has been discussed previously.[9] The immature samples are designated according to the number of days the corn ears were picked after they were pollinated. The 13th day waxy, 14th day sweet, mature waxy and mature dent corn starch granules were dispersed by refluxing in a 6 or 8 *M* lithium bromide solution, the temperature being controlled so that superheating and foaming did not occur. The 20th day sweet and mature sweet corn starch granules[4] were refluxed 24 hours in an aqueous solution buffered with 0.05 *M* phosphate (*p*H 6.4) and saturated with amyl alcohol according to the method described by Lanski, Kooi and Schoch.[10] The 14th day dent corn starch sample was dispersed for 20 hours in phosphate buffered amyl alcohol solution and in addition for various time limits in unbuffered amyl alcohol solutions at the refluxing temperature as above. A 500-ml. glass stoppered flask was used in all dispersions. This flask was equipped with a thermometer and an inlet for helium gas, a glass stirrer, a reflux condenser and a heating mantle. The atmosphere was kept inert by bubbling helium gas through the lithium bromide or buffered amyl alcohol solutions. The 2 to 3% starch solutions were stirred at a moderate rate since it was found that rapid stirring degraded the amylopectin. During refluxing of the lithium bromide, the concentrated lithium bromide solution changes to a darker color with or without the presence of the starch. However, the absence of a blue color when potassium iodide is added to samples of the cooled LiBr–starch mixture indicates that the LiBr is not decomposed to bromine during the refluxing period. The darkening of the lithium bromide is most likely due to impurities in the lithium bromide.

The dispersion of the starch granules in LiBr was followed by withdrawing samples at various times into a stoppered bottle, cooling these samples under a cold water tap, and diluting the cooled solution approximately 25-fold into a light scattering cell. The pipet used in the dilution was rinsed several times with the diluted solution in order to ensure complete delivery. The LiBr–starch solution was held near its refluxing temperature until a steady 90° angle intensity ($i_{90°}$) and dissymmetry (Z) were obtained. The results for the immature and mature waxy, dent and sweet corn starches are shown in Figs. 1, 2, and 3. These results will be discussed below.

In the four lithium bromide-dispersed, 13th day waxy starch samples, the cooled solutions were dialyzed until silver nitrate tests were negative. In all other LiBr dispersions, the hot solution was added with mechanical stirring to 95% ethanol. The precipitated starch was then shaken three or four times with 80% ethanol to remove the LiBr and finally with methanol. The starch was immediately brought back into solution by adding water and shaking. The lithium bromide dispersion method appeared to make the starch more soluble than the amyl alcohol dispersion method. The small amount of methanol remaining in the solution was removed by vacuum distillation between 25 and 40°. It was found that if the *n*-amyl alcohol is vacuum distilled from the solution[6] instead of using the above precipitation method, the solution becomes acid.

The amylose in the case of dent and sweet corn starch samples was removed by reheating the above solution to its refluxing temperature under a helium atmosphere, adding *n*-amyl alcohol, cooling slowly with stirring to room temperature, centrifuging off the precipitated amylose in an International centrifuge and filtering off the remaining precipitated amylose by using a Millipore filter of sufficient size (HA to SM). The amylopectin solution was then precipitated and washed with alcohol, redissolved and vacuum distilled as before.

In the dispersion of the starch granules with buffered aqueous amyl alcohol the *p*H changed from 6.4 to 6.1 during the 20-hour refluxing period. After 20 hours of refluxing, the amylose was removed as described above. The resulting amylopectin solution was dialyzed to remove the phosphate buffer.[11] The amylopectin then was precipitated and washed with alcohol, redissolved in water and the residual alcohols were removed by vacuum distillation as described above.

The resulting 2 to 3% amylopectin solutions from the lithium bromide and amyl alcohol dispersion methods were centrifuged at 21,000 r.p.m. (59,000 × *g*) for one hour or at 42,000 r.p.m. (143,000 × *g*) for 15 minutes in a Spinco preparatory centrifuge. In waxy corn starch samples I, II and IV, the upper three-fourths of the solution was withdrawn slowly with a hypodermic syringe. This latter precaution was found to be unnecessary and was eliminated. Therefore in other clarifications the solution was gently poured off, the impurities adhering to the side of the centrifuge tube. Optical rotation measurements indicated that there was no detectable change in concentration after centrifuging.

Weighed portions of the above 14th day dent corn amylopectin solution (dispersed 20 hours in a buffered aqueous solution saturated with amyl alcohol) and the 13th day waxy I and III amylopectin solutions (dispersed in hot, concentrated LiBr solutions) were diluted with doubly distilled water for ultracentrifuge studies. The sedimentation constants were obtained using a regular analytical cell or a synthetic boundary forming cell for the low concentrations. The concentration of the stock solution was determined by evaporating a weighed portion to dryness under a heat lamp, and then drying in a vacuum oven at 95° to a constant weight (approximately 12 hours).[6] The alkali number (alk. no.) on the immature dent corn amylopectin samples was obtained according to Kerr,[12] and the number-average degree of polymerization ($\bar{X}n$) was obtained from this alk. no. according to Kerr, *et al.*[13]

Acid Hydrolysis of Immature Waxy Amylopectin.—The above resulting 2 to 3% solutions were heated to their refluxing temperature under a helium atmosphere in a 250-ml. three-necked, glass-stoppered flask equipped with a thermometer, refluxing condenser, heating mantle and magnetic stirrer. The buffer was then added. A 0.05 *M* potassium acid phthalate solution (*p*H 4.24 at refluxing temperature[14]) was used to hydrolyze the 13th day waxy starch samples. Samples were extracted at various time intervals and immediately precipitated by adding the hot solution to 95% ethanol with mechanical stirring. They were washed three or four times with 80% ethanol and then with methanol, redissolved and vacuum distilled as described above.

