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The Oxidation of Methyl- β -Glucoside and
Cellulose With an Aqueous Chlorine System

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THE OXIDATION OF METHYL- β -GLUCOSIDE AND CELLULOSE
WITH AN AQUEOUS CHLORINE SYSTEM

A thesis submitted by

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INTRODUCTION

The oxidative degradation of cellulose during bleaching is of great importance to pulp and paper mills, and to textile manufacturers. Much of the work that has been done on this problem has been empirical and the conclusions that were drawn were based upon the indirect evidence from these investigations. There is actually very little evidence as to where and how active chlorine attacks cellulose. This is also true for other carbohydrates in a lesser degree. The present work was undertaken in order to add to the fundamental knowledge of these reactions.

HISTORICAL REVIEW

The bleaching of cellulosic materials with various oxidants has long been practiced. It is well known that the oxidizing agent does not confine its action to the coloring materials present, but that it also attacks the cellulose. This attack on the cellulose manifests itself in the physical properties of the cellulosic material. Chemical examination of the oxidized cellulose also shows that certain chemical changes have occurred within the cellulose. The nature of these changes is dependent upon the oxidant used and the conditions under which the oxidation was conducted. When chlorine has been the oxidant, the pH at which the oxidation was conducted is very important.

The work of Clifford and Fargher (1), Birtwell, Clibbens, and Ridge (2), Davidson (3), Rutherford, Minor, Martin, and Harris (4), and Clibbens and Ridge (5) illustrates the above points. This work gave an over-all picture of the reaction, but it did not tell what the products were or the paths by which the products were formed.

These papers may be summarized as follows:

- A. The oxidized cellulose from acidic oxidations contains more carbonyl groups than carboxyl groups.
- B. The oxidized cellulose from alkaline oxidations contains more carboxyl groups than carbonyl groups.
- C. The rate of reaction is greatest at pH 7.

More recently Kaverzneva (6) has applied new techniques to the

examination of oxidized cellulose and found data from which a better estimate of the oxidation products and their method of formation can be made. Kaverzneva found both aldehydic and ketonic carbonyl groups, and carbonate esters in the various oxidized celluloses which she examined. From these data the following conclusions have been drawn.

1. Aqueous chlorine systems, which are acidic, oxidized the primary hydroxyl group on carbon 6 to an aldehydic carbonyl group, and then to a carboxyl group at a slower rate. There is also a formation of a hydroxyketone at carbons 2 and 3. Further oxidation at this point causes rupture of the pyran ring and yields a carbonic acid ester group with the sugar residue being arabinose.

2. In an alkaline system of aqueous chlorine the oxidation of cellulose begins in the same manner as in the acid system, but then it develops differently. The carbonyl groups at carbon 6 do not accumulate but are rapidly oxidized to carboxyl groups. The hydroxyketone is transformed into the enediol form which is oxidized with the rupture of the pyran ring and the formation of two carboxyl groups.

The oxidation reactions which could occur to cellulose are shown in Figure 1. Meller (7) has recently discussed some of the possible reaction paths. These include the usually postulated attack at carbon 6 to give an aldehydic carbonyl group (X) which could be further oxidized to a carboxyl group (XI). An attack at carbons 2 and 3 might form ketonic carbonyl groups (V), (VI), (VII) or upon cleavage of the carbon to carbon bond might yield a dialdehyde (VIII). The dial-

