[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Molecular Size of Polysaccharides by the Mercaptalation Method; Methylated Potato Starch

BY M. L. WOLFROM AND D. R. MYERS

A new method for the determination of the degree of polymerization of polysaccharides was established in this Laboratory on methylated cellulose (from acetone-soluble cellulose acetate)[1] and when applied to a sample of high-grade, commercial potato starch, yielded the value of 20 ± 4 glucose units,[2] in agreement with the value of 24–28 glucose units found by the application of the Haworth tetramethylglucopyranose end-group assay[3] to numerous samples of methylated starch.[4]

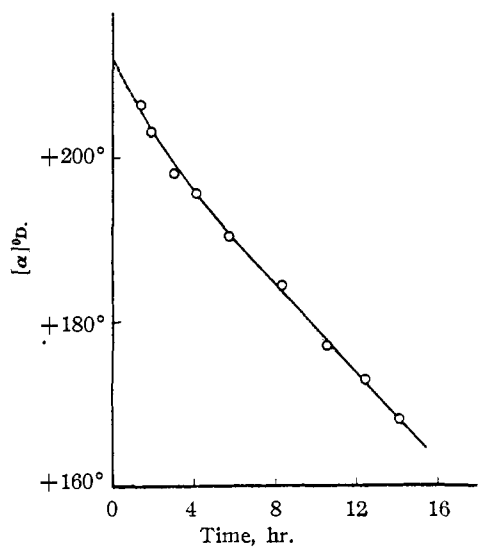


Fig. 1.—Mutarotation of methylated starch (c, 4.80 g. per 100 cc. soln.) in hydrochloric acid (d. 1.19) at 0°.

Since the history of the sample of commercial potato starch used in our work was unknown, it was considered advisable to repeat the work on a sample of starch wherein the isolation from the natural source was controlled. A sample of methylated potato starch was prepared according to the procedure of Hess and Lung.[5] This procedure involves the isolation of the starch from the potato by an extraction with cold, boiled distilled water and methylation of the starch with methyl sulfate and alkali in the absence of oxygen. The starch is thus never subjected to the action of acidity and when in alkaline medium is protected against air oxidation. The methylated starch obtained possesses the solubilities requisite for purification from organic solvents. Our preparation was comparable to that of Hess and Lung in respect to its methoxyl content (42.3%) and viscosity, which was very high.

The mercaptalation assay was then applied to this sample of methylated starch and an estimate of its molecular size was made by a mathematical extrapolation to zero time of the data obtained by subjecting the polysaccharide to hydrolysis at 0° with concentrated hydrochloric acid under conditions of continuous mercaptalation and determining with precision the amount of combined sulfur at stated time intervals.

The course of hydrolysis was followed polarimetrically at 0° by means of a separate sample of the hydrolyzate, withdrawn for this purpose before the addition of the ethyl mercaptan, and the data obtained are diagrammed in Fig. 1. It is seen that a continuous smooth curve was obtained. Extrapolation of the data to zero time (time of addition of the acid to the methylated starch) indicated a specific rotation of +213° for the original methylated starch.

The sulfur analytical data and the degrees of polymerization calculated from them are recorded in Table I. Figure 2 represents a straight line plot of the function $-\log_e [(d - 1)/d]$ against time, wherein d (total number of glucose units ÷ number of polymers) is the average degree of polymerization at time t. The intercept gives the initial degree of polymerization and the slope is $-k$, where k is the specific rate constant for the rate of change in the degree of polymerization with time.

From Fig. 2 the value of the specific rate constant k (hours^{-1}) at 0° was found to be 2.22×10^{-2}, which is very close to the value of 2.66×10^{-2} found[2] at very nearly the same acid concentration for the potato starch with the initial D. P.

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

(2) M. L. Wolfrom, D. R. Myers and E. N. Lassettre, *ibid.*, **61**, 2172 (1939).

(3) W. N. Haworth and H. Machemer, *J. Chem. Soc.*, 2270 (1932).

(4) E. L. Hirst, (Miss) M. M. T. Plant and (Miss) M. D. Wilkinson, *ibid.*, 2375 (1932); W. N. Haworth, E. L. Hirst and (Mrs.) M. D. Woolgar, *ibid.*, 177 (1935); D. K. Baird, W. N. Haworth and E. L. Hirst, *ibid.*, 1201 (1935); E. L. Hirst and G. T. Young, *ibid.*, 951, 1471 (1939); W. Z. Hassid and W. H. Dore, THIS JOURNAL, **59**, 1503 (1937); K. Freudenberg and H. Boppel, *Ber.*, **71**, 2505 (1938).