Light Scattering Studies.—Weighed portions of stock amylopectin solutions were diluted with doubly distilled water to approximately 100 ml. and weighed again on a Sartorius balance. These diluted solutions were then prepared for light scattering by using HA (4500 Å. pore size) or AA (8000 Å. pore size) Millipore filters. The size of the filter used depended on the molecular weight of the sample. It was found by optical rotation that passing the solution through the filter did not change the concentration. In cases where the molecular weight was over approximately

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[9] S. Erlander, *Cereal Chem.*, to be published.

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

[11] In all the dialysis experiments, the dialysis bags were first boiled in a saturated solution of sodium bicarbonate to remove the soluble impurities, and then boiled twice in doubly distilled water to remove the remaining bicarbonate. Extensive washing followed each boiling.

[12] R. W. Kerr, "Chemistry and Industry of Starch," 2nd ed., Academic Press, Inc., New York, N. Y., 1950, pp. 679–680.

[13] R. W. Kerr, F. C. Cleveland and W. J. Katzbeck, THIS JOURNAL, **73**, 111 (1951).

[14] R. G. Bates, "Electrometric *p*H Determinations," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 74.

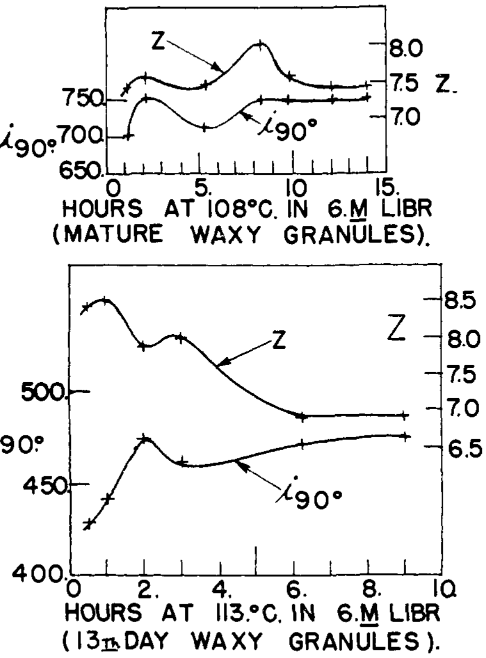

Fig. 1.—The dispersion of 13th day and mature waxy corn starch granules in the 6 *M* LiBr at or near the refluxing temperature as followed by light scattering. The constant intensity of the light scattered at the 90° angle (i_{90°) and the constant dissymmetry (Z) in the figure indicates complete dispersion. The initial increase in i_{90° may be due to the swelling of the starch granules.

100 million, the solutions were not passed through the filter but only the water used in diluting these samples was clarified.

In making the light scattering measurements, 50.00 ml. of HA filtered water was added to a Brice–Phoenix cylindrical cell. The above diluted starch solutions were added to the cell in increments of 1, 5, 15 and 50 ml. This procedure enables one to obtain an accurate blank and avoids the cumulative contamination obtained in going from concentrated to more dilute solutions. This is extremely important since the concentrations used were very low, the lowest being around 10^{-5} or 10^{-6} g./ml. For the first three additions of starch solution to the light scattering cell, the cell was swirled gently to obtain a homogeneous solution. After the last addition it was tipped back and forth. It was found that although a magnetic stirrer can be used to stir the solution, it was more cumbersome and did not give as good results. Light scattering measurements were made over the angle range from 21.6 to 135°.

The weight-average molecular weights of the amylopectins and glycogen and their acid-hydrolyzed products[4] were obtained by plotting C/R_θ *versus* $\sin^2 \theta/2 + kC$ (Zimm plot[15]). Here C equals the concentration and R_θ equals the reduced intensity or Rayleigh's ratio corrected for volume scattering and the use of unpolarized light. These corrections were made by multiplying R_θ by $\sin \theta/(1 + \cos^2\theta)$. The low angle readings were further corrected by using a very dilute solution of fluorescein. Fluorescein gives a constant R_θ reading when corrected for volume scattering (R_θ times $\sin \theta$) and any deviations of these $R_\theta \sin \theta$ values from the R_{90° values were corrected. The constant k was chosen so that kC will be approximately the same order of magnitude as $\sin^2\theta/2$ for the average concentration. The molecular weight was obtained by extrapolating to zero angle and zero concentration and multiplying the intercept by K, that is, $1/\bar{M}_w = KC/R_\theta$ at $\theta = 0$ and $C = 0$. Here K equals $2\pi^2 n_0^2(\mathrm{d}n/\mathrm{d}c)^2/N\lambda^4$. In these studies λ = wave length of light in vacuum = 4358 Å., $N = 6.023 \times 10^{23}$, n_0 = refractive index of solvent (H_2O) = 1.34, and $\mathrm{d}n/\mathrm{d}c$ = change in refractive index with concentration and was found to be 0.156 ml./g. for both the glycogen and the amylopectin

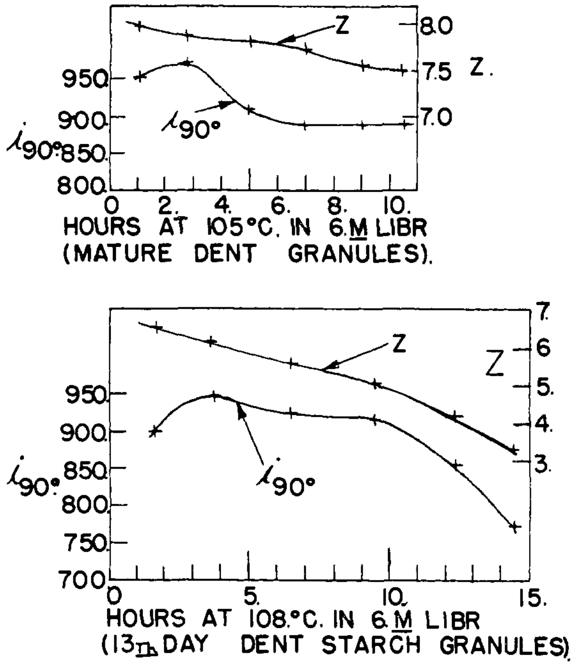

Fig. 2.—The dispersion of 14th day and mature dent corn starch granules in 6 *M* LiBr near the refluxing temperature as followed by light scattering. The constant intensity of the light scattered at the 90° angle (i_{90°) and the constant dissymmetry (Z) indicates complete dispersion (mature dent starch) while the consistently decreasing (immature dent starch) i_{90° and Z indicates that the starch is being hydrolyzed (see text).

