The Molecular Size of Starch by the Mercaptalation Method[1]

BY M. L. WOLFROM, D. R. MYERS AND E. N. LASSETTRE

In a previous publication from this Laboratory[2] we have reported an estimate of the molecular size of methylated cellulose (from acetone-soluble cellulose acetate) made by a mathematical extrapolation to zero time of the data obtained by subjecting the polysaccharide to hydrolysis at 0° with fuming hydrochloric acid under conditions of continuous mercaptalation and determining the amount of combined sulfur at stated time intervals. We wish now to report the extension of this procedure to a sample of high grade commercial potato starch.

Shortly after the publication of the work of Willstätter and Zechmeister[3] on the action of fuming hydrochloric acid (d. 1.20) on cellulose, Daish[4] reported a careful study of the mutarotation of a sample of "soluble" starch at 20° in the same reagent and in ordinary concentrated hydrochloric acid (d. 1.16). He showed that the mutarotation was a continuous function and that the specific rotation changed from an initial value of approximately +200° to a value very close to that of d-glucose in the same solvent. He found that under the same conditions maltose yielded mutarotation data from which a first order specific rate constant could be calculated but that this was not true for starch.

Our sample of commercial potato starch gave a clear solution in hydrochloric acid, showed no detectable Fehling's reduction, and gave a very low alkali number by the Schoch and Jensen[5] procedure.

For the mercaptalation work the starch was suspended in water at 0° (undercooled) and to this was added fuming hydrochloric acid (d. 1.21) so that the resultant mixture was a 5% solution of starch in hydrochloric acid containing 37.5 g. of hydrogen chloride per 100 cc. of solution. An excess of ethyl mercaptan was then added and

samples were withdrawn from the hydrolysis mixture and neutralized at various time intervals during the first twenty-six hours. Since no water-insoluble products were obtained, acetylation was employed in order to effect a separation from inorganic salts. The neutralization mixtures were concentrated to dryness and the mercaptalated hydrolyzed starches converted to their acetates by acetylation with acetic anhydride in pyridine. The acetate mixtures were isolated, dried and precisely analyzed for sulfur. The average chain lengths indicated by the analytical data varied from two to seventeen glucose units. The results are recorded in Table I.

The course of the hydrolysis was followed polarimetrically at 0° by means of a separate sample of the hydrolysis mixture, withdrawn for this purpose before the addition of the ethyl mercaptan. The specific rotations for various time intervals are recorded in Table I. Figure 1 represents the change in rotation with increasing time of hydrolysis. Extrapolation of this curve to zero time (time of addition of the acid to the starch) indicates an initial specific rotation of approximately +203° for the original starch.

Figure 2 represents the degree of polymerization as calculated from the analytical data, plotted against the time of hydrolysis, initial time being taken as the time of addition of the acid. A smooth curve may be drawn through the points but the initial curvature is such that a graphic extrapolation to zero time is uncertain. The data were then subjected to a graphic analysis by application of the equation (2) previously developed for methylated cellulose

$$\frac{d-1}{d} = \frac{d_0 - 1}{d} e^{-kt}$$

where d is the average degree of polymerization in glucose units at time t as defined by the expression

$$d = \frac{\text{total number of glucose units}}{\text{number of polymers}}$$

d_0 is the initial degree of polymerization or the number of glucose units initially present in each polymer; k is the specific rate constant.

When the above equation is satisfied a plot of $\log_e (d-1)/d$ against t will give a straight line,

(1) The experimental data herein reported were presented before the Division of Organic Chemistry at the 96th Meeting of the American Chemical Society, Milwaukee, Wisconsin, September 8, 1938. Original manuscript received December 27, 1938.

(2) M. L. Wolfrom, J. C. Sowden and E. N. Lassettre, THIS JOURNAL, 61, 1072 (1939).

(3) R. Willstätter and L. Zechmeister, Ber., 46, 2401 (1913).

