[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

The Action of an Aqueous Chlorine System on Methyl β-D-Glucopyranoside[1]

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The oxidation of methyl β-D-glucopyranoside with an aqueous chlorine system at *p*H 4.5 has yielded D-glucose, D-arabinose, carbon dioxide and oxalic acid as the major products. Minor products that have been found were 2-ketogluconic acid and 2,5-diketogluconic acid which were tentatively identified on chromatograms. The D-glucose, D-arabinose and unreacted methyl β-D-glucopyranoside were determined quantitatively by a chromatographic procedure. A carbon balance shows that more than 80% of the original methyl β-D-glucoside has been accounted for. The oxidation of D-glucose, gluconic acid, 2-ketogluconic acid, 5-ketogluconic acid and methanol have helped to establish the probable reaction paths. It appears that methyl β-D-glucopyranoside is oxidized directly to D-arabinose carbonate ester which then splits off the ester to give D-arabinose. This product has been found in both the hydrolyzate from the oxidized cellulose and in the oxidation solution itself. In addition, the latter solution contained glucose.

Lindberg, Dyfverman and Wood[2] used methyl β-D-glucopyranoside as a model compound for cellulose. They found that methyl β-D-glucopyranoside was oxidized by an aqueous chlorine system at a *p*H about 1 to gluconic acid and 5-ketogluconic acid. Small quantities of D-glucaric acid were probably present. The oxidation of methyl β-cellobioside by Dyfverman[3] is even more interesting. In addition to D-gluconic acid and 5-keto-D-gluconic acid, cellobionic acid was produced by the reaction. As the reaction progressed, the cellobionic acid was oxidized to D-gluconic acid and 5-keto-D-gluconic acid. Small amounts of D-glucaric acid also were found among the oxidation products.

Recently Whistler, Linke and Kazeniac[4] investigated the oxidation of methyl 4-*O*-methyl-β-D-glucopyranoside with aqueous chlorine solutions buffered at *p*H 9.5. The major product seemed to be a diacid which had been formed by the 2,3 cleavage of the pyranose ring. Upon hydrolysis this product yielded glyoxylic acid and erythronic acid. Starch was also oxidized and the oxidation products examined. The results were similar to those obtained with methyl 4-*O*-methyl-β-D-glucopyranoside.

In the present work the oxidation of methyl β-D-glucopyranoside with an aqueous chlorine system buffered at *p*H 4.5 has been studied.

The major products isolated were D-glucose, D-arabinose, carbon dioxide and oxalic acid. 2-Keto-D-gluconic acid and 2,5-diketo-D-gluconic acid probably were present.

The D-glucose was detected chromatographically, and identified by its optical rotation value, infrared spectra and phenylhydrazone derivative. The D-arabinose was detected chromatographically and was identified by its optical rotation, infrared spectra, phenylhydrazone derivative and diphenylhydrazone derivative. The carbon dioxide was identified as barium carbonate. The oxalic acid was identified as calcium oxalate. The 2-keto-D-gluconic acid and 2,5-diketo-D-gluconic acid were detected chromatographically, but were not isolated because of the small quantity present.

The presence of D-glucose among the oxidation products was not expected since it was demonstrated that methyl β-D-glucopyranoside was not hydrolyzed to a detectable degree under the conditions employed in these oxidations. Even under the more acidic conditions of Lindberg, Dyfverman, and Wood[2] no hydrolysis occurred within 14 days. On the basis of this evidence it can be seen that the oxidation attacks the glycosidic bond as a combined oxidation and hydrolysis reaction, or that the oxidation attacks the methyl aglycone group and yields a new aglycone group which may then be more sensitive to hydrolysis than the original methyl aglycone group.