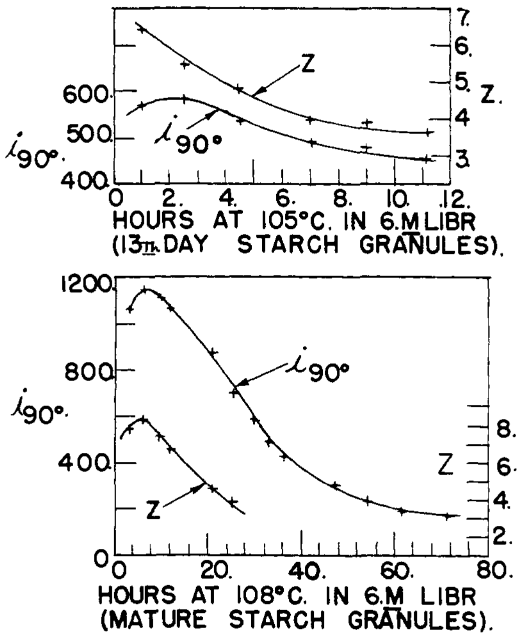

Fig. 3.—The dispersion of 14th day and mature sweet corn starch granules in 6 *M* LiBr near the refluxing temperature as followed by light scattering. The attainment of the constant 90° angle intensity (i_{90°) and constant dissymmetry (Z) in the immature sweet corn starch indicates complete dispersion without hydrolysis while a consistently decreasing i_{90° and Z indicates hydrolysis of the starch (mature sweet corn starch).

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(15) B. Zimm, *J. Chem. Phys.*, **16**, 1099 (1948).

solutions for the above wave length. The calculated value of K was found to be 3.97×10^{-7}. When N KOH is used as a solvent, the value of K using $n_0 = 1.35$ and $dn/dc = 0.152$ at $\lambda = 4358$ A. is $K = 3.94 \times 10^{-7}$. From the Zimm plot the radii of gyration were obtained from the expression[16,17]

$$R_z^2 = \frac{3\lambda^2 \text{ (initial slope)}}{16\pi^2\text{(intercept)}}$$

Witnauer, Senti and Stern[6] found that the measured depolarization for potato amylopectin was approximately 2%. Therefore, it was assumed that the optical anisotropy for the samples studied was also small. The scattering correction[18] for the reflection of the exit beam in the light scattering cell was not made. An assumed 5.3% correction was made, however, on the mature dent corn amylopectin sample ($\bar{M}_w = 45 \times 10^6$), and it was observed that for this molecular weight the scattering correction is negligible.[4,19] The light scattering instrument used in these studies has been described previously.[20,21] Data given by Stamm[22] for refractive indices of NaCl solutions at $\lambda = 4358$ Å. were used to calibrate the differential refractometer.

It was observed that a significant concave-upward curvature occurred in the very low angle region (below the 30 to 35° angles) of the lines of constant concentration for large molecular weights (see Fig. 4). The molecular weights obtained using the angles 30 to 35° as the lowest angle readings were considerably higher, and in some cases they were meaningless; that is, the extrapolated value for C/R_θ was negative. Therefore there is a possibility that some of the molecular weights reported previously in the literature[6,7,23,24] may be too large. It will be shown that a plot of the theoretical value of $1/\bar{X}n$ (obtained from $\bar{M}_w$) *versus* time hydrolyzed gives a straight line. This method provides greater accuracy in obtaining the light scattering molecular weight of the original, unhydrolyzed amylopectin.

Discussion

It is well recognized that certain concentrated aqueous salt solutions are effective swelling agents for starch granules[25] and good dispersing agents for proteins.[26] Certain concentrated salt solutions, in particular lithium bromide, have extremely high activity coefficients.[27] According to Harrington and Schellman,[26] the dispersion of proteins is due to the lowering of the activity of the water by these salts and to the presence of a large anion and small cation. These conditions appear to stabilize the helical form of the protein rather than the crystallized form. If this reasoning can be applied to the dispersion of starch granules, then it appears that any salt which has a high activity coefficient in water, a small cation, and a large anion could act as an excellent solvent for starch granules. Lithium thiocyanate would appear to be a good solvent, but its activity in aqueous solutions has not been studied. A 15% $MgCl_2$ solution which also has a high activity coefficient[27] was found by Staudinger[28] to be a good solvent for glycogen and was later used by Stetten, *et al.*,[5] as a medium for light scattering measurements on glycogen.

It was found that starch granules may be partially dispersed with shaking at room temperature under a nitrogen atmosphere in 6 or 8 M LiBr but that this dispersion is not complete after 3 or 4 days. In order to achieve complete dispersion in a reasonable length of time, it is therefore necessary to heat the solution. The progress of the dispersion of starch granules by concentrated lithium bromide solutions may be followed readily by light scattering measurements (see Figs. 1,2 and 3). If complete dispersion is attained without progressive degradation of the polysaccharide, then the turbidity and dissymmetry should approach a constant value (see for example the behavior of immature and mature waxy starch in Fig. 1). The samples which were hydrolyzed during the dispersion are immature dent and mature sweet corn starch samples. The hydrolysis of these starch samples is most likely due to the lowering of the pH of the 6 M LiBr solution.[29,30]

The molecular weights and other pertinent properties of those samples which attained constant turbidity and dissymmetry on heating in lithium bromide solutions are given in this paper

TABLE I

THE ACID HYDROLYSIS OF 13TH DAY WAXY CORN AMYLOPECTIN

The weight-average molecular weights were obtained from Zimm plots on samples withdrawn during the acid hydrolysis. All of the amylopectin samples were hydrolyzed at 99.0° and pH 4.24 in 0.05 M potassium acid phthalate. The theoretical number-average degrees of polymerization were calculated[3] from these $\bar{M}_w$ assuming $p_6 = 0.058$.

