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

A Study of Cellulose Hydrolysis by Means of Ethyl Mercaptan[1]

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It is difficult to obtain exact information concerning the structure of the cellulose molecule. The classical methods of organic structural determination involve the study of the breakdown of the organic molecule by degradative methods. The cellulose molecule may be degraded by acid hydrolysis and any new information obtained by a study of this hydrolytic process should be of service eventually in interpreting the true structure of the cellulose molecule. The recent studies of Freudenberg and of Freudenberg and Kuhn[3] on the hydrolytic breakdown of cellulose by sulfuric acid and by acetolysis constitute some of the more important recent contributions to the problem of the constitution of cellulose.

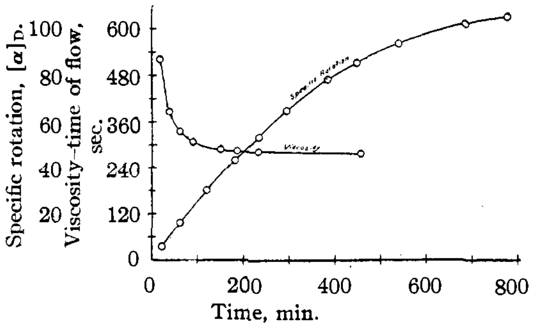

Fig. 1.—Specific rotation and viscosity of 3% solution of cellulose in fuming HCl (d_4^{15} 1.203) at 23°.

In 1913, Willstätter and Zechmeister[4] prepared solutions of cellulose in fuming hydrochloric acid and followed the hydrolysis at 16° by polarimetric and copper reduction methods. With a cellulose preparation that was apparently only solvent-extracted, they obtained an initial induction period and an inflection point at a specific rotation of +14°. In 1923, Sherrard and Froehlke[5] in identifying some purified wood celluloses, demonstrated their similarity to cotton cellulose by applying the polarimetric method of Willstätter and Zechmeister. They also made a run on cotton cellulose as a control. They found the first break in the curve of all celluloses examined at a specific rotation of +43° instead of +14° and found in addition a second break at a specific rotation of +73°. Their temperature of hydrolysis was 20°. It is probable that variations in the type of this polarimetric curve can be ascribed to different methods of cellulose purification.

We have followed polarimetrically the hydrolysis curve at 23° of a sample of chemically purified, high viscosity cotton linters, employing solution concentrations and tube lengths to ensure adequate observed rotations. In agreement with previous work, we find that the initial rotation is zero. With the β-glucose chain conception of the cellulose structure, it is rather difficult to understand why this initial rotation should be zero rather than levo. We did not find any initial induction period, nor did we find any breaks in the curve (Fig. 1). Many intermediate readings other than those shown were obtained. Data were obtained for two concentrations of cellulose and are tabulated in Table I. An analysis of the data (Table I) shows that the calculated monomolecular velocity constants show a slight but definite upward drift. The average value of the velocity constant for the greater part of the reaction is practically independent of the initial cellulose concentration. The polarimetric data so obtained verify the results of Hibbert and Percival,[6] who followed polarimetrically the hydrolysis of cellulose (alkali-purified) in a hydrochloric acid–zinc chloride medium. These workers likewise obtained no breaks in the polarimetric curve and found that the calculated monomolecular velocity constant exhibited a definite upward drift. Their average velocity constant value is in excellent agreement with the one determined in this Laboratory, employing a fuming hydrochloric acid medium at the same temperature.

In agreement with previous workers, we find that the final rotation ($[\alpha]_D$ + 100°) is approximately that of an equivalent amount of *d*-glucose. The equilibrium rotation of *d*-glucose is profoundly affected by the hydrogen chloride concen-

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(2) Du Pont Cellulose Research Fellow.

(3) K. Freudenberg, "Tannin-Cellulose-Lignin," Verlag J. Springer, Berlin, 1933.

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

(5) E. C. Sherrard and A. W. Froehlke, THIS JOURNAL, **45**, 1729 (1923).