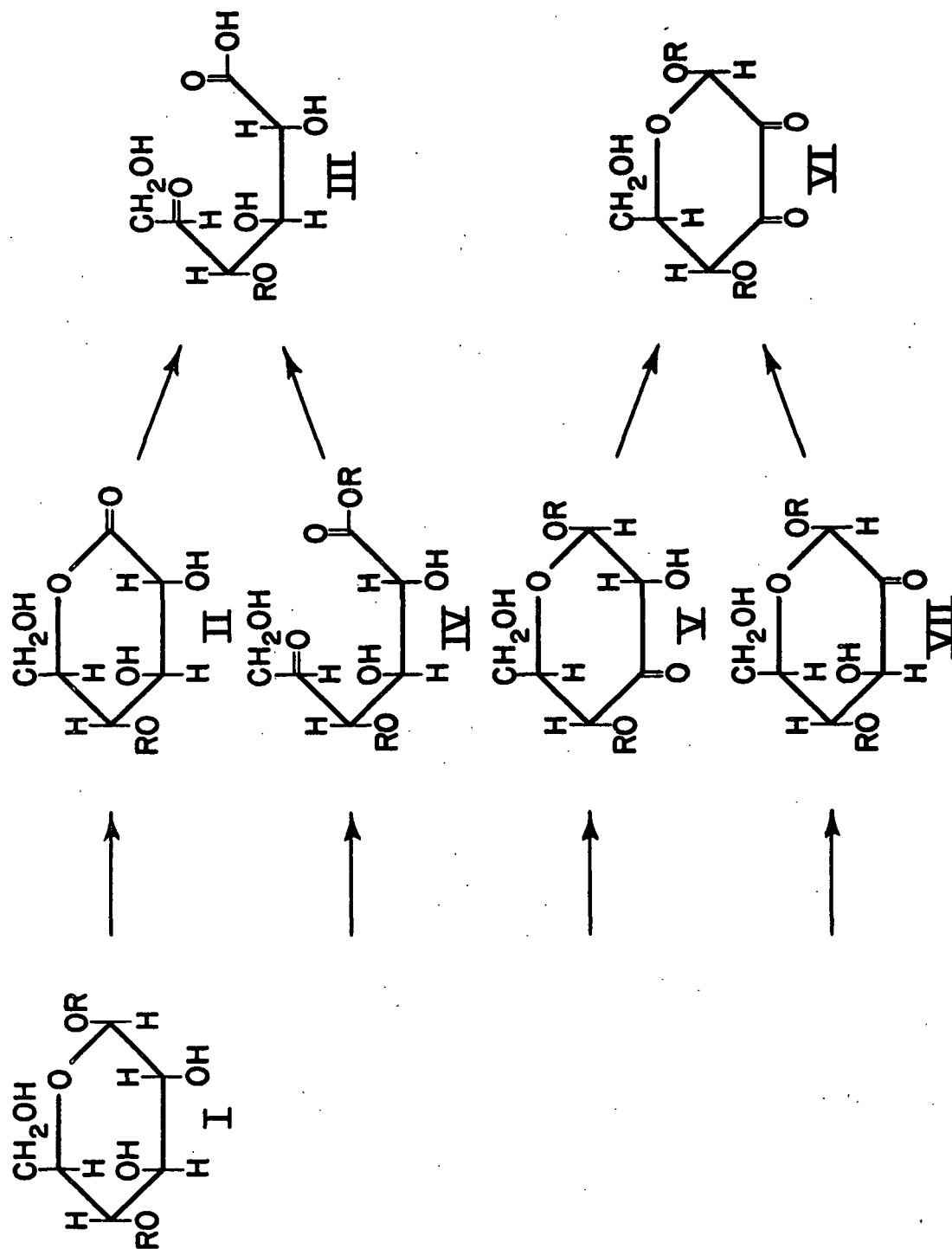


Figure 1. Possible Oxidation Paths and Products of Carbohydrates

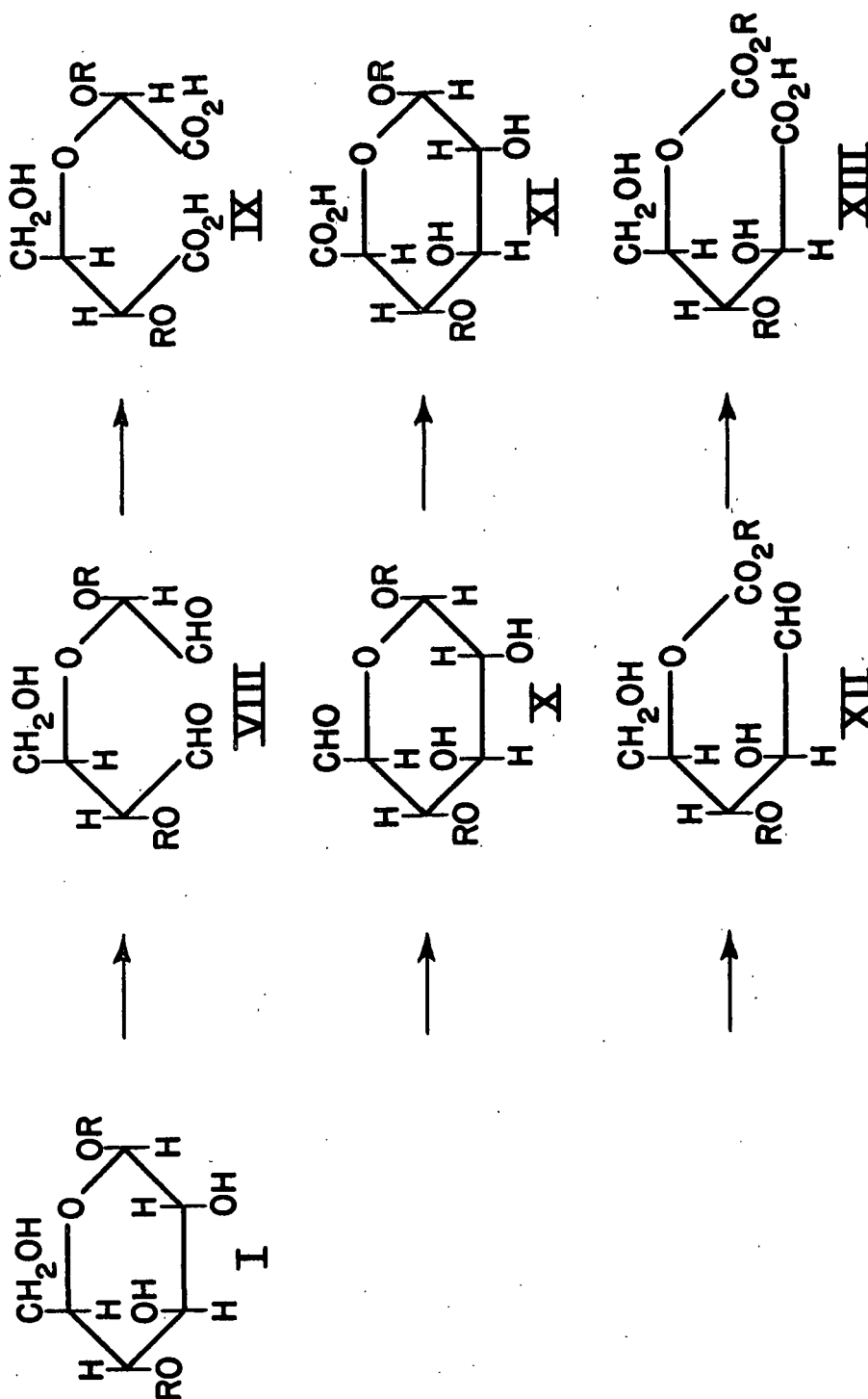


Figure 1. (continued) Possible Oxidation Paths and Products of Carbohydrates

R = H, CH₃, or carbohydrate grouping

dehyde could be oxidized to a diacid (IX). Another postulated reaction involves the cleavage of the bond between carbons 1 and 2, leaving carbon 1 as a carbonate group and carbon 2 as an aldehyde group (XII). The resulting arabinose unit could be further oxidized to arabonic acid (XIII). It must be remembered that many of the postulated reactions could occur simultaneously. Thus, functional group tests on the oxidized cellulose would show the presence of two types of functional groups but these tests would not tell whether the groups were on the same or different glucose units. Functional group tests are often not as specific as they are supposed to be. For these reasons it is impossible to discover the structure of an oxidized cellulose by functional group tests.

Some workers have managed to isolate simple oxidation products from oxidized cellulose. Haskins and Hogsed (8) have oxidized cellulose with peroxide in an alkaline aqueous media and isolated arabonic acid from the oxidation products. Blair and Reeves (9) oxidized a partially hydrolyzed cellulose with hypiodite and isolated gluconic acid from the reaction products. These reactions offer confirmation to some of the proposed oxidation mechanisms discussed previously.

There are problems in hydrolyzing the oxidized cellulose and in separating the oxidation products from the large amount of glucose present. Some of the possible reaction products are sensitive to hydrolysis and might be destroyed. Recently workers in this field have used model compounds which do not present the above problems. The relationship between some of these compounds and cellulose is limited, while in other cases the relationship is very close.