(4) A. J. Daish, J. Chem. Soc., 105, 2065 (1914).

(5) T. J. Schoch and C. C. Jensen, paper presented before the Division of Sugar Chemistry at the 96th Meeting of the American Chemical Society, Milwaukee, Wisconsin, September 8, 1938.
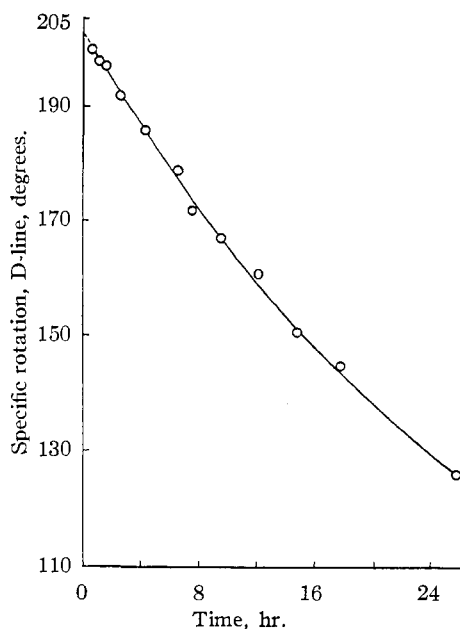
Fig. 1.—Mutarotation of potato starch (c, 4.84) in hydrochloric acid (37.5 g. HCl per 100 cc. soln.) at 0°.

the slope of which is $-k$ and from the intercept of which d_0 can be calculated. The experimental results are shown graphically in Fig. 3. A straight line is obtained and the closely approximate validity of the equation as applied to starch is accordingly confirmed. The points do show a
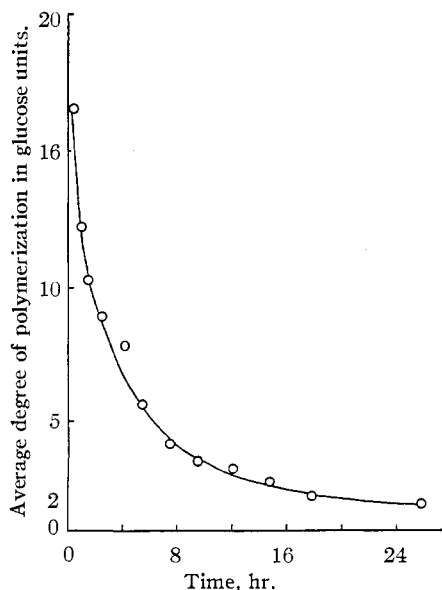

Fig. 2.—Relationship between time of hydrolysis of potato starch and average degree of polymerization as calculated from sulfur content of mercaptalated products.

slight tendency to oscillate about the line but this is of no significance as the polarimetric data (Fig. 1) show no discontinuities. The extrapolated points lie close to the axis. The degree of polymerization reaches such a low value that deviations from linearity might well be expected. However, the accuracy of the present experimental results is such that further refinement of the calculations is not required. The value of the specific rate constant k (hours^{-1}) at 0° is 2.66 × 10^{-2}. The average degree of polymerization, d_0, for the original starch is 20 ± 4, the uncertainty being estimated graphically.
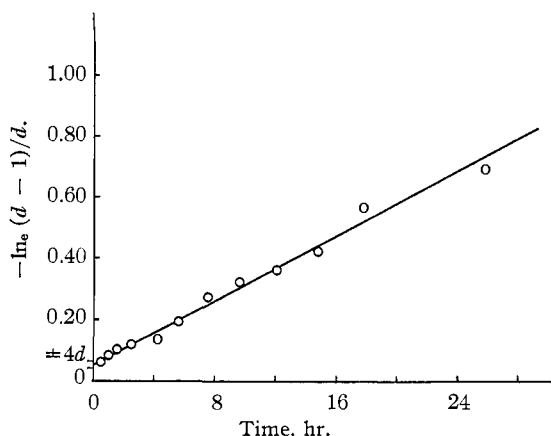

Fig. 3.—Potato starch in hydrochloric acid at 0°.