(6) H. Hibbert and E. G. V. Percival, *ibid.*, **52**, 3995 (1930).

tration. This variation is plotted in Fig. 2 and is in fair agreement with previous data. In agreement with Willstätter and Zechmeister,[4] we find that this rotation change is reversible under the conditions of time and temperature employed and accordingly it must represent a true tautomeric shift.

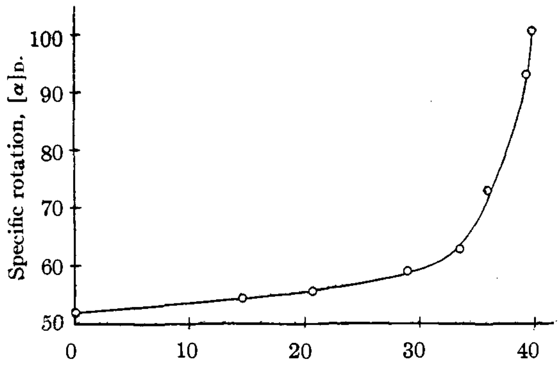

Fig. 2.—Optical rotation of glucose in HCl at 25°.

TABLE I

MUTAROTATION OF CELLULOSE IN FUMING HCl; VELOCITY CONSTANT, TEMPERATURE 23°

c, 1.004; l, 4-dm.				c, 2.857; l, 4-dm.			
t^a (min.)	α_D deg.	$[\alpha]_D$ deg.	k^b	t^a (min.)	α_D deg.	$[\alpha]_D$ deg.	k^b
19	+0.16	+4.0	0.0021	26	+0.65	+5.7	0.0022
24	.19	4.7	.0020	36	0.98	8.6	.0024
44	.41	10.2	.0024	51	1.36	11.9	.0024
79	.76	18.9	.0026	81	2.22	19.4	.0025
119	1.20	29.9	.0030	101	2.80	24.5	.0026
149	1.49	37.1	.0031	126	3.48	30.4	.0027
179	1.80	44.8	.0033	156	4.26	37.3	.0028
199	1.97	49.1	.0034	186	5.02	43.9	.0029
220	2.08	51.8	.0033	216	5.60	49.0	.0029
259	2.42	60.3	.0036	247	6.40	56.0	.0031
286	2.60	64.7	.0036	297	7.38	64.6	.0032
334	2.88	71.7	.0038	339	8.15	71.3	.0034
391	3.17	78.9	.0040	454	9.77	85.5	.0037
476	3.49	86.9	.0043	544	10.66	93.3	.0041
626	3.85	95.9	.0051	694	11.54	100.9	.0048
∞		100.1		∞		104.6	

[a] The initial time was determined here by the backward extrapolation of the curve to zero rotation. [b] $k = \frac{1}{t} \ln_e \frac{\alpha_0 - \alpha_\infty}{\alpha_t - \alpha_\infty}$ (t in minutes; $\alpha_0 = 0$).

We were also interested in following the viscosity changes in the cellulose solutions. We found that at 25° the first measurable viscosity value was quite low and that the solutions attained the viscosity of an apparent equivalent amount of *d*-glucose when the specific rotation was approximately +25°. These data are shown in Figs. 1 and 3.

The main object of our investigation was to insert a carbonyl reagent into the hydrolytic mixture in order to follow the breakdown of the cellulose molecule by means of the intermediate reaction products. Ethyl mercaptan is a suitable reagent for this purpose. This substance will react with the free reducing groups of sugars in concentrated hydrochloric acid to form the sugar mercaptals or thioacetals.[7] At the present stage of our investigations, the mercaptalated cellulose hydrolytic products, other than glucose ethyl mercaptal, were isolated as amorphous mixtures and no attempt was made to separate them into their pure components. They were characterized by means of their sulfur content.

The reaction between a reducing sugar and ethyl mercaptan takes place with extreme rapidity and at low temperatures. Under the Willstätter–Zechmeister hydrolytic conditions, the effective sugar concentration is relatively low and we found it necessary to study the mercaptalation of *d*-glucose under such conditions. A 5% solution of *d*-glucose in fuming hydrochloric acid can be mercaptalated by vigorous mechanical shaking with an excess of ethyl mercaptan for fifteen minutes at 0°. At the end of this period the solution has lost its Fehling reduction capacity and the glucose diethyl mercaptal may be isolated in approximately eighty per cent yield.