An ideal cellulose model compound would be one which had a glucopyranose ring as the basic unit. This unit would be unsubstituted in the 2, 3, and 6 positions but would have glycosidic groups in the 1 and 4 positions. Except for the unsubstituted 4 position methyl- β -glucoside meets these requirements and it is easy to prepare. Other workers have used compounds as models which bore less relationship to cellulose.

Table I is a summary of oxidations conducted on cellulose and various model compounds. Lindberg, Wood, and Dyfverman (10) used methyl- β -glucoside as a model compound for cellulose. They found that methyl- β -glucoside was oxidized by an aqueous chlorine system at a pH 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 (11) is even more interesting. In addition to gluconic acid and 5-ketogluconic acid, cellobionic acid was produced by the reaction. As the reaction progressed, the cellobionic acid was oxidized to gluconic acid and 5-ketogluconic acid. Small amounts of D-glucaric acid were also found among the oxidation products.

Recently Whistler, Link, and Kazeniac (12) investigated the oxidation of methyl 4-O-methyl- β -glucoside with aqueous chlorine in solutions buffered at pH 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.

* D-Glucaric acid is D-gluco-saccharic acid.

Starch was also oxidized and the oxidation products examined. The results were similar to those obtained with methyl 4-O-methyl- β -glucoside. These results confirm one part of the expected mechanism of the alkaline hypochlorite oxidation of cellulose and its model compounds.