The oxidation of methyl β-D-glucopyranoside to yield D-arabinose is somewhat easier to explain. The direct oxidation of the carbon 1-to-2 bond seems to be the most reasonable explanation after examination of the experimental results. Separate oxidations of D-glucose, D-gluconic acid, 2-keto-D-gluconic acid and 5-keto-D-gluconic acid showed that none of these compounds could have produced the quantity of D-arabinose found in the oxidations of methyl-D-glucopyranoside. When D-glucose was oxidized under the same conditions as the methyl β-D-glucopyranoside, 50% of the initial D-glucose was recovered after the reaction had gone to completion and only 1% D-arabinose was produced. In the oxidations of methyl β-D-glucopyranoside there was always about twice as much D-arabinose as D-glucose. Thus, the high ratio of unreacted glucose to arabinose in the glucose oxidation solution makes the glucose only a minor intermediate in the formation of D-arabinose. D-Gluconic acid yielded only a trace of D-arabinose upon oxidation and approximately 80% 2-keto-D-gluconic acid. The high yield of 2-keto-D-gluconic acid in this case is not compatible with the low yield found from the oxidation of methyl β-D-glucopyranoside. The oxidation of 2-keto-D-gluconic acid and 5-keto-D-gluconic acid yielded no D-arabinose; thus, they have been eliminated from the list of possible intermediates.

The carbon dioxide and oxalic acid which have been found among the oxidation products cannot be attributed to any definite source. It is probable that part of the carbon dioxide originated from the methyl aglycone groups and that another part came from the carbon 1 of the glucopyranose ring after cleavage of the carbon 1-to-2 bond. The remainder of the carbon dioxide came from the fur-

(1) Paper presented before the Division of Carbohydrate Chemistry at the 131st meeting of the American Chemical Society at Miami, Florida, April, 1957.

(2) B. Lindberg, A. Dyfverman and D. Wood, *Acta Chem. Scand.*, **5**, 253 (1951).

(3) A. Dyfverman, *ibid.*, **7**, 280 (1953).

(4) R. L. Whistler, E. G. Linke and S. Kazeniac, THIS JOURNAL, **78**, 4704 (1956).

ther degradation of the glucopyranose rings. The possible formation of oxalic acid by oxidative fission of carbons 2 and 3 was considered; however, the presence of erythronic acid which would also be formed in this reaction could not be established on the paper chromatogram.

The 2-keto-D-gluconic acid detected in the oxidation products probably came from the oxidation of D-glucose. Whether or not D-gluconic acid was an intermediate in the formation of 2-keto-D-gluconic acid is difficult to say. Since no D-gluconic acid has been found in the oxidation solutions it would not appear to be a likely intermediate. The 2,5-diketo-D-gluconic acid most likely comes from the oxidation of the 2-keto-D-gluconic acid and is reasonably stable in solution. The compound appears to be quite reactive and is very difficult to isolate.[5]

Figure 1 illustrates the possible reaction paths that have been discussed.

Cellulose has been oxidized under similar conditions and the oxidation solution and oxidized cellulose examined. D-Arabinose has been found in both the oxidation solution and the hydrolyzate from the oxidized cellulose. The yield of D-arabinose is about 0.3% in the oxidation solution and 1% in the oxidized cellulose on the basis of the initial weight of cellulose.

The oxidation solution also contained D-glucose. The conditions used for the oxidation were again checked and it was found that cellulose did not undergo hydrolysis in this environment. Therefore, the D-glucose found from the oxidation of cellulose must have been formed in a manner similar to that described for the formation of D-glucose from methyl β-D-glucoside. Such a reaction might be called an oxidative hydrolysis. The D-arabinose found from the oxidation of cellulose probably was produced in the same manner as that from methyl β-D-glucopyranoside.

This work on cellulose is only preliminary in nature and is being continued at the present time. It does seem to confirm the work of Kaverzneva[6] on cellulose oxidized under the same conditions as the present work. She has postulated the cleavage of the carbon 1-to-2 bond with the formation of a carbonyl group at carbon 2 and a carbonate group at carbon 1. This postulate was based on her finding carbonate groups in the oxidized cellulose. Until the present work the presence of the D-arabinose unit had not been proven. The actual isolation of D-arabinose gives confirmation to her postulate for the cleavage of the carbon 1-to-2 bond since such a reaction is the only way by which D-arabinose could be produced.