(5) K. Hess and K. H. Lung, *ibid.*, **71**, 815 (1938).

(degree of polymerization in glucose units) of 20 ± 4, and is considerably different from the value 1.02×10^{-3} found[1] for methylated cellulose at an even greater acid concentration. From this it can be inferred that both the methylated and the unmethylated starch have the same type of hydrolyzable bond which is in turn hydrolyzed at a greater rate than the cellulose bond.

The intercept of Fig. 2 gives a value of approximately 150 for the initial D. P. The accuracy of the data is such that an exact extrapolation is difficult. The value is very certainly not as low as 25. It is at least 150 and may be higher. It would therefore appear that the results of this work and of the previous assay[2] of commercial potato starch made in this Laboratory by the mercaptalation method, would support the conclusion that a unit of approximately 25 glucose units is a component of the starch molecule but does not represent the entire molecule of native starch, a conclusion reached by Hirst and Young[4] and by Freudenberg and Boppel[4] by other methods. The values obtained by us for the specific rate constants would indicate that there is no great difference in the ease of hydrolysis of the bonds constituting the starch molecule, as might be possible if units of *ca.* 25 D. P. were joined together. An alternative interpretation is that our assay of 20 ± 4 glucose units on the sample of commercial potato starch was merely in fortuitous agreement with the values in the same range obtained by the tetramethylglucopyranose end-group assay. In view of the numerous determinations by the latter method which have yielded this value, this viewpoint does not appear reasonable and the entity of *ca.* 25 glucose units would appear to have a real significance. Our work on methylated starch does show, however, that the degree of polymerization of a polysaccharide may be profoundly altered by its method of isolation.

It is to be noted that the mercaptalation assay is independent of branching and merely requires that the bonds hydrolyze into two entities with no significant differences in rate. It is thus a dynamic end-group assay and is not limited to an end-group initially present, as is the case with the tetramethylglucopyranose end-group assay which, although based upon a hydrolytic product, gives true molecular size only in the case of a straight-chain molecule. Our method also assays the

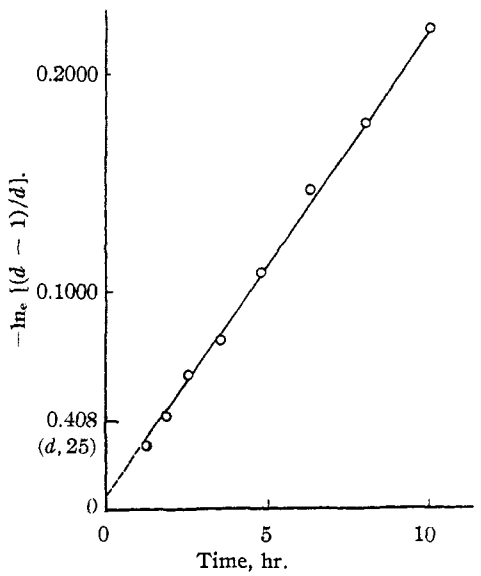


Fig. 2.—Methylated starch in hydrochloric acid at 0°.

polysaccharide while it is being broken down in solution and thus avoids the difficulties inherent in other methods that search for an end-group in the polysaccharide in its original colloidal state.

The relative viscosity of our sample of methylated starch as determined in chloroform at 20° was found to be 6.16, as compared with the highest value of 7.71 found by Hess and Lung[5] for their preparations. Using the value[6] of 0.5×10^{-4} for the K_m of methylated starch in chloroform in the Staudinger viscosity equation, $MK_m = (\eta_\gamma - 1)/c$ (M is molecular weight; η_γ is relative viscosity; c is concentration in $C_9H_{16}O_5$ gram units per liter), this viscosity corresponds to a degree of polymerization of 7000 glucose units. This wide divergence in the degrees of polymerization given by the viscosity method (7000) and the mercaptalation method (150) is certainly outside of our error of extrapolation and can only be explained by the hypothesis that the same entity is not represented by the two values. This is in contrast with the situation found with methylated cellulose (from acetone-soluble cellulose acetate), wherein the mercaptalation assay (400 ± 70)[1] was in agreement with the viscosity D. P. (350 ± 35 determined on the acetate) as evaluated from the Kraemer[7] modification of the Staudinger viscosity formula. Our value also is much lower than that obtained recently

(6) H. Staudinger and E. Husemann, *Ann.*, **527**, 218 (1937).

(7) E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 164 (1935); E. O. Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938).

by Beckmann and Worstall[8] for methylated starch by ultracentrifugal analysis.