TABLE I

CARBOHYDRATE OXIDATIONS

Carbohydrate	Oxidant	Acidity or Alkalinity	Products	Reference
Cellulose	H ₂ O ₂	OH ⁻	Arabonic acid	(8)
Partially hydrolyzed cellulose	OI ⁻	OH ⁻	Gluconic acid	(9)
Methyl- β -glucoside	Cl ₂	H ⁺	Gluconic acid 5-Ketogluconic acid	(10)
Methyl- β -cellobioside	Cl ₂	H ⁺	Gluconic acid 5-Ketogluconic acid Cellobionic acid D-Glucaric acid	(11)
Methyl 4-O-Methyl- β -glucoside	OCl ⁻	OH ⁻	Glyoxylic acid Erythronic acid	(12)
Gluconic acid	HOCl	H ⁺	2-Ketogluconic acid	(13)

The oxidations of simple carbohydrates with various oxidants have been used and studied by sugar chemist. Although these compounds were not studied as cellulose model compounds, they bear some similarity to cellulose and are of interest.

Shilov and Yasnikov (13) used glycollic aldehyde, glucose, and gluconic acid. These studies were primarily concerned with the kinetics of the reactions and not with the isolation of organic products. It was shown that gluconic acid was oxidized to 2-ketogluconic acid with aqueous chlorine at neutral pH's.

Bromine oxidations in strongly acidic solutions have been used extensively. Glycosides have been oxidized to glycuronides. Strong oxidation of glycosides has been shown to give cleavage of the pyran ring at carbons 2, 3, and 4. The final product was a diacid. D-glucaric acid has also been found among the oxidation products from D-glucose.

Much work has been done in producing aldonic acids from aldoses. These reactions are best done with bromine. The aldonic acid is the major product produced, but small amounts of saccharic and ketoaldonic acids are also produced. The ketoaldonic acid seems to be 5-ketogluconic acid rather than the 2-ketogluconic acid. The original Ruff degradation (14) of aldonic acids to aldoses employed bromine, but peroxide was found to give higher yields, so it became the commonly used reagent. Chlorine water has received very little attention. Glucose has been shown to give gluconic acid and possibly 5-ketogluconic acid when oxidized with aqueous chlorine (15).

In the pH range of 4 to 6, bromine has again received much attention. The rate of reaction is greater and the products similar to those from the low pH oxidations with bromine. The oxidations at the higher pH are useful for oxidizing disaccharides and glycosides since

hydrolysis of the glycosidic bonds does not occur. Methyl- β -D-glucoside has been oxidized with bromine in the presence of calcium carbonate, and methyl glucuronide was produced (29).

Alkaline oxidations are usually more drastic than those in acid media. Under closely controlled conditions hypiodite can be used to determine carbonyl by oxidizing it to carboxyl. Prolonged oxidation of glucose with aqueous alkaline halogens is said to give gluconic acid, 2-ketogluconic acid, and arabonic acid (16). Other workers (17), and (18) have reported finding 5-ketogluconic acid, and only small traces of 2-ketogluconic acid.

From the preceding survey of past work it can be seen that a number of compounds expected from the oxidation of cellulose and simple carbohydrates have been found and in some cases the reaction paths determined. There are other expected products that have not been found and many reaction paths that have not been demonstrated.

PRESENTATION OF THE PROBLEM

Because of the uncertainties which exist as to how and where cellulose is attacked during bleaching with aqueous chlorine systems further studies on model compounds seem to be desirable. The work of Lindberg, Dyfverman, and Wood was done at very low pH's. The present work was initiated with the belief that methyl- β -glucoside would react differently at pH 4.5. Therefore the problem was set forth as follows:

The oxidation of methyl- β -glucoside with aqueous chlorine systems at a pH of 4.5 yields compounds, the more important of which will be identified, the reaction paths established so far as possible and the results related to general carbohydrate chemistry.

SUMMARY AND DISCUSSION OF RESULTS

The major products of the oxidation of methyl- β -glucoside at pH 4.5 were found to be D-glucose, D-arabinose, carbon dioxide, and oxalic acid. Minor products that were found were 2-ketogluconic acid and 2,5-diketogluconic acid which were tentatively identified on chromatograms.

It was shown that the D-glucose, which was found among the oxidation products, was not produced by a direct hydrolysis of the methyl- β -glucoside; therefore, it must have been produced by an oxidative hydrolysis of the glycosidic bond. The term oxidative hydrolysis refers to a reaction which involves simultaneous oxidation and hydrolysis of a glycosidic bond or to a pair of reactions involving an oxidation of either the glycoside or aglycone followed by a hydrolysis of the glycosidic bond.

The D-arabinose found must have been produced by an oxidative reaction. It appears that the D-arabinose was produced by the direct oxidation of methyl- β -glucoside. The only compounds found among the oxidation products which could be intermediates in the formation of D-arabinose were glucose and 2-ketogluconic acid. The oxidation of 2-ketogluconic acid clearly showed that this compound did not yield D-arabinose under the oxidation conditions employed in this work. The oxidation of gluconic acid demonstrated that it probably did not play a role in the formation of D-arabinose. The oxidation of gluconic acid did yield small amounts of D-arabinose, but the major

product was 2-ketogluconic acid. The absence of gluconic acid and the very small amount of 2-ketogluconic acid found among the products from the oxidation of methyl- β -glucoside were inconsistent with the relatively large amount of D-arabinose that was found.