Waxy I sample			Waxy II sample			Waxy 111 sample		
Hr. refl.	$\bar{M}_w \times 10^{-6}$	Theor. $\bar{X}_n$	Hr. refl.	$\bar{M}_w \times 10^{-6}$	Theor. $\bar{X}_n$	Hr. refl.	$\bar{M}_w \times 10^{-6}$	Theor. $\bar{X}_n$
0.5	105	2270	2.0	55.6	1765	0.0	168	3070
1.0	83.5	2160	5.0	18.8	1021	3.0	43.0	1550
2.0	53.7	1731	7.0	13.3	858	6.0	18.3	1009
3.0	37.6	1450	10.0	7.91	660	10.0	9.1	709
5.0	21.5	1093	13.0	6.02	575	14.0	5.25	536
7.0	15.8	936	16.0	3.67	447			
			27.0	1.48	281			

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[16] P. Debye, *J. Phys. Colloid Chem.*, **51**, 18 (1947).

[17] A. Peterlin, *Record Chem. Progr.*, **17**, 109 (1956).

[18] H. Sheffer and J. C. Hyde, *Can. J. Chem.*, **30**, 817 (1952).

[19] These corrections[18] are made by subtracting 5.3% of the forward angle intensity (less than 90°) from the corresponding backward angle intensity and then by subtracting 5.3% of this corrected backward angle intensity from the corresponding forward angle intensity. For large dissymmetries the only significant correction occurs for those angles greater than 90°. Since the molecular weight is obtained by extrapolating to zero angle and zero concentration, the molecular weight for those amylopectins having a high dissymmetry remains unchanged when a reflection correction is made. This correction may, however, be more significant at lower angles for the more extensively hydrolyzed amylopectins where the dissymmetries are much smaller.

[20] T. G. Northrup and R. L. Sinsheimer, *J. Chem. Phys.*, **22**, 703 (1954).

[21] G. R. Hopkins and R. L. Sinsheimer, *Biochim. et Biophys. Acta*, **17**, 476 (1955).

[22] R. F. Stamm, *J. Opt. Soc. Amer.*, **40**, 788 (1950).

[23] B. H. Zimm and C. D. Thurmond, THIS JOURNAL, **74**, 1111 (1952).

[24] C. J. Stacy and J. F. Foster, *J. Polymer Sci.*, **25**, 39 (1957).

[25] C. E. Mangels and C. H. Bailey, THIS JOURNAL, **55**, 1981 (1933); J. A. Radley, "Starch and Its Derivatives," Chapman and Hall, Ltd., London, 1953, Vol. I, pp. 85–87.

[26] W. F. Harrington and J. A. Schellman, *Compt. rend. Lab. Carlsberg sér. chim.*, **30**, 167 (1956).

[27] R. A. Robinson and R. H. Stokes, "Electrolyte Solutions," Academic Press, Inc., Publishers, New York N. Y., 1955, pp. 482 and 489.

[28] H. Staudinger, *Makromol. Chem.*, **2**, 88 (1948).

[29] Yount and Metzler[30] observed that an acetate buffer which normally buffers water near pH 7 changed the pH of a 6 M lithium bromide solution from a neutral pH to a pH of around 2. It was then observed by the authors that a phosphate buffer (pH 6.5) lowered the pH of a neutral 6 M LiBr solution to a pH of 2.0. The exact nature of this change in pH is not clearly understood. The change can be observed using both a glass electrode and pH paper. It might be possible to remove the acidity by dialyzing a cold mixture of starch granules and 6 M LiBr against 6 M LiBr so that subsequent heating of the starch–lithium bromide mixture would not lead to degradation. It was observed that a concentrated solution of lithium bromide will dissolve a dialysis bag within an hour when placed under a heat lamp. However, in 6 M LiBr and at 0° the dialysis bag does not appear to be affected within 24 hours.

[30] R. Yount and D. Metzler, private communication.

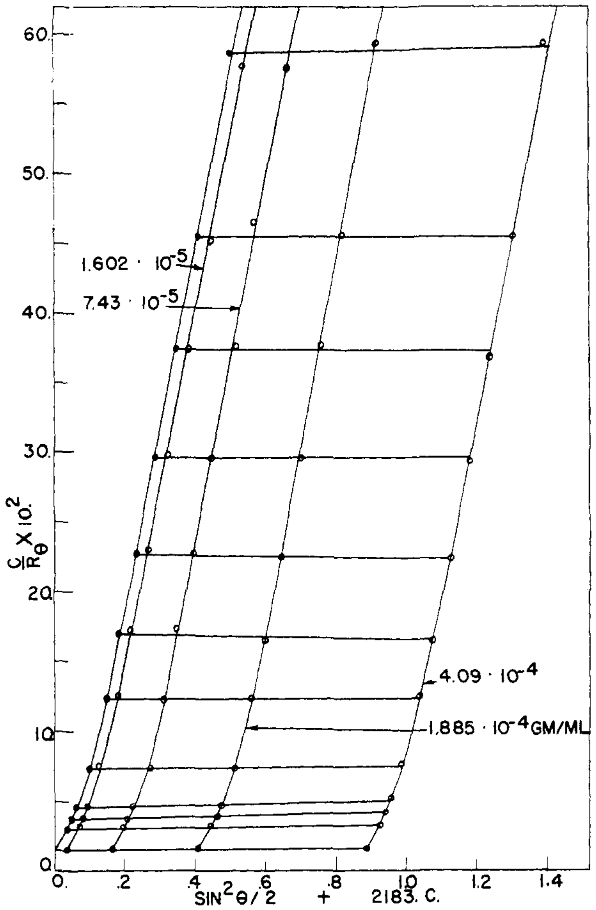

Fig. 4.—A Zimm plot[15] of the 14th day dent corn amylopectin; M_w = 168 × 10^6. The starch granules from which the amylopectin was obtained were dispersed by refluxing for 20 hours in a buffered solution saturated with amyl alcohol. The concave-upward curvature in the low-angle region (the 21.6°, 25.2°, and 28.8° angle readings) occurred in the Zimm plot of all high molecular weight amylopectins (see text).

