TABLE III

THERMAL MUTAROTATION OF TETRAMETHYL α-*d*-GALACTOSE FROM 25 TO 0.0°

0.7514 g. of sugar in 20 ml. of water

Time, min. and sec.	Rotation obsd.	$(k_1 + k_2) \times 10^6$ calcd.
9′15″	8.73	
10′15″	8.74	
37′40″	8.78	2040
74′0″	8.81	1622
157′0″	8.88	1631
397′0″	8.92	866
638′0″	8.97	826
1406′0″	9.05	872
1408′0″	9.04	745
∞	9.07	

than the difference between the velocity constants of the first and second parts of the reaction and the consistent nature of this difference of the various determinations is such that the authors consider it a real difference.

The tetramethyl α-*d*-galactopyranose cannot have a furanose ring since the fourth carbon is methylated. In consequence if the differences in the mutarotation velocity constants of Tables II and III are real, then the complex mutarotation of tetramethyl α-*d*-galactose cannot require a galactofuranose as one of the sugars in the interconversion. This does not imply, however, that galactofuranose may not be involved in the complex mutarotation of unmethylated *d*-galactose.[13]

Summary

The mutarotations of solutions of tetramethyl α-*d*-galactopyranose have been studied for 25 and 0°. Velocity constants for the first part of the mutarotation at 0.0° show slightly but consistently higher values than for changes nearer the equilibrium.

"Thermal mutarotation" for tetramethyl α-*d*-galactopyranose for a 25 to 0° change has been investigated. The rotation constant, $k_1 + k_2$, is consistently larger at the first than at the last of the change by an amount which is considered in excess of the experimental error.

It is suggested that the assumed pyranose–furanose interconversion as a part of the mechanism of complex mutarotation can hardly be perfectly general.

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(13) Diacetone mannose has been reported by Irvine and Skinner [*J. Chem. Soc.*, 1095 (1926)] as having a complex mutarotation. The authors repeated their work at 17° and found agreement with that report. The changes in specific rotation go from 9 to −3° back to 1°. This change hardly could be attributed to a pyranose–furanose interconversion.

LINCOLN, NEBRASKA RECEIVED SEPTEMBER 6, 1938

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

Studies of Cellulose Hydrolysis by Means of Ethyl Mercaptan. III(1)

BY M. L. WOLFROM AND JOHN C. SOWDEN[2]

Previous publications[3] from this Laboratory have described a study of the course of hydrolysis of the cellulose molecule. The method consisted of allowing a solution of high viscosity cotton linters in fuming hydrochloric acid to hydrolyze at 16° and following the course of hydrolysis by mercaptalation with ethyl mercaptan at various time intervals. Sulfur analyses on the resulting mercaptalated hydrolyzed celluloses then gave an estimate of their average molecular size. Cuprammonium viscosity measurements on the non-mercaptalated hydrolyzed products, isolated after corresponding periods of hydrolysis in the fuming hydrochloric acid, were also employed to estimate the average chain lengths by application of the formula of Kraemer and Lansing.[4] A comparison of the average molecular sizes as determined by the two methods was thus available.

The studies at 16° indicated that the sulfur analytical method gave appreciably lower values for the chain lengths than those calculated from cuprammonium viscosity data, for hydrolyzed products of above 100 glucose units in size. Thus, it is of interest to study further the earlier stages of the hydrolysis, in order to determine the nature of this difference in chain lengths as calculated by the two methods for products having degrees of polymerization greater than 100 glucose units.

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(1) Presented before the Division of Organic Chemistry at the 96th meeting of The American Chemical Society, Milwaukee, Wisconsin, September 8, 1938.

(2) Du Pont Cellulose Research Fellow.

(3) M. L. Wolfrom and Louis W. Georges, THIS JOURNAL, **59**, 282 (1937); M. L. Wolfrom, Louis W. Georges and John C. Sowden, *ibid.*, **60**, 1026 (1938).

(4) E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 164 (1935).

In the work herein reported, the course of hydrolysis of the same sample of cotton linters previously studied was followed during the first eight and one-half hours in fuming hydrochloric acid at 0°. The lower temperature resulted in a much slower rate of hydrolysis than that previously observed at 16°, and the products isolated had average chain lengths in the range of 100 to 300 glucose units.