The oxidation of D-glucose yielded small amounts of D-arabinose, but D-glucose was most likely not an intermediate in the formation of D-arabinose from methyl- β -glucoside. The oxidation of one mole of D-glucose with 2.5 moles of active chlorine gave 1% D-arabinose and 50% unreacted D-glucose, in addition to other products. In the oxidations of methyl- β -glucoside the yield of D-arabinose was always about twice that of D-glucose. If D-glucose were the precursor of D-arabinose there probably would have been more D-glucose than D-arabinose, and this was not the case. Thus, it appears that the direct oxidation of methyl- β -glucoside at carbons 1 and 2 yielded D-arabinose. The carbon 1 was probably left in the form of a carbonate ester group.

The presence of 2-ketogluconic acid was indicated by the chromatograms. It was shown that the oxidation of D-glucose yielded some 2-ketogluconic acid, while the oxidation of gluconic acid gave a high yield of 2-ketogluconic acid. Since no gluconic acid was found among the oxidation products and the quantity of 2-ketogluconic acid was very small, it seems most likely that D-glucose was the intermediate in the formation of 2-ketogluconic acid. This does not completely rule out gluconic acid as a possible intermediate. The 2,5-diketogluconic acid which seems to be present probably came from the oxidation of 2-ketogluconic acid.

Figure 2 shows the paths by which these reactions could occur.

The oxalic acid found was too small a fragment to be attributed to any definite source. The carbon dioxide found in the reaction products was probably due in part to the methyl aglycone groups and to the carbonate groups formed from the carbon 1 of the glucose unit. The remainder of the carbon dioxide must have come from further degradation of the carbohydrate material.

The major reactions which have been found in the course of this work occurred on carbons 1 and 2 of the glucopyranose ring. These results were not surprising when one considered other recent work on the oxidation of carbohydrates. It now seems that reactions at carbons 1 and 2 may be more common than had previously been realized. Lindberg *et al.*, (10) and Dyfverman (11) have shown that methyl- β -glucoside was oxidized to gluconic acid and methyl- β -cellobioside to cellobionic and gluconic acids. Perlin (19) has shown that 1 mole of lead tetraacetate or sodium will oxidize 1 mole of glucose to arabinose under acidic conditions, and that 2 moles of oxidant yields erythrose. Huffman, Lewis, Smith, and Spriestersbach (20) have shown that a periodate oxidation of 3-O-methyl-glucose under mild acid conditions gives cleavage between carbons 1 and 2 yielding 2-O-methyl-arabinose. The well known Ruff (14) degradation is an older example in which oxidation occurs at carbons 1 and 2. This reaction converts aldonic acids to sugars of one less carbon atom. Thus, the results obtained in this work seem quite in line with the work of other modern investigators.