The value which we have obtained for our sample of potato starch lies within the range of the value of 23–24 glucose units (5% tetramethylglucopyranose in final hydrolysis mixture) found by Hirst, Plant and Wilkinson[6] for both the amylose and amylopectin fractions of potato starch. Hirst and co-workers employed the tetramethylglucopyranose end-group assay method developed by Haworth and Machemer.[7] Application of this end-group assay method to other samples of starch from several plant sources have all yielded values within the range of 25–30 glucose units.[8] Hassid and Dore employed an extraction technique for the tetramethylglucopyranose isolation instead of the distillation technique of Haworth and Machemer.

Neumann and Hess[9] have developed a method

(6) E. L. Hirst, M. M. T. Plant and M. D. Wilkinson, J. Chem. Soc., 2375 (1932).

(7) W. N. Haworth and H. Machemer, ibid., 2270 (1932).

(8) (a) W. N. Haworth, E. L. Hirst and M. D. Woolgar, ibid., 177 (1935); (b) K. D. Baird, W. N. Haworth and E. L. Hirst, ibid., 1201 (1935); (c) W. Z. Hassid and W. H. Dore, THIS JOURNAL, 59, 1503 (1937).

(9) F. Neumann and K. Hess, Ber., 70B, 721 (1937).

for tetramethylglucopyranose end-group assay which is different from that of Haworth and Machemer[7] and for which a high degree of accuracy is cited. Application of this procedure to potato starch by Hess and Lung[10] yielded a value of 52.0–52.4 glucose units. It is rather surprising that a refinement in the end-group assay method should yield a higher rather than a lower value. The results of Hess and Lung are not directly comparable with other values on potato starch as these workers isolated the starch from the plant source by their own method. The end-group assay of Neumann and Hess[9] has been criticized by Hirst and Young[11] and by Averill and Peat.[12]

Evidence that the starch particle in dilute solution is a molecular aggregate rather than a single molecule has been presented frequently.[13] The ultracentrifuge results so far obtained[14] show a wide distribution of particle size ranging from an apparent molecular weight of 60,000 to 1,000,000. The Staudinger viscosity methods yield a value of more than 1000 glucose units for starch.[15]

This discrepancy between values for molecular size by the viscosity and chemical methods is explained by Staudinger by postulating a branched chain structure for the starch molecule. One of the arguments for such a structure is the isolation of dimethyl glucose from the hydrolysis products of methylated starch by Irvine.[16]

Hess[10] claims that the amount of dimethylglucose isolated corresponds to the degree to which the methylation of the original starch is incomplete.

It is interesting to point out that a branched structure would not affect the value for molecular size obtained in the present work. In order for this value from the mercaptalation technique to represent the true molecular size, it is only necessary that a reducing group be produced whenever a bond is broken by hydrolysis.

Experimental

Characterization of Starch.—The sample of starch used in this work was a high grade commercial (J. L. Hopkins Co., New York) potato starch. It showed no detectable

(10) K. Hess and K. H. Lung, *Ber.*, **71B**, 815 (1938).

(11) E. L. Hirst and G. T. Young, *J. Chem. Soc.*, 1247 (1938).

(12) F. J. Averill and S. Peat, *ibid.*, 1244 (1938).

(13) M. Samec, B. Lavrenčič and S. Premrl, *Kolloid-Beihefte*, **37**, 91 (1933); refs. 8a and 8b.

(14) O. Lamm, *Kolloid-Z.*, **69**, 44 (1934); T. Svedberg, *Ber.*, **67A**, 117 (1934).

(15) H. Staudinger and E. Husemann, *ibid.*, **70B**, 1451 (1937); *Ann.*, **527**, 195 (1937); *Ber.*, **71B**, 1057 (1938); E. Husemann, *Papier-Fabr.*, **36**, Tech. Wiss. Th., 559 (1938).