A cuprammonium viscosity measurement on a sample of our stock of high viscosity cotton linters showed that no degradation had occurred during storage. In order to assure comparable conditions in the preparation of the mercaptalated and non-mercaptalated products, a stock solution of the linters in the fuming acid was prepared at 0° and divided into two equal parts. An excess of ethyl mercaptan, to provide continuous mercaptalation of the hydrolyzed celluloses, was added to one part and the hydrolysis allowed to progress at 0°. Simultaneous removal and neutralization of samples from the two hydrolysis mixtures at various time intervals then resulted in mercaptalated and non-mercaptalated products which had been subjected to comparable hydrolytic conditions in the fuming acid.

As in the previous studies at 16°,[3] ash analyses were made on the washed products to determine their sodium chloride content. Precise sulfur analyses were then made on the mercaptalated hydrolyzed products by the Parr bomb method and the viscosities of both the mercaptalated and non-mercaptalated hydrolyzed products were determined in cuprammonium solution at a concentration of 0.1%. These data are recorded in Tables II and III. Due to the opacity of the hydrolysis mixture at 0°, it was impossible to follow the course of the hydrolysis polarimetrically. Thus, the time of addition of the fuming hydrochloric acid to the linters was taken as the zero time of the hydrolysis.

The copper numbers (Hägglund–Bertrand) of the mercaptalated and non-mercaptalated hydrolyzed celluloses were determined and are recorded in Table IV. No explanation is yet at hand for the fact that the mercaptalated products, which would be expected to have a zero copper number, exhibit a low but definite reducing power. The original cotton linters were found to have a very low reducing power, as evidenced by a copper number of approximately 0.2.

In Table I are recorded the degrees of polymerization in glucose units, calculated from cuprammonium viscosity data by the formula of Kraemer and Lansing,[4] for both the mercaptalated and non-mercaptalated hydrolyzed celluloses, and also those calculated for the mercaptalated products from sulfur analytical data. The degrees of polymerization, as calculated from viscosity data, show fair agreement, the values for the mercaptalated products tending to be approximately twenty glucose units greater than those for the non-mercaptalated products.

Table I

Degree of Polymerization (D. P.) in Glucose Units of Mercaptalated and Non-mercaptalated Hydrolyzed Celluloses Calculated from Sulfur Analyses and Cuprammonium Viscosity Data

Time of hydrolysis, min.[a]	Mercaptalated products D. P. by S content	D. P. by viscosity	Non-mercaptalated products D. P. by viscosity
270	190	276	260
310	148[b]	255	233
350	104	228	229
390	87	226	206
430	95	218	196
470	101[b]	204	187
510	98[b]	198	174

[a] Initial time taken as time of addition of acid to the cellulose.

[b] The results of two closely agreeing sulfur analyses (see Table II) were averaged and the D. P. calculated from this average.

The average degrees of polymerization of the mercaptalated product mixtures as calculated from their sulfur content are definitely lower than those of the corresponding non-mercaptalated products as calculated from cuprammonium viscosity data. The degree of polymerization calculated from sulfur analytical data on a nonhomogeneous mixture constitutes a number average and would thus be expected to be somewhat lower than that calculated from cuprammonium viscosity based on ultracentrifuge measurements, which represents a weight average (4). This difference will not be appreciable if the degrees of polymerization of the components of the mixture do not extend over a wide range.

In the calculation of the average degree of polymerization (D. P.) from the sulfur analyses, the assumption is made that all free reducing groups have reacted completely to form thioacetals (mercaptals). Any correction of the data for incompleteness of reaction or for the reaction resulting in the formation of thioglycosides instead of thioacetals, would lead to still lower degrees of polymerization as calculated from the sulfur content. The presence of adsorbed ethyl mercaptan would lead, however, to a calculated degree of polymerization that was too low. The absence of adsorbed ethyl mercaptan in the products analyzed was accordingly demonstrated. Several of the samples were analyzed before and after extensive drying at 56.5° in high vacuum. Also, one mercaptalated product was analyzed before and after prolonged soaking in absolute ethanol. Neither of these treatments, which would be expected to remove any adsorbed ethyl mercaptan, had any appreciable effect on the observed sulfur content.

The relationship between the degrees of polymerization by the two methods with increasing time of hydrolysis is shown in Fig. 1. The difference in the values obtained by the two methods, which was observed in the range of products of 100 to 200 glucose units in size during the previous work at 16°,[3] apparently does not tend to increase in the range of degraded celluloses from 200 to 300 glucose units in size. The nature of the curves in Fig. 1 is such that extrapolations to zero time cannot be made on the basis of the data at hand.