Experimental

Preparation and Characterization of the Methylated Starch.—The methylated starch sample was prepared from a lot of California new potatoes according to the procedure of Hess and Lung[5] except that the methylation was performed under oxygen-free nitrogen instead of hydrogen. As far as possible, all air was displaced with oxygen-free nitrogen. The product was purified, as described by Hess and Lung, from methyl acetate by the addition of low-boiling petroleum ether.

Anal. Moisture, 3.60; ash, 0.29; OCH_3, 42.3; P_2O_5, 0.07.

An amount of 0.2514 g. (moisture free basis; moisture determined on separate sample) of the methylated starch in 25.02 g. of U. S. P. chloroform solution required 376.4 sec. to flow through an Ostwald type viscometer at 20° and the solvent required 61.1 sec. to flow through the viscometer at the same temperature; relative viscosity, 6.16 (*c* 1% by wt.); absolute viscosity, 3.7 centipoises; D. P. by Staudinger formula,[6] 7000.

The values cited by Hess and Lung[5] for their preparation of highest viscosity were: methoxyl, 41.6; relative viscosity, 7.71 ($CHCl_3$, *c* 1% by wt.).

Mercaptalation Assay of the Methylated Starch.—The methylated starch (31.2 g.) 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 concentrated hydrochloric acid (d^0_4 1.198, 600 cc.), previously cooled to 0°, was added. As soon as solution (d^0_4 1.199, 4.80 g. methylated starch, ash and moisture free basis, per 100 cc. soln.) was complete, polarimetric and density samples were withdrawn and maintained at 0°. Well purified ethyl mercaptan (100 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 a suspension of 51 g. of sodium bicarbonate in 150 cc. of water.

The mercaptalated, hydrolyzed products were separated from the neutralized aqueous solutions by three extractions with equal volumes of chloroform. The chloroform solutions were washed three times with equal volumes of distilled water, dried over calcium chloride and concentrated under reduced pressure at 37–40°. All of the products thus obtained were amorphous solids except the product with the lowest degree of polymerization (Table I) which was a highly viscous sirup. The polarimeter sample from the hydrolysis mixture, withdrawn before the addition of the ethyl mercaptan, was maintained at 0° and its optical rotation observed at various time intervals as the hydrolysis progressed. The polarimetric data are recorded in Table I and are plotted in Fig. 1.

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 previously described. In one case the value was checked by a determination according to the Carius procedure and excellent agreement was obtained.

(8) C. O. Beckmann and C. M. Worstall, paper presented before the Division of Agricultural and Food Chemistry at the 100th Meeting of the American Chemical Society, Detroit, Michigan, September 13, 1940.

TABLE I

DEGREE OF POLYMERIZATION OF METHYLATED POTATO STARCH (*c*, 4.80) AFTER HYDROLYSIS WITH CONCENTRATED HYDROCHLORIC ACID (d. 1.19) AT 0° FOR VARIOUS TIME INTERVALS

Time of hydrolysis, hours[a]	$[\alpha]^0D$	Mercaptalated product from 2.42 g. methylated starch, g.[b]	S, %	D. P. by S content[c]
0	+212.5°[d]	...	..	150[d]
1.30	206.0	2.1	0.90	34.3
1.80	204.0	2.4	1.29	23.8
2.55	201.3	2.1	1.81	16.8
3.55	197.5	2.0	2.26	13.3
4.80	193.5	2.1	3.04	9.7
6.30	189.0	2.0	3.99	7.3
8.05	184.3	2.2	4.66	6.1
10.05	178.8	2.1	5.54	5.1
12.30	172.8	2.1	6.34	4.4[f]
20.55	...	2.3	8.75	3.0[f]
Final	113.6[e]	...	..	...

[a] Initial time taken as time of addition of the acid to the methylated starch. [b] Calculated on the moisture-free basis. [c] Average degree of polymerization in glucose units. [d] By extrapolation. [e] Not included in Fig. 1. [f] Not included in Fig. 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.) was calculated from the sulfur content by the equation

$$\text{D. P.} = 2 + \left(\frac{6412}{\%\text{S} \times \text{C}_9\text{H}_{16}\text{O}_5}\right) - 2\left(\frac{\text{C}_9\text{H}_{17}\text{O}_5 + \text{SC}_2\text{H}_5}{\text{C}_9\text{H}_{16}\text{O}_5}\right) = \frac{31.42}{\%\text{S}} - 0.61$$

where $C_9H_{17}O_5$ represents the molecular weight of the end structural units and $C_9H_{16}O_5$ represents the molecular weight of the intermediate units.