(16) J. C. Irvine, *Nature*, **129**, 470 (1932).

Fehling's reduction. According to Schoch and Jensen,[5] Fehling's solution procedures are not reliable when applied to starch and they have devised a measure which they term "alkali number." This value is determined by heating the starch under controlled conditions with 0.1 N sodium hydroxide for one hour at the temperature of boiling water. The amount of alkali consumed is then determined and the "alkali number" is expressed as the number of cc. of 0.1 N alkali consumed by one gram of starch during one hour of digestion. Dr. Schoch has kindly determined the alkali number of our sample of potato starch in comparison with those of several other starches and reports as follows:

	Alkali number
Your sample	5.7
Imported potato starch	6.6
Domestic potato starch	6.9
Corn starch	9.8

Dr. Schoch states that "these results indicate that your material is of exceptionally high quality, and that manufacturing processes have produced little or no hydrolytic degradation."

Hydrolysis and Mercaptalation.—The starch (71 g., moisture content 12.22%) was placed in a 2-liter, 3-necked flask at 0°, equipped for rapid mechanical stirring and for rapid removal of samples through a delivery tube, and was treated with water (100 cc.) previously undercooled to 0°. The starch was suspended by rapid stirring and then fuming hydrochloric acid (1150 cc., d^0_4 1.212), previously cooled to 0°, was added. As soon as solution (d^0_4 1.214, 4.84 g. starch (moisture free) per 100 cc. soln. at 0°) was complete, polarimeter and density samples were withdrawn and maintained at 0°. Well-purified ethyl mercaptan (200 g.), previously cooled to 0°, was then added and the stirring continued at 0°. Samples of 50 cc. were withdrawn after the time intervals recorded in Table I, and immediately poured, with stirring, into mixtures of sodium bicarbonate (54 g.) and water (185 cc.). The neutralization mixtures were then concentrated to dryness under reduced pressure at 37–40°, and the resulting mixtures of salt and hydrolyzed starch mercaptals were dried in the vacuum oven at 40°.

Acetylation of Mercaptalated Products.—The mixtures of salt and mercaptalated products were individually acetylated for four days with acetic anhydride (20 cc.) and pyridine (40 cc.). The resulting pyridine solutions were poured into ice and water (1000 cc.) and the insoluble acetates extracted with chloroform. The chloroform extracts were washed successively with hydrochloric acid (5%), saturated sodium bicarbonate solution, and water. Concentration of the dried extracts under reduced pressure at 37–40° produced the acetates, usually as amorphous solids, but as sirups in a few instances. Several of these products were analyzed for acetyl content by the Kunz procedure. The results are tabulated in Table I.

Optical Rotation of Potato Starch in Hydrochloric Acid at 0°.—The polarimeter sample from the hydrolysis mixture was maintained at 0° and its optical rotation observed at various time intervals as the hydrolysis progressed. The concentration of starch in the solution was 4.84 g. per 100 cc. and the concentration of hydrogen chloride, 37.5 g. per 100 cc. The hydrolysis solution had a density of

1.214 at 0°. The polarimetric data are recorded in Table I and are plotted in Fig. 1.

TABLE I

DEGREE OF POLYMERIZATION OF POTATO STARCH (c, 4.84) AFTER HYDROLYSIS WITH HYDROCHLORIC ACID (37.5 G. HCl PER 100 CC. SOLN.) AT 0° FOR VARIOUS TIME INTERVALS