(the 13th day waxy starch samples I, II and III) and in another paper[4] (the 13th day waxy IV, mature waxy, mature dent and 14th day sweet corn starch samples). The four different dispersions of the 13th day waxy corn amylopectin were acid hydrolyzed with phthalate as described in the Experimental section. The data obtained from these studies on samples I, II and III are listed in Table I. It is shown elsewhere[4] that a plot of $1/\bar{X}_n$ *versus* time hydrolyzed should give a straight line for A–R–B$_2$ type random condensation polymers. Here $1/\bar{X}_n$ is the reciprocal of the number-average degree of polymerization and can be interconverted to the weight-average molecular weight using the previously developed equations.[3] In Fig. 5 and in another paper[4] it is seen that a straight line is obtained for all the 13th day waxy starch samples when the reciprocal of the number-average degree of polymerization as obtained from the weight-average molecular weight is plotted against time hydrolyzed.[31] This indicates that amylopectin has a broad size distribution such as that found in A–R–B$_2$ type polymers.[3]

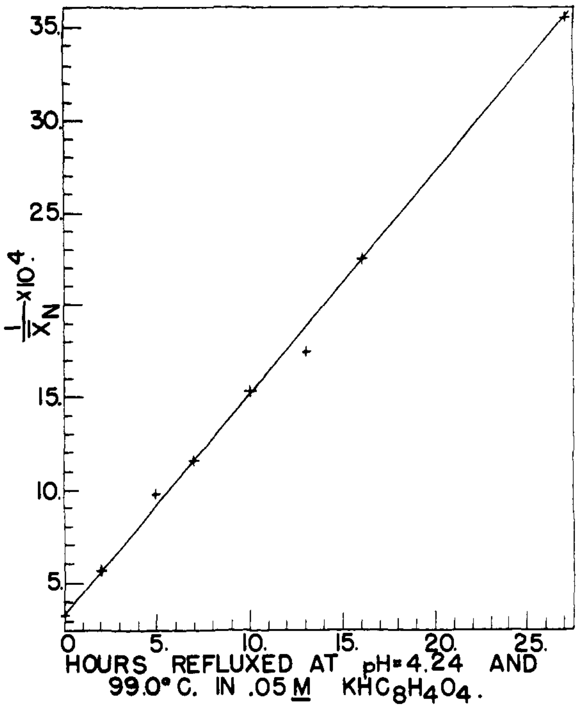

Fig. 5.—The acid hydrolysis of the 13th day waxy corn amylopectin, sample II. The theoretical number-average degrees of polymerization (see Table I) were obtained from the weight-average molecular weight using p_6 = 0.058 for the statistical model.[3] The straight line indicates that amylopectin has a broad size distribution as found in A–R–B$_2$ type polymers (see text).

It is apparent that the initial weight-average molecular weight can be obtained from the extrapolated $\bar{X}_n$. These extrapolated $\bar{M}_w$ for the waxy amylopectin and also the temperature and length of time used in dispersing the starch granules with lithium bromide are listed in Table II. The extrapolated $\bar{M}_w$ for the 13th day waxy I amylopectin is smaller than the other values. This sample was clarified by centrifugation and by removing the upper portion of the centrifugate.

TABLE II

THE MOLECULAR WEIGHTS OF VARIOUS PREPARATIONS OF 13TH DAY WAXY CORN AMYLOPECTIN

The extrapolated weight-average molecular weights of the 13th day waxy corn amylopectin listed in this table were calculated from the extrapolated values of $1/\bar{X}_n$ of Table I and another paper[4] (see text). The solvent, temperature and length of time used in dispersing the starch granules are given.

Sample	LiBr concn., M	Refluxing temp., °C.	Hours refl.	Extrapolated $\bar{M}_w \times 10^{-6}$
I	6	113	9	146
II	6	113	2	168
III	8	125	6	168
IV	8	125	6	168

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(31) Because of theoretical complications, the change in the observed $\bar{X}_n$ (as observed by end-group assay or osmotic pressure measurements) during hydrolysis is compared to the corresponding change in the theoretical $\bar{X}_n$ in another paper.[4] In the present paper the acid hydrolysis lines are used to obtain a more precise light-scattering molecular weight and to show that a given sample of amylopectin constantly behaves as a statistically formed A–R–B$_2$ type polymer.