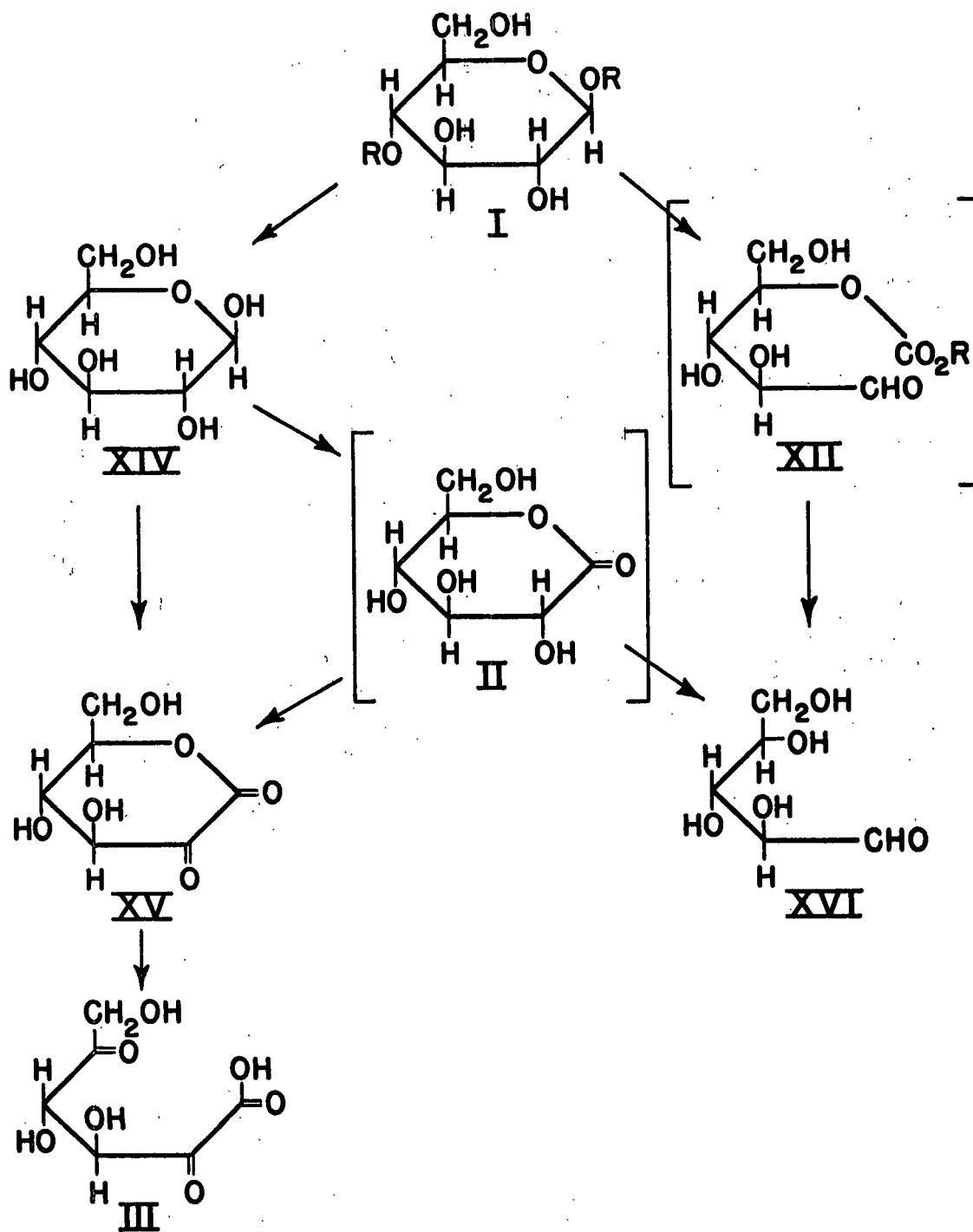


Figure 2. The Probable Paths of Oxidation of Methyl- β -glucoside in an Aqueous Chlorine System at pH 4.5

Cotton cellulose was oxidized in the same manner as methyl- β -glucoside. The spent oxidation solution contained D-glucose and D-arabinose. The hydrolyzate of the oxidized cellulose contained D-glucose, D-arabinose, and 2-ketogluconic acid; thus, the oxidation of cellulose seems to be quite similar to that of methyl- β -glucoside. The hydrolyzate of the cotton cellulose which was used as a starting material contained no detectable D-arabinose. Therefore, the D-arabinose in the oxidized cellulose and the oxidation solution was produced by the oxidation reaction. The presence of D-arabinose also helped to explain certain anomalies which have been observed when using functional group analyses. Dorée (21) presented furfural and uronic acid determinations on oxidized cellulose samples. Although uronic acids give less than the theoretical amount of furfural, they do give theoretical quantities of carbon dioxide. Therefore, the quantity of carbon dioxide liberated by cellulose under the same conditions has been considered to be a measure of the uronic acid content. In the examples of Dorée there was always an excess of furfural which could not be explained. It now seems that this may be due to D-arabinose units which were present in the oxidized cellulose samples.

The conditions under which the reaction was conducted did not give a measurable quantity of glucose by hydrolysis of cellulose in the period of time used for the oxidation. Therefore, the D-glucose in the spent oxidation solution must have been produced by an oxidative hydrolysis reaction.

In addition to the products identified there were three other

products from the hydrolyzed oxidized cellulose that were not identified. These compounds were found on the chromatograms, but they had R_g^* values which did not correspond to any of the known samples used in this work.

The results of the oxidation of cellulose are only of a preliminary nature and further work is to be carried out by another student. Some conclusions can be drawn from the work at this time. The preceding results show that cellulose suffers an oxidative hydrolysis of the glycosidic bonds when oxidized with aqueous chlorine at pH 4.5. There is also an oxidative cleavage of the carbon bond between carbons 1 and 2 of the glucopyranose unit. The carbon 2 is left as a carbonyl group, and this becomes the carbon 1 of the arabinose unit. The original carbon 1 of the glucopyranose unit is left as a carbonate ester, which is easily hydrolyzed off the arabinose unit.

The possible oxidation reactions of cellulose have been discussed previously. Both Kaverzneva (6) and Meller (7) have suggested that the bond between carbons 1 and 2 of the glucopyranose unit could be cleaved during oxidation. Kaverzneva has some evidence indicating the presence of a carbonate grouping in oxidized cellulose. The isolation of D-arabinose in the present work gives full confirmation to the postulate of Kaverzneva that a cleavage of the carbon 1 to 2 bond could occur. Such a cleavage is the only way that D-arabinose could be formed from methyl- β -glucoside or cellulose.