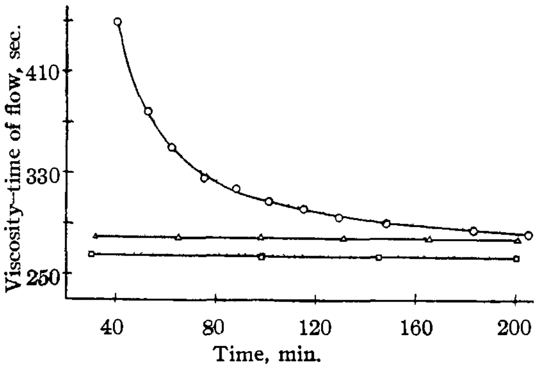

Fig. 3.—Comparison of viscosity at 25°: ○, 3% solution of cellulose in fuming HCl; △, 3% solution of glucose in fuming HCl; □, fuming HCl.

The glucose mercaptal that separates, after sodium bicarbonate neutralization, is contaminated with appreciable amounts of sodium chloride. For quantitative purposes, it was found desirable to leave this salt in the product and correct for its presence by an ash determination. We are probably dealing here with an addition compound

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[7] E. Fischer, *Ber.*, **27**, 673 (1894).

similar in nature to the well-known *d*-glucose–sodium chloride compound. The mother liquor could then be investigated by concentration to dryness and acetylation, the acetylated product being purified by pouring into a large volume of water. Difficulties were here encountered in acetylating quantitatively, a small amount of material adhering to a relatively large amount of crystalline salt that was insoluble in the acetylating mixture.

The optimum mercaptalation procedure thus established for *d*-glucose was then applied to the cellulose hydrolytic solutions and the course of hydrolysis of cellulose (5%) in fuming hydrochloric acid solutions at 16° was studied by means of the mercaptalated products obtained from the reaction of ethyl mercaptan with the cellulose solutions at various stages of hydrolysis. Simultaneous polarimetric data were obtained. Sulfur analyses served to determine the average molecular size of the mixtures of mercaptalated products isolated from the cellulose hydrolysis. The pertinent data are tabulated in Table II. An analysis of these data is given in Table III and shows that the cellulose molecule fragments with extreme rapidity under these conditions. Glucose ethyl mercaptal first appears when the hydrolysis is approximately two-thirds completed. Its amount gradually increases. When the hydrolysis was approximately at the half-period, the

TABLE II

MERCAPTALATED PRODUCTS ISOLATED FROM CELLULOSE SOLUTIONS (c, 4.669) IN FUMING HYDROCHLORIC ACID (d^{16}_4 1.203) AT 16°

			Water-insoluble mercaptalated products (from 2.80 g. cellulose)			Acetylated water-soluble products		
Expt.	Time of hydrolysis	$[\alpha]^{16}_D$	% NaCl	Wt. (NaCl-free basis), g.	% S (NaCl-free basis)	Expt.	Wt.	S, %
a	17 min.	..	19.4	2.7	0.33	*a'*	Trace	
b	3 hrs.	+17.5°	0.5	1.5	2.03	*b'*	1.1	4.6
c	6	36.7	0.6	0.7	2.39	*c'*	1.8	6.7
d	9	46.6				*d'*	3.5	7.4
e	12	61.0				*e'*	1.6	6.0
f	15	70.1	0	0.4		*f'*	2.9	6.2
g	18	79.8	5.8	1.1	21.3	*g'*	2.2	6.0
h	21	..	6.4	1.7	21.1	*h'*	1.4	5.7
i	24	91.5	9.5	1.8	21.9	*i'*	2.7	7.1
j	27.5	99.8	17.9	2.1	21.6	*j'*	2.3	6.8
k	34.5	103.0	18.8	2.5	21.1	*k'*	1.2	7.7
l	38.7	104.9				*l'*		
m	51.7	..	11.4	2.5	21.4	*m'*	1.3	6.2
n	55.0	..	20.9	2.4	21.7	*n'*	1.3	6.7

(*a*) The addition of 5 cc. of water preceding the mercaptan addition was omitted.