Experimental

Starting Materials.—Methyl β-D-glucopyranoside was prepared by the method of Raymond and Schroeder.[7] The melting point of the product was 105° and the rotation was −34.5°. When this compound was chromatographed, several minor spots were found. One of these was D-glucose

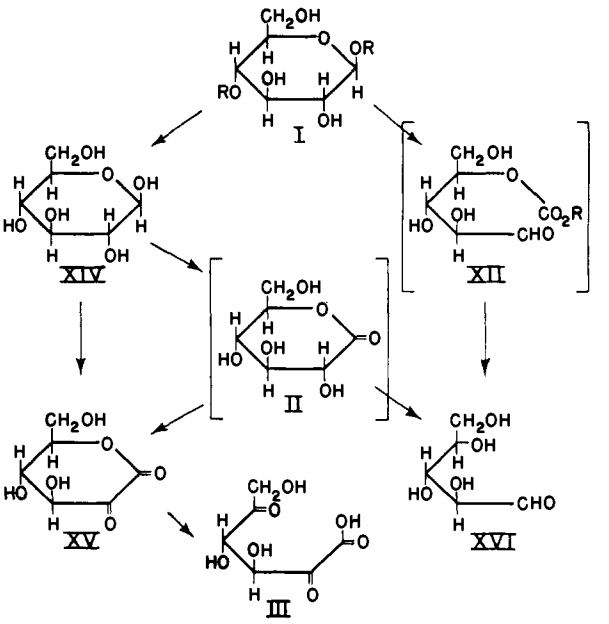

Fig. 1.—The probable paths of oxidation of methyl β-D-glucoside in an aqueous chlorine system at *p*H 4.5.

and the other two were unidentified. The quantity of D-glucose present was approximately 1%. One of the unidentified spots had a blue-white fluorescence. The methyl β-D-glucopyranoside was purified by dissolving it in a small quantity of methanol and forming the potassium acetate complex again. The complex was decomposed and the methyl β-D-glucopyranoside crystallized. This process was then repeated. The final product was obtained in 20% yield. The methyl β-D-glucopyranoside contained 0.1% glucose, and the other impurities could no longer be detected. The melting point of the final product was 106° and the rotation was −34.6°.

The cellulose used in the present work was obtained in the form of a handpicked cotton.[8] The cotton fibers were removed from the seeds by hand. Three grams of cotton were refluxed in 180 ml. of 1% potassium hydroxide for 1 hour. The cellulose was then filtered off and washed with hot alcohol and then with 1 liter of water, and air-dried for two days.

The 2-keto-D-gluconic acid and the 5-keto-D-gluconic acid were obtained as the calcium salts[9] and were found to be chromatographically pure.

Analytical Methods.—The aqueous chlorine systems were examined using the methods of White.[10] The anions in the oxidation solutions were determined by passing the solution through an Amberlite IR-120 column,[11] and titrating with 0.1 *N* sodium hydroxide to a phenolphthalein endpoint.

The chromatographic technique of Pridham[12] was used to determine glucose and arabinose quantitatively. The method was found to be reliable only in the range from 5 to 50 μg. of sugar per spot.

Fractionation Procedures.—The initial oxidations were treated with silver carbonate as described by Lindberg, *et al.*,[2] to remove the chloride ions from the oxidation solutions. It was decided to search for new methods when it was found that the silver ions reacted with some of the oxidation products in the original reaction mixture. The chlorine ion was difficult to remove completely by this method, and the sample had to be handled several times. Amberlite IR-45[11] in the acetate form was found to be very

(5) H. Katznelson, S. W. Tanenbaum and E. L. Tatum, *J. Biol. Chem.*, **204**, 43 (1953).

(6) E. D. Kaverzneva, *Comm. 13th Intern. Congr. Pure and Appl. Chem., Stockholm*, 1953, pp. 328–360.

(7) A. L. Raymond and E. E. Schroeder, THIS JOURNAL, **70**, 2789 (1948).

(8) The cotton was furnished through the courtesy of Dr. H. D. Barker of the Field Crops Research Branch, U. S. Department of Agriculture.

(9) The 2-ketogluconic acid and the 5-ketogluconic acid were obtained from Dr. I. A. Wolff of the Northern Regional Research Laboratories.

(10) J. F. White, *Am. Dyestuff Reptr.*, **31**, 485 (1942).