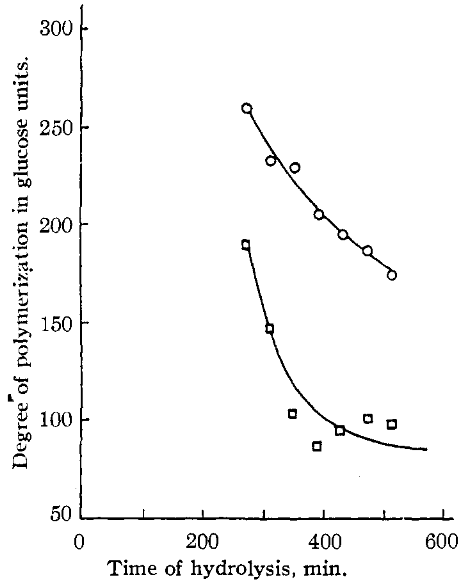

Fig. 1.—Rate of change of degree of polymerization (D. P.) of cellulose (4%) with time of hydrolysis by fuming hydrochloric acid ($d^{16}{}_4$ 1.205) at 0°: ○, D. P. by cuprammonium viscosity; □, D. P. by sulfur content.

Experimental

Cellulose Characterization.—The degree of polymerization of our stock cotton linters[5] was redetermined by measuring the viscosity of a 0.1% solution in cuprammonium solvent and applying the formula of Kraemer and Lansing:[4]

Average time of flow, solution	402.5 sec. at 25°
Average time of flow, solvent	184.9 sec. at 25°
D. P.	2023

This compares with the values 1980 and 2008 found for the same stock in the previous work.[3]

Hydrolysis and Mercaptalation of Linters.—The cotton linters (75.0 g., moisture-free basis) in a glass-stoppered bottle at 0° were treated with fuming hydrochloric acid (1500 cc.; $d^{16}{}_4$ 1.205; % HCl, approx. 41) previously cooled to 0°. The mixture was maintained at 0° with occasional shaking. After three hours the resulting opaque, homogeneous mixture was equally divided and placed in two 1-liter, 3-necked flasks at 0°, equipped for rapid mechanical stirring and for rapid removal of samples through a delivery tube. One flask contained well-purified ethyl mercaptan (100 g.) at 0° to provide continuous mercaptalation of the degraded cellulose as the hydrolysis progressed. The two mixtures were stirred rapidly and maintained at 0° throughout the duration of the hydrolysis. After two hundred and seventy minutes of hydrolysis, samples of approximately 85 cc. were withdrawn simultaneously every forty minutes from each flask. The samples were withdrawn to calibrated bottles by application of suction and immediately poured, with stirring, into separate mixtures of sodium bicarbonate (96 g.) and water (300 cc.). In this way, seven samples each of mercaptalated and non-mercaptalated hydrolyzed celluloses were obtained, the time of contact with the fuming acid varying from 270 to 510 minutes. The products were collected and washed in the manner described in our previous work.[3]

TABLE II

MERCAPTALATED PRODUCTS FROM COTTON LINTERS (c, 5 G. PER 100 CC. SOLN.) IN FUMING HYDROCHLORIC ACID ($d^{16}{}_4$ 1.205) AT 0°

Time of hydrolysis, min.[a]	% ash, as NaCl	Mercaptalated product from 4.25 g. cellulose wt., g.[b]	% S		Cuprammonium viscosity, 25°, c : 0.1, time of flow in sec. Soln.	Solvent	D. P. by viscosity
270	5.29	3.62	0.210[d]		204.4	183.8	276
310	5.14	3.51	.270[d]	0.272	203.4	184.4	255
350	4.97	3.60	.384[d]		201.1	184.2	228
390	5.67	2.42[c]	.461[d]		202.0	185.2	226
430	4.24	3.55	.419[d]		201.4	185.2	218
470	4.45	3.37	.441[d]	.354	200.3	185.2	204
510	4.30	3.42	.431	.386[e]	199.5	184.9	198

[a] See footnote a, Table I. [b] Calculated on vacuum-dried (63°) and sodium chloride free basis. [c] Part of product lost mechanically during washing operations. [d] Sample previously dried sixteen hours at 56.5° and 0.001 to 0.0005 mm. [e] Sample previously soaked seven days in absolute ethanol.