(*d* to *n*) The acid solution was poured into a suspension of 68 g. of sodium bicarbonate in 100 cc. of water.

(*d*, *e*) No product precipitated.

(*f*) After standing for one day, 0.4 g. of a crystalline product separated. This material was characterized as *d*-glucose diethyl mercaptal. On recrystallization the substance melted at 127° (unchanged on admixture with an authentic specimen of *d*-glucose diethyl mercaptal).

(*g to n*) Crystalline products were obtained which were mixtures of glucose diethyl mercaptal and sodium chloride. They were removed by filtration and were not washed. The sodium chloride content was determined by conversion to sodium sulfate in a platinum crucible with concentrated sulfuric acid. Calcd. for *d*-glucose ethyl mercaptal: S, 22.4. The glucose ethyl mercaptal was isolated and identified as its pentaacetate for one typical run. A portion (1.5 g.) of the crude water-insoluble product from (*k*) was acetylated overnight with acetic anhydride (10 cc.) and pyridine (5 cc.) and poured into ice and water (400 cc.); yield, 1.6 g. On recrystallization from methanol–water the substance was identified as *d*-glucose diethyl mercaptal pentaacetate; m. p. 45–47° (mixed m. p. unchanged); $[\alpha]^{24}_D$ +13.3° (c, 3.6; $CHCl_3$).

(*j* to *n*) A second solution of cellulose (30 g.) was prepared as previously described and used after it had been allowed to hydrolyze at 16° for twenty-seven and one-half hours.

(*a'*) A trace of amorphous material was obtained.

(*b'*) A solid, amorphous product was obtained.

(*c'*, *d'*) These products separated as gummy masses contaminated with salt. They were taken up in chloroform and the extract washed with sulfuric acid (1 molar), aqueous sodium bicarbonate and water. On solvent removal and trituration with ether the products were obtained as granular solids.

(*e'* to *h'*) The products separated as viscous sirups which hardened to a solid mass on standing.

(*i'* to *n'*) These sirupy products were treated as described for (*c'*) and (*d'*) and were obtained as glassy solids after drying in a vacuum desiccator.

isolated mixtures of products corresponded to the trisaccharide stage. The amount of this type of material gradually decreased but appreciable quantities were still present at the last point determined.

TABLE III

MOLECULAR SIZE OF ISOLATED MERCAPTAL PRODUCTS

5% Cellulose in fuming hydrochloric acid; temp., 16°. Each experiment equivalent to 2.80 g. cellulose.

Expt.	Time of hydrolysis, hours	$[\alpha]^{16}D$	Water-insoluble product Amount, g.	D. p.[a]	Water-soluble product as acetate Amount, g.	D. p.[a]
a	1/4	..	2.7	121	...	...
b	3	+17.5°	1.5	20	1.1	4
c	6	36.7	0.7	17	1.8	2.5
d	9	46.6	0	.	3.5	2.5
e	12	61.0	0	.	1.6	3
f	15	70.1	0.4	1	2.9	3
g	18	79.8	1.1	1	2.2	3
h	21	..	1.7	1	1.4	3
i	24	91.5	1.8	1	2.7	2.5
j	27.5	99.8	2.1	1	2.3	2.5
k	34.5	103.0	2.5	1	1.2	2
m	51.7	..	2.5	1	1.3	3
n	55	..	2.4	1	1.3	2.5

[a] D. p. = degree of polymerization or number of glucose units in the chain. For (a), (b) and (c), this was calculated by the following close approximation: d. p. = $\frac{100 \times 2S}{\%S \times C_6H_{10}O_5} = \frac{40}{\%S}$.

Further work is in progress in this Laboratory.