(11) Product of Rohm and Haas Co., Philadelphia, Pa.

(12) J. Pridham, *Anal. Chem.*, **28**, 1803 (1956).

good for removing the acidic materials while allowing the neutral compounds to pass through the column. The neutral solutions were passed over an Amberlite IR-120 column to remove any cations present.

The acidic compounds were eluted from the anion exchange resin as salts by washing the column with dilute ammonium hydroxide. A further elution of the column with sulfuric acid removed no more material. The salts of the acidic compounds were passed over an Amberlite IR-120 column to regenerate the free acids. The free acids were treated with silver carbonate for a short time with rapid stirring in order to remove the chloride ions and no adverse reaction was observed at this stage. The solution was then passed over an IR-120 column to remove the silver ions in solution. After this the acid solution was concentrated to the desired volume.

Chromatographic Methods.—The following chromatographic developers were found to be the most generally useful: A, butanol, acetic acid, water (4:1:5); B, ethyl acetate, acetic acid, formic acid, water (18:3:1:4); C, isobutyric acid saturated with water. The major sprays used were: a, ammoniacal silver nitrate; b, *p*-anisidine hydrochloride; c, methyl orange; d, periodate–permanganate. Table I is a compilation of the R_g values obtained for various known compounds in developers A, B and C.

TABLE I

R_g VALUES OF KNOWN COMPOUNDS

	Developers[a] A	B	C
Cellobiose	0.28	0.30	0.60
Glucose	1.0	1.0	1.0
Arabinose	1.42	1.56	1.32
Ribose	2.0	2.3	1.6
Mannose	1.25	1.25	1.15
Xylose	1.64	1.7	1.35
Rhamnose	2.62	2.68	1.80
Erythrose	1.75	1.6	0.76
	(2.7)	(2.3)	(1.0)
	(3.7)	(3.0)	(1.4)
2-Ketogluconic acid	1.35	1.4	0.8
5-Ketogluconic acid	2.05	1.95	0.92
Gluconic acid	1.16	1.3	0.96
		3.1	
Glucuronic acid	2.15	2.8	1.51
Methyl β-glucoside	1.95	2.6	1.95
Erythronic acid	2.92	4.2	2.09

[a] A is butanol:acetic acid:water (4:1:5); B is ethyl acetate:acetic acid:formic acid:water (18:3:1:4); C is isobutyric acid saturated with water.

Preparation of Oxidizing Agent.—The oxidations were all performed with an aqueous chlorine system. The oxidizing agent was prepared by passing chlorine gas into a 1 *N* solution of sodium hydroxide until the *p*H of the solution fell to 9.5. The oxidations were made by adding a known volume of this oxidizing solution to an aqueous solution of methyl β-D-glucopyranoside or other carbohydrate. Acetic acid was used to reduce the *p*H to the desired level. In the initial oxidations the *p*H was maintained at the desired level by adding sodium hydroxide as necessary. In the final oxidations which were conducted at a *p*H of 4.5 a sodium acetate–acetic acid buffer system was employed. The reactions were allowed to continue until a negative starch–iodide test was obtained, usually a period of 12–24 hours.

The temperature of the reactions was 20 ± 2°. The reaction gave the same products whether conducted in the presence or absence of light or exposed to the air. The effect of chlorate as a possible oxidant was not studied.

The Oxidation of Methyl β-D-Glucopyranoside.—The physical conditions under which the reactions were conducted varied widely. Some of the oxidations were made in open beakers whereas others were conducted in closed systems. The conditions under which the reactions were conducted did not seem to have any effect upon the path of the reaction.

The oxidation solutions were examined for their inorganic constituents and a material balance was made over the system. Oxidation 5, Table II, contained 9.2 mmoles of OCl^-, and 9.2 mmoles of Cl^-; total chlorine content of 18.4 mmoles initially. After the reaction had gone to completion, 18.3 mmoles of Cl^-, and 0.06 mmole of ClO_3^- were present; total chlorine content of 18.36 mmoles.