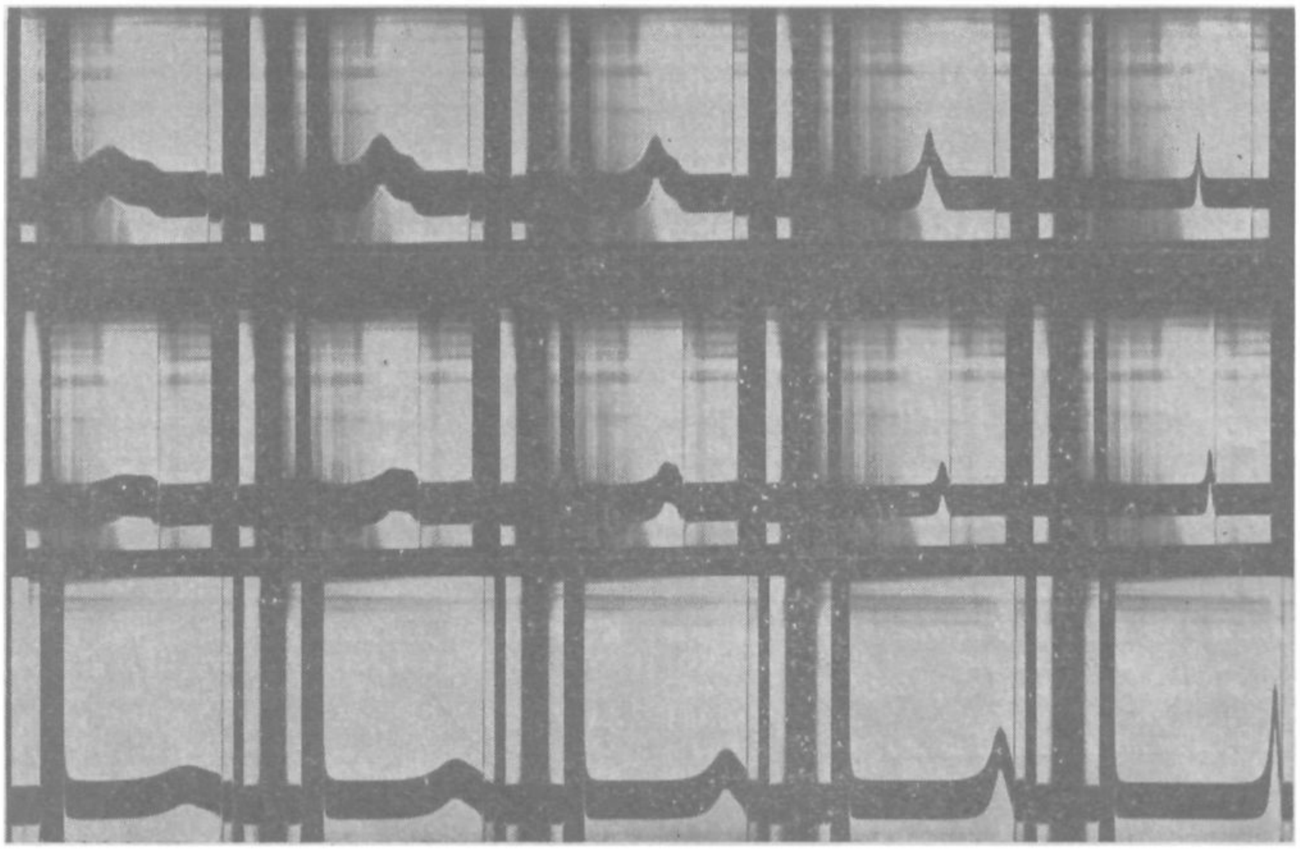

Fig. 6.—Sedimentation picture showing two components in 13th day waxy corn amylopectin and one in immature dent corn amylopectin. The pictures read from right to left. Top frame: waxy I starch, total concn. 0.501%, 12,590 r.p.m., pictures taken at 0, 8, 16, 24 and 32 minutes, $s_{20,w}$ (fast component) = 141S., $s_{20,w}$ (slow component) = 20S. Middle frame: waxy I starch, total concn. 0.200%, 8,210 r.p.m., pictures taken at 0, 2, 10, 18 and 26 minutes, $s_{20,w}$ (fast component) = 296S.; $s_{20,w}$ (slow component) = 94S. Bottom frame: 14th day dent corn amylopectin, concn. 0.529%, 12,590 r.p.m., pictures taken at 0, 8, 16, 24 and 32 minutes; $s_{20,w}$ = 106S.

Therefore this smaller molecular weight may have been due to a loss of high molecular weight material. The constant values for $\bar{M}_w$ indicate that complete dispersion with no detectable degradation has been achieved.

The dispersion of 14th day immature dent corn starch granules was studied in unbuffered and buffered solutions which were saturated with amyl alcohol. The molecular weights on the resulting amylopectins are listed in Table III. It is seen that the amylopectin is degraded to a greater extent in a 3-hour, unbuffered dispersion than in a 20-hour, buffered dispersion.[32] The molecular weights obtained in water and in N KOH on the same samples agree quite well. The molecular weight of the amylopectin obtained by refluxing for 20 hours in a buffered Pentasol solution had a weight-average molecular weight of the same order of magnitude in both 6 M LiBr and water.[33]

If hydrogen bonded aggregates are present, then the above variations in the method of dispersion by LiBr should produce drastically different results in the resulting amylopectin. The consistency of the extrapolated molecular weights, the consistency of the linear $1/\bar{X}_n$ *versus* time graphs, and the similar molecular weights obtained in water, N KOH and 6 M LiBr strongly indicates that no physical aggregates are present.

The sedimentation constants were obtained for the 13th day waxy I and III (both dispersed with LiBr) and the 14th day dent corn amylopectins (dispersed 20 hours in buffered Pentasol) using water as a solvent in all cases. Two components were found in immature waxy starch (see Fig. 6). The sedimentation coefficients for the two components of immature waxy starch and for the single component of immature dent corn amylopectin are listed in Tables IV and V. The extrapolation of these sedimentation coefficients to zero concentration is shown in Figs. 7 and 8. It was observed that a straight line is obtained using $1/s_{20,w}$ *versus* concentration, and a curved line is obtained using $s_{20,w}$ *versus* concentration. Here $s_{20,w}$ is the

TABLE III

THE DISPERSION OF 14TH DAY DENT CORN STARCH GRANULES IN BUFFERED AND UNBUFFERED AQUEOUS SOLUTIONS SATURATED WITH AMYL ALCOHOL

The number-average molecular weight as obtained from the alkali number,[13] the weight-average molecular weight and the Z-average radii of gyration are listed for the resulting amylopectin. The theoretical $\bar{X}_n$ was obtained[3] from the $\bar{M}_w$ assuming p_6 = 0.068 in the formula: $\bar{M}_w = 162 (1 - p^2_4 - p^2_6)/(1 - p_4 - p_6)^2$. Either N KOH or water was used as a solvent in obtained $\bar{M}_w$.