Experimental

Cellulose Characterization.—The cellulose used throughout this research was a sample of purified cotton linters[8] of high viscosity which had been subjected to the customary alkali kier and mild bleaching process. The material showed the following characterization: alpha cellulose, 99.2%; ash, 0.04%; iron, 10 p. p. m.; moisture, 4.0%. The material was analyzed for pectin by the Link[9] modification of the Lefèvre–Tollens[10] procedure and was found to be pectin-free by this method. The cuprammonium viscosity of the cellulose was determined in 0.1% concentration at 25 ± 0.02°: $n = 2.732$ centipoises; $[\eta]$ (intrinsic

$$\text{viscosity}) = \frac{\ln_e \eta_r}{C} = \frac{\ln_e \frac{409.5}{192.3}}{0.1} = 7.559.^{11}$$

Preparation and Measurement of Cellulose Solutions.—Fuming hydrochloric acid (98.74 cc.; d^{15}_4 1.203) was added to the cotton linters (1.000 g.; moisture content, 4.0%) in a glass-stoppered flask and the mixture shaken until the cellulose was completely dissolved (eight minutes) with occasional cooling in an ice-bath. The density of the solution after three hours of standing was found to be 1.2003; 25°/4°. This made a 1% solution of cellulose (moisture-free basis) in fuming hydrochloric acid. A portion of the solution was used for the polarimetric observations and another portion was used for the direct determination of viscosity in an Ostwald type viscometer. A similar procedure was used in obtaining data for a 3% cellulose concentration. The results obtained for one typical cellulose concentration are shown in Fig. 1. The data shown in Fig. 3 were obtained for comparative purposes. The calculated velocity constant data are tabulated in Table I. The optical rotations of *d*-glucose in various concentrations of hydrochloric acid are shown in Fig. 2.

Reversibility of the Optical Rotation of *d*-Glucose in Concentrated Hydrochloric Acid.—The experiment to be described was performed to determine whether the rotation of *d*-glucose in fuming hydrochloric acid was due to a permanent change in structure or to a reversible tautomeric equilibrium. *d*-Glucose (1.0623 g.) was dissolved in fuming hydrochloric acid (d^{15}_4 1.202) to make 50.00 cc. of solution; $[\alpha]_D$ +100.3°. This solution was then diluted to 99.90 cc. with water. This lowered the hydrogen chloride percentage to approximately 20.1% and the concentration of *d*-glucose from *c*, 2.125 to *c*, 1.063; $[\alpha]_D$ +57°. The specific rotation for *d*-glucose in acid of the same concentration was found to be +56°. This indicates that the equilibrium is essentially reversible and that the rotation changes are measures of true tautomeric shifts.

Mercaptalation of a 5% *d*-Glucose Solution.—*d*-Glucose (3.3 g.) was dissolved in 60 cc. of hydrochloric acid (d^{20}_4 1.184) and the solution cooled to 0°. Purified ethyl mercaptan (8 cc.; cooled to 0°) was added and the mixture vigorously shaken by mechanical means for fifteen minutes at 0°. The faint yellow solution was poured with stirring into a suspension of 60 g. of sodium bicarbonate in 100 cc. of water. The heat change was negative and the neutralization could thus be effected at low temperatures. The mixture was cooled in an ice–salt bath and the crystalline product removed by filtration, washing being omitted; yield, 4.9 g. or 4.0 g. on a salt-free basis (77%).

Anal. Ash as Na_2SO_4: 21.0. Calcd. as NaCl, 17.3. S (NaCl-free basis): 21.6. Calcd. for $C_6H_{12}O_5(SC_2H_5)_2$, 22.4.

A portion (1.5 g.) of the above salt-containing product was acetylated overnight with acetic anhydride (10 cc.) and pyridine (5 cc.) and poured into 400 cc. of ice and water. A crystalline product was obtained that was characterized as glucose diethyl mercaptal pentaacetate by its melting point of 45–47° (mixed m. p. unchanged); $[\alpha]^{24}D$ +12.8° (*c*, 3.6; $CHCl_3$); yield, 2.1 g. (calcd., 2.17 g.). Wolfrom[12] recorded for this substance the constants: m. p. 45-47°; $[\alpha]^{29}D$ +11° ($CHCl_3$).