Time of hydrolysis, hrs.[a]	$[\alpha]^0_D$	Acetylated mercaptalated product from 2.42 g. starch, wt., g.	%S	D. P.[c] by S content	Acetyl content (cc. 0.1 N NaOH) Calcd.	Found
0	+203°[b]	...	..	..	...	...
0.55	200	2.5	1.29	16.5	...	...
1.05	198	3.7	1.72	12.2	...	...
1.55	197	4.0	2.03	10.2	10.37	10.43
2.55	192	4.0	2.31	8.9	10.35	10.51
4.25	186	5.0	2.59	7.9	...	...
5.55	179	4.5	3.46	5.7	10.32	10.39
7.55	172	4.0	4.51	4.2	10.29	10.06
9.55	167	4.2	5.23	3.5	...	...
12.05	161	4.7	5.56	3.3	...	...
14.80	151	3.9	6.31	2.8	10.24	10.01
17.80	145	3.7	7.59	2.2	...	...
25.80	126	4.2	8.35	2.0	10.20	10.13

[a] Initial time taken as time of addition of the acid to the starch. [b] By extrapolation. [c] Average degree of polymerization in glucose units, assuming complete acetylation.

Sulfur Analyses of Acetylated Mercaptalated Products.

—The sulfur analyses were performed by the Parr bomb method, employing total samples of approximately 1 g., on a moisture-free basis, in the manner described previously.[2] In Table I are recorded the sulfur analytical data and the corresponding average degrees of polymerization calculated from them. The degree of polymerization (D. P.) may be calculated from the sulfur content by the equation

$$D. P. = 2 + \left[\frac{6412}{\%S + C_{12}H_{16}O_8}\right] - 2\left[\frac{C_{14}H_{19}O_9 + C_2H_5S}{C_{12}H_{16}O_8}\right]$$

where $C_{14}H_{19}O_9$ is the molecular weight of the end structural units and $C_{12}H_{16}O_8$ is the molecular weight of the intervening units.

Summary

1. Potato starch has been hydrolyzed with a concentrated solution of hydrochloric acid at 0° in the presence of an excess of ethyl mercaptan. The resulting mercaptalated mixtures of hydrolyzed products were isolated as their acetates at various time intervals, during the first twenty-six hours of the hydrolysis.

2. Sulfur analytical data indicated that the average degree of polymerization of the mercaptalated products varied from 17 glucose units after one-half hour to 2 glucose units after twenty-six hours.

3. The course of the hydrolytic reaction (without mercaptalation) at 0° was followed by optical rotation measurements.

4. A graphic analysis of the data yields a value of 2.66×10^{-2} for the specific rate constant (hours^{-1}) of the rate of change of the degree of polymerization in concentrated hydrochloric acid at 0°.

5. By graphic analysis the value 20 ± 4 glucose units is obtained for the initial average degree of polymerization of the potato starch.

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The Action of Isobutylmagnesium Bromide on 3,4,5-Trimethoxybenzonitrile

BY H. L. HALLER AND P. S. SCHAFFER[1]

A recent communication by Suter and Weston[2] describes the preparation of some 5-alkylresorcinols. As work along similar lines has been under way for some time in this Laboratory, occasion is taken to present some of the results that are closely related to the studies of Suter and Weston. This work had for its object the preparation of 5-isoamylresorcinol[3] in order that its properties

might be compared with those of 4-isoamylresorcinol[4] and 2-isoamylresorcinol.[5]

4-Isoamylresorcinol was first prepared by Dohme[4] in connection with his studies on the bactericidal action of 4-alkylresorcinols, and 2-isoamylresorcinol was first synthesized by Haller[5] and shown to be identical with tetrahydrobutanol,[6] a degradation product of rotenone.[7]

The reaction of 3,5-dimethoxybenzonitrile with

(1) Present address: Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

(2) Suter and Weston, THIS JOURNAL, 61, 232 (1939).

(3) Because the junior author has been assigned to new duties, the work was interrupted before its completion.

(4) Dohme, Cox and Miller, THIS JOURNAL, 48, 1688 (1926).

(5) Haller, ibid., 55, 3032 (1933).

(6) Haller and LaForge, ibid., 53, 4460 (1931); 54, 1988 (1932).

(7) LaForge, Haller and Smith, Chem. Rev., 12, 181 (1933).