Amylopectin sample	Solvent for $\bar{M}_w$	$\bar{M}_w \times 10^{-6}$	Theor. $\bar{X}_n$	$\bar{X}_n$ (from alk. no.)	$(\bar{R}z^2)^{1/}$ Å.
20 hr., buff.	H_2O	168	2880	. .	4680
3 hr., unbuff.	N KOH	141	2630	2670	4160
6 hr., unbuff.	N KOH	99	2190	1305	3850
6 hr., unbuff.	H_2O	98	2180	1305	3840
8 hr., unbuff.	N KOH	24.0	1080	801	2344
8 hr., unbuff.	H_2O	24.4	1081	801	2145
10 hr., unbuff.	H_2O	12.2	764	740	1440
14 hr., unbuff.	H_2O	3.97	432	392	828

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[32] On refluxing an unbuffered solution of amylopectin in aqueous amyl alcohol, the pH of the solution drops to about 4 after four or five hours. Consequently, the amyl alcohols appear to be oxidized to the acid. The use of pure n-amyl alcohol or amyl alcohol which has been vacuum distilled over NaOH did not prevent this production of acidity.

[33] The exact molecular weight was not obtained in lithium bromide because of the difficulty in getting an accurate dn/dc for the 6 M LiBr solutions.

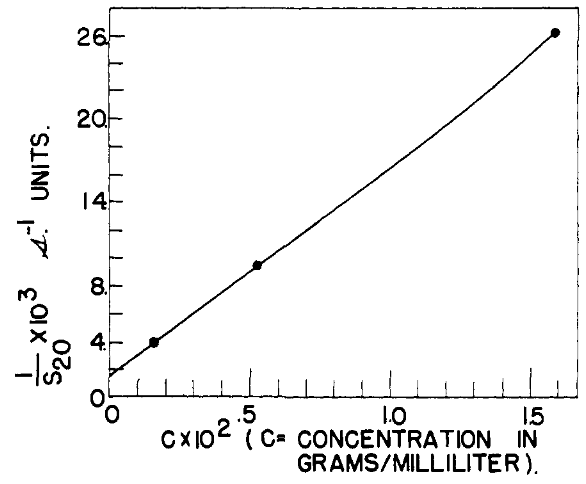

Fig. 7.—Extrapolation of the sedimentation coefficients (in reciprocal Svedberg units) of the 14th day dent corn amylopectin to zero concentration.

sedimentation coefficient in Svedberg units corrected to 20°. The curved line is extremely dependent on concentration and no accurate extrapolation to zero concentration can be made. Therefore the $1/s_{20,\mathrm{w}}$ *versus* concentration line was used to obtain the sedimentation constant. The extremely high sedimentation constants suggest that the magnitude of the light scattering molecular weights is correct. Large sedimentation coefficients on other amylopectins also have been reported.[24,34]

TABLE IV

SEDIMENTATION COEFFICIENTS FOR THE 14TH DAY DENT CORN AMYLOPECTIN USING WATER AS A SOLVENT

The amylopectin was obtained by dispersing the starch granules for 20 hours in a buffered aqueous solution saturated with amyl alcohol.

$C \times 10^2$, g./ml.	1.586	0.529	0.1586
$s_{20,\mathrm{w}}$, Svedbergs	36.2	106	257
R.p.m. of run	29,500	12,590	4197
Extrapol. value	$s_{20,\mathrm{w}} \approx 625$ Svedbergs		

TABLE V

SEDIMENTATION COEFFICIENTS (IN SVEDBERG UNITS) FOR THE 13TH DAY WAXY CORN STARCH USING WATER AS A SOLVENT

The 13th day waxy corn starch samples I and III were dispersed as indicated in Table II.

	Waxy I starch						Waxy III starch			
$C \times 10^2$, g./ml.	1.008	0.501	0.454	0.200	0.0454	0.0454	0.574	0.491	0.1648	0.0404
$s_{20,\mathrm{w}}$ (fast compon.)	95	141	223	296	331	552	114	131	386	610
$s_{20,\mathrm{w}}$ (slow compon.)	17	20	...	94	...	...	42	44	...	...
R.p.m. of run	29,500	12,590	12,590	8210	8210	5993	8210	12,590	8210	5993
Extrapol. value for fast compon.	$s_{20,\mathrm{w}} = 720$ Svedbergs						$s_{20,\mathrm{w}} = 870$ Svedbergs			

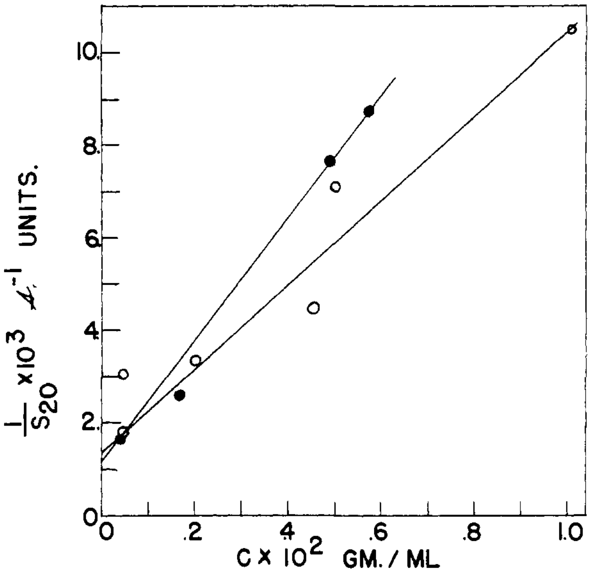

Fig. 8.—Extrapolation of the sedimentation coefficients (in reciprocal Svedbergs) of the fast component in the 13th day waxy I (O) and waxy III (●) corn amylopectin samples.