TABLE II

THE OXIDATION OF METHYL-β-D-GLUCOSIDE

	Experimental Runs 1	2	3	4	5	6
Methyl β-D-glucoside, mmoles						
Initial	45	10	5	2	5	1
Residual[a]	26	8.0	3.6	1.4	3.5	0.65
NaOCl, meq.	45	25	25	10	19	4
Volume, ml.	1000	65	100	50	100	25
Oxidation products, %						
D-Glucose	3.8	6.0	1.5	4.0	5.3	3.7
D-Arabinose	7.7	17.8	2.9	10.0	12.1	10.0
Carbon dioxide	....	..	75	65	...	56
Oxalic acid	....	..	...	..	...	19.1

[a] The value for methyl β-D-glucoside in run 1 was determined by recrystallizing the unreacted material and weighing. The values in 2 through 6 were determined by the modification of Pridham's technique[12] which was described in the text.

The theoretical acidity of the oxidation solution after being passed over an Amberlite IR-120 column was calculated to be 68.2 mmoles on the basis of chlorine and acetate put into the reaction. The acidity determined experimentally was 67.6 mmoles. A blank oxidation was made under similar conditions and the initial chlorine content was 3.86 mmoles and the acidity 13.82 mmoles. The final chloride content was 3.78 mmoles and the acidity 13.92 mmoles. Within the accuracy of the determinations there is a good material balance of the chlorine in the oxidation solution and also of the acidity. Thus, the oxidation does not seem to have produced any titratable organic acids from methyl β-D-glucopyranoside. A hydrolysis of methyl β-D-glucopyranoside was attempted with the sodium acetate–acetic acid buffer system. Under the conditions employed in these reactions no detectable hydrolysis occurred in 14 days.

In some of the final oxidations the oxidation flask was connected to a gas buret and the volume of gas produced was measured. This gas was later passed through a barium hydroxide solution and barium carbonate precipitated, thus showing that the gas was at least partially carbon dioxide. In other cases the gas from the oxidation solution was bubbled through a solution of potassium iodide in order to measure the active chlorine lost as gas, which was found to be negligible.

Chromatograms of the neutral fraction showed that it contained glucose, arabinose and unreacted methyl β-D-glucopyranoside. The quantity of glucose and arabinose was determined by Pridham's technique.[12] Two 7 by 24 inch sheets of 3-mm. paper were spotted with 5 ml. of the neutral fraction of the oxidation solution. These chromatograms were developed for 18 hours with developer B. The glucose and arabinose regions were cut out and eluted with water. The eluates were diluted to 3 ml. and rotation values were determined. The amount of sugar was calculated from the concentration of the solution and from the volume of the solution applied to the chromatogram.

The glucose was found to have a rotation of 56 ± 5° (*c* 0.2, water) and that of arabinose −106 ± 5° (*c* 0.6, water). Hence both sugars have the D-configuration and the D-arabinose must have been formed from D-glucose.

The D-glucose was further identified as the phenylosazone, with a m.p. of 208°, known 210°. Preparation of the osatriazole could not be conducted on the small sample available. Another sample of D-glucose was eluted from a chromatogram into a solution of potassium bromide, and this solution was evaporated and dried. The mixture was ground up and made into a pellet for infrared analysis. The spectra of the unknown and of known D-glucose corresponded exactly.

The D-arabinose has been identified as the phenylhydrazone with a m.p. of 158°, known 160°. The arabinose diphenylhydrazone was prepared from the chromatographi-

cally purified D-arabinose and from the neutral fraction of the oxidation solution which contained D-glucose and methyl β-D-glucopyranoside as well as D-arabinose. The white needles had a m.p. of 196.5°, known 197°. The infrared spectra of D-arabinose was compared to a known sample of L-arabinose and was found to be the same.

Part of the methyl β-D-glucopyranoside in the neutral fraction of an oxidation solution was recovered by recrystallization. The yield was 30% and the final product had a melting point of 106°. A quantitative measurement of the residual methyl β-D-glucopyranoside was made by hydrolyzing it to D-glucose and determining the increase in D-glucose concentration by the chromatographic technique of Pridham,[12] and then converting this glucose value to an equivalent amount of methyl β-D-glucopyranoside. The hydrolysis of 0.05 g. of methyl β-D-glucopyranoside was made with 10 ml. of 10% sulfuric acid. The solution was refluxed for 5 hours. Then the sulfate was removed with barium carbonate, and the solution deionized by treatment with Amberlite IR-120, and concentrated to an appropriate volume. The validity of this modification of Pridham's technique was tested by analyzing purified methyl β-D-glucopyranoside and obtaining values of more than 99%.