Mercaptalation of Cellulose Hydrolytic Products.—An amount of 30 g. of cellulose was dissolved in 600 cc. of fuming hydrochloric acid (d^{15}_4 1.203) by shaking in a 1-liter glass-stoppered bottle. After seventeen minutes a clear solution (d^{15}_4 1.219) was obtained that contained 4.669 g. of cellulose per 100 cc. of solution. This solution was kept in a thermostated constant temperature bath held at 16 ± 0.1°. The water in the bath was cooled by means of an immersed coil which was connected with a

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(8) Furnished through the courtesy of the Hercules Powder Co., Hopewell, Va.

(9) A. D. Dickson, H. Otterson and K. P. Link, THIS JOURNAL, **52**, 775 (1930).

(10) K. U. Lefèvre and B. Tollens, *Ber.*, **40**, 4513 (1907).

(11) E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 157 (1935).

(12) M. L. Wolfrom, THIS JOURNAL, **51**, 2188 (1929).

refrigeration unit. Samples of the solution under investigation were removed at stated intervals for mercaptalation. Polarimetric readings at 16° were taken simultaneously and when plotted showed again a continuous curve. The mercaptalation products were isolated and analyzed. In the experiments herein reported, no attempts were made to isolate the various mercaptalated oligosaccharides in pure form. The data are tabulated in Table II. The various mercaptalations are denoted by letter in this table and will be referred to by these designations. The mercaptalation procedure employed was that described for *d*-glucose, unless otherwise specified. An amount of 60 cc. (equivalent to 2.80 g. of cellulose) of the solution was transferred to a glass-stoppered pressure bottle, 5 cc. of water and 8 cc. of purified ethyl mercaptan were added and the whole shaken mechanically for fifteen minutes at 0°. The addition of a small amount of water at this point lowered the acid concentration to that of ordinary concentrated hydrochloric acid. The product was precipitated by pouring the solution gradually into a suspension of 68 g. of sodium bicarbonate in 200 cc. of water. The excess bicarbonate was neutralized with acetic acid. The material was centrifuged and the residue washed successively with water, ethanol, acetone and petroleum ether. The washed product was dried in a vacuum oven at 50°.

The mother liquors from the precipitates were tested with Fehling's solution and negative results were obtained in each case. They were then concentrated at 55°, under reduced pressure to a moist salt and this transferred to a Büchner funnel, washed with acetone and dried at 40°. The salt residue was then acetylated for two days at room temperature (occasional shaking) with acetic anhydride (36 cc.), and pyridine (18 cc.) and the acetylation product isolated by pouring into ice and water (800 cc.). The data obtained are tabulated in Table II.

The data tabulated in Table II were calculated to their average degrees of polymerization by means of the sulfur analyses and these calculated results are shown in Table III.

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Summary

1. Ethyl mercaptan has been used as a reagent to follow the course of hydrolysis of the cellulose molecule in fuming hydrochloric acid.

2. The hydrolysis of cellulose (chemically purified linters of high viscosity) in fuming hydrochloric acid at 23° was followed polarimetrically and by viscosity measurements. The rate of increase of the optical rotation from 0° to that of *d*-glucose ($[\alpha]_D$ + 100°) was found to be a continuous function. The calculated monomolecular velocity constants showed a slight but definite upward drift. At an early stage ($[\alpha]_D$ + 25°, approx.) of the hydrolysis the viscosity of the solution approached that of a solution containing an equivalent amount of apparent *d*-glucose.

3. The course of hydrolysis of cellulose in fuming hydrochloric acid solutions (5%) at 16° was studied by means of the mercaptalated products obtained from the reaction of ethyl mercaptan with the cellulose solutions at various stages of hydrolysis. Simultaneous polarimetric data were obtained. Control experiments were made to determine the optimum procedure for the mercaptalation of *d*-glucose and this procedure was used in the cellulose reactions. Sulfur analyses served to determine the average molecular size of the mixtures of mercaptalated products isolated from the cellulose hydrolysis.

4. The data indicate a rapid hydrolysis to surprisingly small units. When the hydrolysis was approximately at the half-period, the isolated products corresponded to the trisaccharide stage. Glucose diethyl mercaptal first appeared when the hydrolysis was approximately two-thirds completed and gradually increased in amount.

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