Summary

1. A sample of methylated starch prepared from potatoes according to the procedure of Hess and Lung[5] has been hydrolyzed with concentrated hydrochloric acid at 0° in the presence of an excess of ethyl mercaptan. The resulting mixtures of mercaptalated, hydrolyzed products were isolated at various time intervals during the hydrolysis.

2. Sulfur analytical data indicated that the average degree of polymerization of the mercaptalated products varied from 34 glucose units after 1.3 hours to 3 after 20.55 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 yielded a value of 2.22×10^{-2} (hours^{-1}) for the specific rate constant of the rate of change of the degree of polymerization in concentrated hydrochloric acid at 0°.

5. By graphic analysis the minimum value of *ca.* 150 was obtained for the initial average degree of polymerization of the methylated starch.

6. The degree of polymerization of a starch product is highly dependent on the method of preparation of the product.

COLUMBUS, OHIO RECEIVED MARCH 12, 1941

[CONTRIBUTION FROM THE GEORGE HERBERT JONES LABORATORY OF THE UNIVERSITY OF CHICAGO]

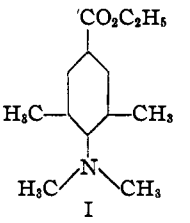
The Effect of Resonance on Reaction Velocity[1]

BY F. H. WESTHEIMER AND RICHARD P. METCALF

In previous publications,[2] one of us has computed that part of the effect of substituents on the velocity of saponification which can reasonably be considered electrostatic in origin. Many cases are explicable on an electrostatic basis; the extraordinarily slow rates of saponification of ethyl *p*-aminobenzoate[3] and ethyl *p*-dimethylaminobenzoate, however, cannot be ascribed exclusively to the effect of the dipole moment of the amino group. The assumption was therefore made that resonance interaction of the amino and carbethoxy groups stabilized the ester, and thereby caused a decrease in the rate of saponification. This assumption is consistent with the fact that ethyl *m*-aminobenzoate, where such resonance interaction is impossible, saponifies twenty times faster than the para isomer.

It was realized that this hypothesis could be subjected to a severe test. Birtles and Hampson[4] have shown that the introduction of alkyl substituents ortho to the dimethylamino group prevents that group from lying in the plane of the benzene ring, and thereby "damps" its resonance. It is a necessary condition imposed by our theory that elimination of the resonance involving the amino group should result in a rate of saponification approaching that of the unsubstituted ester. On the other hand, in those cases in which the effect of the substituent on the rate of saponification can be explained on an electrostatic basis alone, "damping" the resonance should have only a minor effect on the rate.

The theory was examined experimentally by measuring the rates of saponification of the following esters: ethyl 3,5-dimethylbenzoate, ethyl 3,5-dimethyl-4-nitrobenzoate, ethyl 3,5-dimethyl-4-aminobenzoate and ethyl 3,5-dimethyl-4-dimethylaminobenzoate. (I). To supplement



I

Kindler's data the rate of saponification of ethyl *p*-dimethylaminobenzoate was also obtained.

All the compounds needed for the investigation with the exception of ethyl 3,5-dimethyl-4-dimethylaminobenzoate were known. The acid corresponding to this ester was synthesized by two methods. First 2,6-dimethylaniline was brominated, and the bromoamine methylated with dimethyl sulfate. The 3,5-dimethyl-4-dimethylaminobromobenzene was converted into the corresponding lithium compound, and carbonated to the acid. The second method of synthesis consisted of methylating the methyl ester of 3,5-dimethyl-4-aminobenzoic acid. The resultant crude ester was hydrolyzed to the desired acid.

Experimental

Materials.—3,5-Dimethylbenzoic acid was made both by the oxidation of mesitylene[5] and, probably for the first time, by a Grignard reaction from 3,5-dimethylbromobenzene. The latter reaction gave more than an 80% yield of recrystallized acid melting at 168°. The ester[6] boiled at 74–75° at 2 mm. Ester obtained from both samples of acid saponified at the same rate.

(1) Presented on April 9, 1941, at the St. Louis meeting of the American Chemical Society.

(2) Westheimer and Shookhoff, THIS JOURNAL, **62**, 269 (1940); Westheimer, *ibid.*, **62**, 1892 (1940).

(3) Kindler, *Ann.*, **450**, 1 (1926).

(4) Birtles and Hampson, *J. Chem. Soc.*, 10 (1937); Ingham and Hampson, *ibid.*, 981 (1939).

(5) Fittig, *Ann.*, **141**, 129 (1867).

(6) Fittig and Brueckner, *ibid.*, **147**, 42 (1868).