The acidic fraction of the oxidation solution was found to contain very little material, but when this fraction was concentrated to a thick sirup in a rotating vacuum concentrator at 30°, some materials could be detected on the chromatograms. Traces of 2-ketogluconic acid and 2,5-diketogluconic acid were indicated by the chromatograms.

Upon warming and careful treatment of the acidic solution with calcium carbonate the solution yielded a white precipitate. The precipitate was then filtered off and washed. The solid gave a yellow solution when treated with sulfuric acid and resorcinol, and the color changed to blue upon standing. After neutralization with ammonium hydroxide the solution became of a faint pink color. Another sample of the solid was treated with sulfuric acid and pyrogallol, and a dirty green color resulted, which turned orange upon standing. Neutralization of this solution with ammonium hydroxide developed a blue color which turned to a brown upon standing. These color tests indicate that the solid was calcium oxalate.[13] The white solid dissolved in dilute hydrochloric acid with no evolution of gas. After a sample of the white solid had been ignited in a crucible, the residue dissolved with the evolution of gas. The calcium content of the original white solid was found to be 27.1%. Calcium oxalate monohydrate has a calcium content of 27.4%.

The filtrate from the calcium oxalate precipitation was treated with Amberlite IR-120 to remove the calcium ions, concentrated, and chromatographed. There was a spot which corresponded to known 2-ketogluconic acid using developers B and C. The reactions of known and unknown spots were the same with sprays a, b, c and d. The quantity of 2-ketogluconic acid present was extremely small and isolation was impossible.

No authentic sample of 2,5-diketo-D-gluconic acid was available. The only previous report of this compound has been by Katznelson, Tanenbaum and Tatum.[5] The R_g value reported for this compound in developer C was 0.59. The values observed in this work ranged from 0.57 to 0.61. The pale yellow color which resulted when the chromatograms were sprayed with *p*-anisidine hydrochloride was characteristic.

Oxidation of Cellulose.—The oxidation of 0.75 g. of cotton cellulose was performed with 50 ml. of 1 *N* sodium hypochlorite in a system buffered at *p*H 4.5 with sodium acetate–acetic acid. The reaction was allowed to proceed until all of the active chlorine had been consumed, and then the solution was filtered from the cellulose. The oxidized cellulose was washed and dried *in vacuo*.

The filtrate was deionized in the manner used for methyl β-D-glucopyranoside oxidation solutions. The neutral fraction was chromatographed and found to contain 1% glucose and 0.3% arabinose based upon the original cellulose oxidized. The acidic fraction was not examined.

A part of the oxidized cellulose, 0.6 g., was hydrolyzed with 10 ml. of 70% sulfuric acid for 15 minutes, and then diluted to 1% acid and refluxed for 1 hour. The oxidized cellulose appeared to have gone into solution after the first 15 minutes. After the hydrolysis solution had cooled, it was treated with barium carbonate, filtered and passed over Amberlite IR-120 resin. The solution was concentrated *in vacuo* and chromatographed. Glucose, arabinose and 2-ketogluconic acid were identified chromatographically. Three other products could not be identified. When the chromatograms were sprayed with *p*-anisidine hydrochloride all of the spots were visible. The first two unidentified spots had a reddish color and R_g values of 1.8 and 2.45, while the third spot was yellow and had an R_g value of 3.9 in developer C. No further attempt was made to identify these compounds which were probably lower sugars.