The slow component found in the 13th day waxy corn starch samples but not in the mature waxy corn starch sample or other starch samples studied cannot be precipitated with Pentasol. Iodine titration studies indicate that only 0.92% amylose (an iodine binding capacity of 0.175 mg. of iodine per 100 mg. of sample) is present. Furthermore, this iodine binding capacity may only be due to the iodine binding capacity of the exterior chains of the amylopectin. A 6 *M* LiBr solution was dialyzed against water for 24 hours at room temperature in the same manner as the LiBr-dispersed immature waxy starch samples. This blank solution gave no sedimentation pattern when examined at both low and high speeds in the ultracentrifuge indicating that the slow peak is not due to impurities obtained from the dialysis bag. This slower component may be a glycogen[4,35] or an amylopectin type polymer. Other studies by the authors[4] suggest that the large molecules of corn amylopectin and corn glycogen are chemically aggregated in groups of four, perhaps to a protein nucleus. It is postulated therefore that this slower sedimenting component may be amylopectin or glycogen molecules that have not been chemically aggregated or it may be a lower molecular weight amylopectin or glycogen impurity from the ovary or the pericarp. It is also suggested that the "thymol amylopectin" of Cowie and Greenwood[36] may be a glycogen or an amylopectin which is not chemically aggregated.

Upon inspection of Fig. 6 it appears that the relative concentration of the slower component increases with dilution. This effect is not due to a variation in the method of preparing the sample since both runs were made on dilution of the same stock solution. While this effect might be due to an equilibrium between the fast and slow components, it is much more likely that the apparent increase in the relative amount of the slower com-

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[36] J. M. G. Cowie and C. T. Greenwood, *J. Chem. Soc.*, 4640 (1957).

ponent is a concentration anomaly.[37–39] This anomaly can be explained if one assumes that the sedimentation coefficient of the slower component is greatly dependent on the concentration as observed above for that of the faster moving component.

For simplicity let us assume that the equation obtained by Johnston and Ogston[40] is valid[37]: $C_s^{obs}/C_s^0 = (S_f - S_{s,mixt})/(S_f - S_s)$ where C_s^{obs} and C_s^0 are the observed and initial concentrations of the slow component; $S_{s,mixt}$ and S_s are the sedimentation coefficients of the slow component in the presence of the fast component and alone; and S_f is the sedimentation coefficient of the fast component. Again for simplicity, let us assume that in the 0.5% solution the value of $S_{s,mixt}$ is equal to that found in the 1% solution, that is, 17 Svedbergs, and that in the 0.2% solution the value of $S_{s,mixt}$ is equal to that found in the 0.5% solution, that is, 20 Svedbergs. Using the values for the fast and slow components of waxy I amylopectin listed in Table V, the value of C_s^{obs}/C_s^0 for the 0.5% solution is $(141 - 17)/(141 - 20) = 1.03$ and for the 0.2% solution is $(296 - 20)/(296 - 94) = 1.37$. Thus the observed relative concentrations for the slower component in the 0.2% solution may be as much as 4/3 times as large as in the 0.5% solution.

Conclusions

1. Starch granules can be completely dispersed by refluxing in neutral 6 or 8 M LiBr solutions without degrading the amylopectin to any detectable extent. 2. In dispersing starch granules by refluxing in amyl alcohol solutions, a buffer must be used. An amylopectin obtained from starch granules dispersed in a buffered, amyl alcohol-saturated solution for 20 hours was less hydrolyzed than an amylopectin obtained from starch granules dispersed for three hours in an unbuffered solution. 3. The above results illustrate that (a) similar light-scattering molecular weights are obtained using N KOH, water and 6 M LiBr as solvents; (b) sedimentation constants of immature corn amylopectins are extremely large; (c) immature waxy corn amylopectin consistently behaves as a statistically branched polymer (acid hydrolysis studies); and (d) variations in the method of dispersing starch granules with lithium bromide do not appear to alter the molecular weight of the amylopectin. These results indicate that intermolecular hydrogen bonding is absent in the dispersed amylopectin samples and that amylopectins have broad size distributions such as found in A–R–B_2 type polymers. 4. Immature waxy corn starch has two components. The fast moving component may be a chemically aggregated amylopectin while the slower moving component may be an amylopectin or glycogen which does not exist as a chemically aggregated "tetramer" or which is an impurity from the pericarp or ovary. The apparent increase in the relative concentration of the slower component when the starch solution is diluted is most likely due to a concentration anomaly in the schlieren ultracentrifuge pattern.

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Ames, Iowa

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[Contribution from the Department of Chemistry, University of British Columbia]

The Constitution of the Hemicelluloses of Sitka Spruce (*Picea sitchensis*). I. Composition of the Hemicellulose and Identification of 2-*O*-(4-*O*-Methyl-D-glucopyranosiduronic Acid)-D-xylose[1]

By G. G. S. Dutton and K. Hunt

Received March 13, 1958

The hemicelluloses of Sitka spruce which were extracted from the chlorite holocellulose with 10% aqueous potassium hydroxide gave upon hydrolysis D-xylose, D-mannose, D-galactose, D-glucose, (?)L-arabinose and an aldobiouronic acid. All of the neutral sugars except arabinose were identified as crystalline derivatives. The aldobiouronic acid has been identified as 2-*O*-(4-*O*-methyl-α-D-glucuronosyl)-D-xylose.

Sitka spruce sawdust was delignified by treatment with sodium chlorite and the hemicellulose was obtained from the holocellulose by alkaline extraction. Upon hydrolysis the hemicellulose gave an aldobiouronic acid and a mixture of neutral sugars. The acidic component was separated by means of ion-exchange resins and the neutral sugars separated on a cellulose column using 1-butanol saturated with water at 5°. All of the sugars with the exception of arabinose were characterized as crystalline compounds. The aldobiouronic acid

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[1] A preliminary account of this work was presented at the 40th Annual Conference, Chemical Institute of Canada, in Vancouver, Canada, June, 1957, and at the 133rd A. C. S. Meeting in San Francisco, Calif., April, 1958. The Sitka Spruce sawdust was obtained from the Powell River Co. Ltd. through the courtesy of Dr. R. F. Patterson to whom we express our thanks. This work is abstracted from a thesis submitted by K. Hunt for the M.Sc. degree, April, 1957, and was supported by the National Research Council of Canada to whom we express our thanks.