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(5) Supplied through the courtesy of the Hercules Powder Company.

TABLE III

NON-MERCAPTALATED PRODUCTS FROM COTTON LINTERS (*c*, 5.0 G. PER 100 CC. SOLN.) IN FUMING HYDROCHLORIC ACID (d^{16}_4 1.205) AT 0°

Time of hydrolysis, min.[a]	% ash, as NaCl	Product from 4.25 g. cellulose, wt., g.[b]	Cuprammonium viscosity, 25°, *c* : 0.1, time of flow in sec. Soln.	Solvent	D. P. by viscosity
270	7.93	4.41	203.1	183.8	260
310	5.70	4.18	201.0	183.8	233
350	7.71	4.12	201.9	184.9	229
390	6.84	4.13	200.5	185.2	206
430	6.54	4.05	199.7	185.2	196
470	6.37	4.09	199.0	185.2	187
510	5.47	3.63[c]	197.7	184.9	174

[a] See footnote *a*, Table I. [b] Calculated on vacuum-dried (63°) and sodium chloride free basis. [c] Part of product lost mechanically during washing operations.

Ash and Sulfur Analyses.—The ash content of the mercaptalated and non-mercaptalated hydrolyzed celluloses was determined by ashing 0.3- to 0.4-g. samples with 2 cc. of concentrated sulfuric acid in platinum crucibles. The results were calculated in terms of sodium chloride and are recorded in Tables II and III.

The sulfur analyses were performed by the Parr bomb method in the manner described in the previous publications.[3] In the present work, somewhat larger samples were employed for the sulfur determinations, however. Two samples of approximately 0.5 g. each, on an ash free, moisture free basis, were fused separately and then dissolved in the same solution for precipitation of the barium sulfate.

From the sulfur analyses, the degree of polymerization in glucose units was calculated from the approximation

$$\text{D. P.} = \frac{100 \times 2\text{S}}{\%\text{S} \times \text{C}_6\text{H}_{10}\text{O}_5} = \frac{40}{\%\text{S}}$$

The sulfur content of the various mercaptalated products is recorded in Table II, and the degrees of polymerization calculated from these data, in Table I.

Cuprammonium Viscosities.—The viscosities of the mercaptalated and non-mercaptalated hydrolyzed celluloses were observed in cuprammonium solution[6] at 25 ± 0.03° as described previously.[3] In all instances, a concentration of 0.1% was employed. The constants of the Ostwald type viscometer used were given in the former publications.[3]

From the viscosity measurements, the degree of polymerization in glucose units was calculated by the formula of Kraemer and Lansing.[4] The results are tabulated in Tables I, II, and III.

In order to determine the effect of marked non-homogeneity on the degree of polymerization (D. P.) as determined by cuprammonium viscosity, the following experiments were performed. A mixture consisting of 49.1% of a hydrolyzed cellulose product of observed D. P. 266 and 50.9% of observed D. P. 181 gave an observed D. P. of 226 (calcd. by weight average, 223; calcd. from theoretical sulfur content, 215). A mixture consisting of 40.8% of the hydrolyzed cellulose product of observed D. P. 266 and 59.2% anhydrous glucose gave an observed D. P. of 108 (calcd. by weight average, 109).

Copper Numbers.—The copper numbers of the mercaptalated and non-mercaptalated products were determined by the Hägglund–Bertrand[7] method. Samples of 0.3 to 0.5 g., on an ash free, moisture free basis, were employed. The results are recorded in Table IV.

The copper number of the stock cotton linters, used as starting material, was also determined, in duplicate. A copper number of approximately 0.2 was observed for the original linters.

TABLE IV

COPPER NUMBER[a] (HÄGGLUND–BERTRAND) OF MERCAPTALATED AND NON-MERCAPTALATED HYDROLYZED CELLULOSES

Time of hydrolysis, min.[b]	Mercaptalated products, copper no.[c]	Non-mercaptalated products, copper no.[c]
0		0.2[d]
270	0.90	5.26
310	1.03	5.96
350	0.49	6.75
390	1.27	7.64
430	0.58	7.89
470	.59	7.59
510	.50	8.12

[a] Copper no. is grams of copper reduced from cupric to cuprous state by 100 g. of sample. [b] See footnote *a*, Table I. [c] Calculated to a moisture free and sodium chloride free basis. [d] Original linters.

The authors are indebted to E. I. du Pont de Nemours and Company, Inc., for the fellowship grant which made this research possible.