* R_g is the R value with respect to glucose

EXPERIMENTAL

STARTING COMPOUNDS

Methyl- β -glucoside was prepared by the method of Raymond and Schroeder (22). The melting point of the product was 105°C. and the rotation was -34.5°. When this compound was chromatographed, several spots were found. One of these was D-glucose and the other two were unidentified. The quantity of glucose was approximately 1%. One of the unidentified spots had a blue-white fluorescence. The methyl- β -glucoside 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 -glucoside crystallized. The process was then repeated. The final product was obtained in 20% yield. The methyl- β -glucoside contained 0.1% glucose, and the other impurities could no longer be detected. The melting point was 106°C. and the rotation was -34.6°.

The cellulose used in the present work was obtained in the form of handpicked cotton through the courtesy of Dr. H. D. Barker of the Field Crops Research Branch, U.S. Department of Agriculture.

The cotton fibers were removed from the seeds by hand and stored in screw cap bottles before purification. Three grams of cotton were refluxed in 180 ml. of 1% potassium hydroxide for one hour. The cellulose was then filtered off and washed with hot alcohol and then with 1 liter of water. The cellulose was air dried for two days before being stored in a screw cap bottle.

The glucose used in the course of this work was a reagent grade sample obtained from the Baker Chemical Company.

The gluconic acid used was obtained from the Pfanstiehl Chemical Company in the form of the impure lactone. This compound was purified by recrystallization from 80% ethanol.

The ketoaldonic acids, 2-ketogluconic acid and 5-ketogluconic acid, were obtained from Dr. Wolff of the Northern Regional Research Laboratories. All of these samples were found to be chromatographically pure. A sample of 5-ketogluconic acid from the Chas. Pfizer Company contained some 2-ketogluconic acid.

ANALYTICAL METHODS

The amount and nature of the chlorine compounds were determined in all aqueous hypochlorite solutions used and in many of the solutions oxidized. The methods used were those of White (23). The total potential acidity of the oxidation solutions was determined by passing through an Amberlite IR-120* column and then titrating with N/10 sodium hydroxide to a phenolphthalein end point.

The sodium hypiodite method (24) was employed to determine the aldehydic carbonyl present in the oxidation solutions. Due to the small amount of sample available for this determination, the quantities of reagents were reduced to a tenth of the prescribed amount. The total carbonyl was determined by the sodium borohydride technique (25). Again, only a small amount of sample was available for analysis, and the results suffered from this.

* Amberlite resins are produced by Rohm and Haas Co.

The technique of Pridham (26) was used to determine glucose and arabinose quantitatively. This is a method in which a known volume of the unknown solution is spotted on a chromatogram. After the chromatogram was developed, it was sprayed with p-anisidine hydrochloride and heated in an oven for ten minutes to develop the spots. The spots were cut out of the chromatogram and placed in test tubes. The colored material was eluted from the paper with a methanolic solution of stannous chloride. The absorbancy of the solution was measured in a colorimeter and the value found compared to that of known amounts of the same compound from the same chromatogram. The method is good only in the range from 5 to 50 μ g. of sugar per spot.

FRACTIONATION PROCEDURES

The initial oxidations were treated with silver carbonate as described by Lindberg et al., (10) 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. The chloride ion was difficult to remove completely by this method, and the sample had to be handled several times.

The use of ion-exchange resins seemed attractive and the field was investigated. IR-4B, IR-45, IR-400, and IR-410 were used in their carbonate, bicarbonate, acetate, and free acid forms. IR-45 in the acetate form was found to be very good for removing the acidic materials while allowing the neutral compounds to pass through the column.

The neutral compounds were passed over IR-120 to remove any cations present. Finally the neutral solution was concentrated to a known volume.

The acidic compounds were eluted from the anion exchange column as salts by washing the resin 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 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. 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

A large number of chromatographic developers was investigated in the course of this program. The behavior of known compounds and of mixtures was studied. These have served to identify the products from the oxidation of methyl- β -glucoside and the related compounds that were used in the course of this study. The following ones 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

Many spray reagents were used to detect compounds on the chro-

matograms, but some sprays were of greater utility than others. The major sprays used were:

- A. Ammoniacal silver nitrate (30)
- B. p-Anisidine hydrochloride (26)
- C. Methyl orange (0.1% in water)
- D. Periodate-permanganate (31)

Table II is a compilation of the R_g values obtained for various known compounds in developers A, B, and C.

EXPERIMENTAL PROCEDURES AND RESULTS

The oxidations were all performed with an aqueous chlorine system. The oxidizing agent was prepared by passing chlorine gas into a normal solution of sodium hydroxide until the pH 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 -glucoside or other carbohydrate. Acetic acid was used to reduce the pH to the desired level. In the initial oxidations the pH was maintained at the desired level by adding sodium hydroxide as necessary. In the final work which was conducted at a pH of 4.5 a sodium acetate-acetic acid buffer system was employed.

THE OXIDATION OF METHYL- β -GLUCOSIDE

Table III is a summary of the oxidation conditions used for various oxidations.