Another oxidation of cellulose was made with 5 g. of cotton cellulose and 140 ml. of 1 *N* sodium hypochlorite at *p*H 4.5. After the reaction had gone to completion the oxidized cellulose was filtered off, washed, and dried. A 1-g. sample of the oxidized cellulose was hydrolyzed with 10 ml. of 70% sulfuric acid, and then diluted to 1% and refluxed for 1 hour. The sulfate was removed with barium carbonate, and after filtration the cations were removed with Amberlite IR-120 resin. The solution was concentrated *in vacuo* and dried overnight in a vacuum desiccator. The solid was extracted with hot absolute methanol. The methanolic solution was concentrated *in vacuo* to dryness. The solid was dissolved in water and diluted to 3 ml. This solution was found to contain 10 mg. of arabinose and 1 mg. of glucose when analyzed by Pridham's technique.[12] The extraction with methanol probably removed most of the arabinose from the solid.

Two 7 by 24 inch sheets of 3-mm. paper were spotted with 1.5 ml. of the above solution and developed in developer B for 16 hours. After the position of glucose and arabinose had been determined, the arabinose area was cut out and eluted with water. The solution was diluted to 3 ml. A rotation value, $[\alpha]^{23}D$ $-105 \pm 5°$ (*c* 1.7, water) was obtained, showing the D-configuration.

The rotation solution was dried *in vacuo* and put into a potassium bromide pellet for infrared analysis. The spectra obtained were the same as that for a known sample of L-arabinose.

Oxidation of Possible Intermediate Compounds.—The oxidation of 1.6 g. of methanol with 50 ml. of 1 *N* sodium hypochlorite at *p*H 4.5 was performed. After 1 hour, formaldehyde was found in the solution and identified as the dimedone derivative. After 5 hours a test for formaldehyde was no longer obtained. Formic acid was believed to be present at this time. Reduction of the solution with zinc and hydrochloric acid followed by treatment with dimedone gave the dimedone derivative of formaldehyde. After 8 hours no formic acid could be detected by this test. The only possible compound that could be formed from the oxidation of formic acid would be carbon dioxide, and this was identified by passing the gas from the reaction into a barium hydroxide solution and obtaining a barium carbonate precipitate.

D-Glucose was oxidized and fractionated in the same manner as methyl β-D-glucopyranoside. The neutral fraction contained about 50% of the initial glucose and about 1% arabinose. These were determined by Pridham's[12] technique. Traces of 2-ketogluconic acid and chlorate ion were found in the acidic fraction of the oxidation solution.

The oxidation of D-gluconic acid seems to be rather simple. The major product seems to be 2-keto-D-gluconic acid which was obtained as the calcium salt. This salt had a m.p. of 153° dec., known 153° dec. The free acid from this calcium salt had the same chromatographic properties as authentic 2-keto-D-gluconic acid. Very small quantities of arabinose were detected on the chromatograms, but no quantitative measure could be made because of the small sample quantity.

The oxidation of 50 mg. of 2-keto-D-gluconic acid proceeded very slowly, and after two days the excess active chlorine was reduced to chloride by treatment with sodium sulfite or hydrogen peroxide. The oxidation solutions were fractionated in the same manner as the methyl β-D-glucopyranoside oxidation solutions. The neutral and acidic fractions were concentrated and chromatographed. The chromatograms indicated that the acidic fraction contained about 80% of the original 2-keto-D-gluconic acid. A small quantity of 2,5-diketogluconic acid seemed to be the only product. The neutral fraction contained no detectable compounds.

The oxidation of 50 mg. of 5-keto-D-gluconic acid was

(13) "Allen's Commercial Organic Analysis," 5th ed., Vol. I, P. Blakiston's Son and Co., Philadelphia, Pa., 1923, p. 641.

made. The conditions and results were similar to those for 2-ketogluconic acid. The presence of 2,5-diketogluconic acid was again indicated by the presence of a yellow spot on chromatograms developed in developer C. The spot had an R_g value of 0.6 and gave a yellow color with *p*-anisidine hydrochloride. The acid was very unstable and neither the free acid nor the calcium salt could be isolated. Therefore positive identification was impossible. The quantity of this acid in the oxidation solutions was probably very small.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY, AND FROM THE WOOD CHEMISTRY DIVISION, PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

Assignment of Structure to Cellulose 3,6-Dinitrate[1]

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A cellulose dinitrate, degree of substitution (D.S.) 1.72 (N, 10%), was prepared by the action of pyridine–hydroxylamine on the trinitrate (D.S., 2.88; N, 13.8%), and was methylated to a mono-*O*-methylcellulose dinitrate. Almost complete denitration then yielded an *O*-methylcellulose, D.S., 1.12, which on hydrolysis gave 2-*O*-methyl-D-glucose (84%) and 2,3-di-*O*-methyl-D-glucose (11%). Hence the nitrate groups in the original dinitrate were exclusively or almost exclusively in the third and sixth positions of the cellulose.