Summary

1. High viscosity cotton linters have been hydrolyzed with fuming hydrochloric acid at 0° in the presence of an excess of ethyl mercaptan. The resulting mixtures of mercaptalated hydrolyzed celluloses were isolated at various time

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[6] Prepared according to the standards of the Cellulose Division of the American Chemical Society.

[7] E. Hägglund, *Celluloseckemie*, 11, 1 (1930).

intervals during the first eight and one-half hours of the hydrolysis.

2. Corresponding non-mercaptalated hydrolyzed celluloses were prepared under exactly similar conditions, except that the ethyl mercaptan was omitted.

3. Sulfur analytical data indicated that the average degrees of polymerization of the mercaptalated products varied from 190 glucose units after 270 minutes to approximately 100 glucose units after 510 minutes.

4. The degrees of polymerization calculated, according to the formula of Kraemer and Lansing, from the cuprammonium viscosity data for the corresponding non-mercaptalated hydrolyzed celluloses were consistently higher than those calculated from the sulfur analytical data, and varied from 260 glucose units after 270 minutes to 174 glucose units after 510 minutes.

5. The mercaptalated hydrolyzed celluloses showed low but definite copper numbers, varying from 0.5 to 1.3. The non-mercaptalated celluloses had copper numbers increasing in value with increasing time of hydrolysis, and ranging from 5.3 to 8.1.

COLUMBUS, OHIO RECEIVED OCTOBER 8, 1938

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CALIFORNIA FRUIT GROWERS EXCHANGE]

The Bitter Constituents of Navel and Valencia Oranges

BY RALPH H. HIGBY

The production of oranges in California is largely of two varieties, the Valencia, a seeded summer orange, and the Washington Navel, a seedless winter ripening variety. The Valencia and Navel are equally good for general use but the juice from this latter variety is not used extensively for canning or for the manufacture of other juice products because of the bitter taste and astringency which develops in the juice when it is allowed to stand exposed to air, or when it is pasteurized. The degree of bitterness is largely dependent upon the maturity of the fruit, most pronounced in early season, but usually disappearing entirely with warm weather in March and April. Bitterness is found also in the juice of unripe Valencia oranges, but never in fruit of this variety which has reached commercial maturity.

Although the presence of a bitter constituent in the edible portion of the Navel orange has long been recognized, no record of its isolation or identification has appeared in the literature. Naringin, the bitter glucoside of grapefruit has long been known, it is commercially available and its properties and structure are fairly well established.

The bitter substances of citrus seeds have also been investigated rather extensively. As early as 1841, Bernay[1] isolated a strongly bitter substance from the seeds of several varieties of citrus (*C. Aurantium Risso*, *C. Limonum Risso*, and *C. Bigardia Loisl.*) which he named limonin and erroneously thought to be an alkaloid. Since that time, various other workers, C. Schmidt,[2] Paternò and Oglialoro,[3] and Peters and Frerich,[4] have prepared this substance and made some study of its properties. In a recent publication, Koller and Czerny[5] have reported the results of a rather comprehensive investigation of limonin, from orange seeds, and have announced the isolation of a second bitter substance, from the same source, which they have named isolimonin. Shortly thereafter Feist and Schulte[6] separated what was thought to be a third bitter substance from lemon seeds, calling it citrolimonin. Similarity in equivalent weight and specific optical rotation have led Koller and Czerny[7] to believe that limonin and citrolimonin are identical despite a considerable discrepancy in the reported melting point.

As one part of a research program directed toward the commercial utilization of Navel orange juice, a study has been made of the bitter constituents of the orange, particularly those contained in the edible portion. Bitter crystalline substances have been extracted from the pulp of the Navel orange, and from both pulp and seeds of the Valencia orange. These substances have

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[1] Bernay, *Ann.*, **40**, 317 (1841).

[2] C. Schmidt, *ibid.*, **51**, 388 (1844).

[3] Paternò and Oglialoro, *Ber.*, **12**, 685 (1879).

[4] Peters and Frerich, *Arch. Pharm.*, **240**, 661 (1902).

[5] C. Koller and H. Czerny, *Monatsh.*, **67**, 248–268 (1936).

[6] K. Feist and H. Schulte, *Ber.*, **69**, 1322 (1936).

[7] C. Koller and H. Czerny, *Monatsh.*, **70**, 26–29 (1937).