Although cellulose trinitrate was rapidly degraded to an amorphous powder by solution in dry pyridine at room temperature, the presence of an excess of free hydroxylamine base markedly altered the course of the reaction[2]; almost exactly one molar equivalent of nitrogen gas of 99% purity was evolved, and the near-white, fibrous product had the composition of a dinitrate (D.S. 1.7). Since the product had a degree of polymerization of about 120, not more than 1.7% of the original glucosidic bonds had been cleaved, and the reaction was therefore a denitration rather than a degradation. This dinitrate was remarkable not only in being soluble in an unusually wide range of organic liquids, but also in its relatively great stability toward alkali. It could be methylated with dimethyl sulfate and 30% sodium hydroxide to soluble, brittle white fibers with unchanged nitrate substitution and only a moderate decrease in intrinsic viscosity (Table I). The same stability, however, defeated early attempts to determine the structure of the mono-*O*-methylcellulose dinitrate by denitration to the corresponding methylcellulose. The nitrate groups could not be removed by hydrogenation over a palladium–calcium carbonate catalyst,[2,3] while reduction with iron and acetic acid,[4] or reductive acetylation with zinc and acetic anhydride,[5] yielded highly degraded gums. Very little denitration was achieved by employing a low concentration (3%) of ammonium hydrosulfide in aqueous acetone at less than 20°, as recommended by Bock,[6] Riechel[7,8] and their respective coworkers. An increase in the concentration of the hydrosulfide led to increased decomposition, and to the formation in large amounts of mercaptans and other sulfur compounds (perhaps condensation products of the acetone and ammonium hydrosulfide) which could not be effectively separated from the product. These experiments have been omitted from the present article.

TABLE I

YIELDS, VISCOSITIES AND DEGREES OF SUBSTITUTION IN THE CELLULOSE TRINITRATE, *O*-METHYLGLUCOSE SEQUENCE

Cellulose	Yield,[a] %	NO_3 D.S.	OCH_3 D.S.	$[\eta]$,%[b]
Trinitrate	..	2.88	0	18.7
3,6-Dinitrate	99	1.72	0	1.22[c]
Methyl dinitrate	95	1.70	1.22	0.24
Monomethyl	91	0.14	1.12	0.21[d]
Methylglucoses	89	..	1.16	..
2-*O*-Methyl[e]	84	..	1.0	..
2,3-Di-*O*-methyl[e]	11.3	..	2.0	..
2,6-Di-*O*-methyl[e] (?)	1.7	..	1.97	..

[a] From preceding substance, corrected for change in D.S. [b] Intrinsic viscosities in ethyl acetate uncorrected for variability in nitrate substitution; observed relative viscosities corrected for kinetic energy effect. [c] After renitration, presumably without degradation, to D.S. 2.75. [d] In cuprietylenediamine, according to K. Wilson, *Svensk Papperstidn.*, **55**, 125 (1952). [e] As percentage of methylglucose mixture.

Satisfactory denitrations eventually were achieved in aqueous dioxane by using a large excess of ammonium hydrosulfide in high concentration, and by adding water to the system to keep the product in solution for the most part as denitration proceeded. The reaction was slow and required more than 24 hours, perhaps because it was not catalyzed sufficiently by polysulfide ions.[9] The methylcellulose was isolated as a light yellow, degraded powder which was partially soluble in water and completely so in 4% sodium hydroxide. The intrinsic viscosity (Table I), however, suggested that the additional degradation incurred during the denitration was not severe. No way was found to eliminate a residual nitrogen content of about 1% from the product, and this negative result was in accord with those of other denitra-

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