The physical conditions under which the oxidations were conducted

TABLE II

R_g VALUES OF KNOWN COMPOUNDS

	Developers		
	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 (2.7) (3.7)	1.6 (2.3) (3.0)	0.76 (1.0) (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 3.1	0.96
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. 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

TABLE III

OXIDATION CONDITIONS

No.	Methyl- β - glucoside, mmol.	NaOCl, meq.	pH	Volume, ml.	Buffer used
1	0.125	0.10	7.0	10	no buffer
2	0.025	0.005	4.5	25	
3	0.025	0.001	4.5	25	
4	0.025	0.005	7.0	25	
5	0.025	0.001	7.0	25	
6	0.025	0.005	9.0	25	
7	0.025	0.001	9.0	25	pH maintained by NaOH addition
8	10	1.0	7.0	100	
9	10	1.0	4.5	20	
10	10	20	4.5	25	NaHCO ₃
11	0.025	10	6.5	25	
12	10	9.7	7.0	75	pH maintained by NaOH addition
13	10	9.7	9.0	75	
14	10	9.7	4.5	75	
15	2.5	25	4.5	350	NaOAc-HOAc
16	1.0	15	4.5	65	
17	45	45	4.5	1000	
18	10	25	4.5	65	
19	5	25	4.5	100	
20	2	10	4.5	50	
21	5	25	4.5	100	
22	5	19	4.5	100	
23	1	4	4.5	25	
24	0	4	4.5	25	

varied widely. Some of the oxidations were made in open beakers whereas others were conducted in closed systems. The conditions under which the oxidations were conducted did not seem to have any effect upon the path of the reaction. A brown glass-stoppered bottle was used as a container for the largest oxidation made. Gas formed during the course of the reaction caused the stopper to bump occasionally.

The oxidation solutions were examined for their inorganic constituents and a material balance was made over the system. Oxidation 22, Table III contained 9.2 mmol. of OCl^- , total chlorine content 18.4 mmol. initially. After the reaction had been allowed to go to completion, 18.3 mmol. of Cl^- , were present and 0.06 mmol. of ClO_3^- , total 18.36 mmol. of chlorine.

After being passed over an IR-120 column the potential acidity of the oxidation solution was calculated to be 68.2 mmol. on the basis of chlorine and acetate put into the reaction. The acidity determined experimentally was 67.6 mmol. A blank oxidation was made under the same conditions and the initial chlorine content was 3.86 mmol. and the acidity 13.82 mmol. The final chloride content was 3.78 mmol. and the acidity 13.92 mmol. 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 organic acids from methyl- β -glucoside. An attempt to hydrolyze methyl- β -glucoside was made using the sodium acetate-acetic acid buffer system, under the con-

ditions employed in these reactions, but no detectable hydrolysis of the methyl- β -glucoside occurred in 14 days.

In some of the final oxidations the oxidation flask was connected to a gas buret and the volume of gas was measured. This gas was later passed into 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. The amount of active chlorine lost as a gas was found to be negligible.

The oxidation solutions were fractionated by the procedures described previously. The fractions were then chromatographed on Whatman no. 1 paper using the various developers which have been described.

The neutral fraction of the oxidation solution was found to contain glucose, arabinose, and unreacted methyl- β -glucoside. The quantity of glucose and arabinose was determined by Pridham's technique (26). In one case the yield of glucose was 1.5% and that of arabinose 3.5%. Approximately the same values were found for all the other oxidations. Two seven by twenty-four inch sheets of 3-mm. paper were spotted with 5 ml. of the neutral fraction of the oxidation solution. The solution was calculated to contain 1.67 mg. of arabinose and 0.72 mg. of glucose. 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 micro-rotation values were determined. The amount of sugar as given above 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 $+56^{\circ} \pm 5$ (c 0.2, water) and that of arabinose $-106^{\circ} \pm 5$ (c 0.6, water). Hence, both sugars have the D- configuration, and the D-arabinose must have been formed from D-glucose.

In order to prove the identity of these compounds, derivatives were prepared from the fractions which were eluted from the chromatograms. The D-glucose was identified as the phenylosazone, with a mp. of 208°C. , known 210°C. Further proof was sought by attempting to prepare the osotriazole, but this reaction could not be conducted on such a small scale. A sample of the 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 known D-glucose correspond exactly. Figure 3 contains the infrared spectra curves of both glucose and arabinose. On the basis of this evidence it has been concluded that D-glucose was formed during the oxidation reaction.

The D-arabinose has been identified as the phenylhydrazone with a m.p., of 158°C. , known 160°C. The arabinose diphenylhydrazone was prepared from the chromatographically purified D-arabinose and from the neutral fraction of the oxidation solution which contains D-glucose and methyl- β -glucoside as well as D-arabinose. The white needles had a m.p., of 196.5°C. , known 197°C.

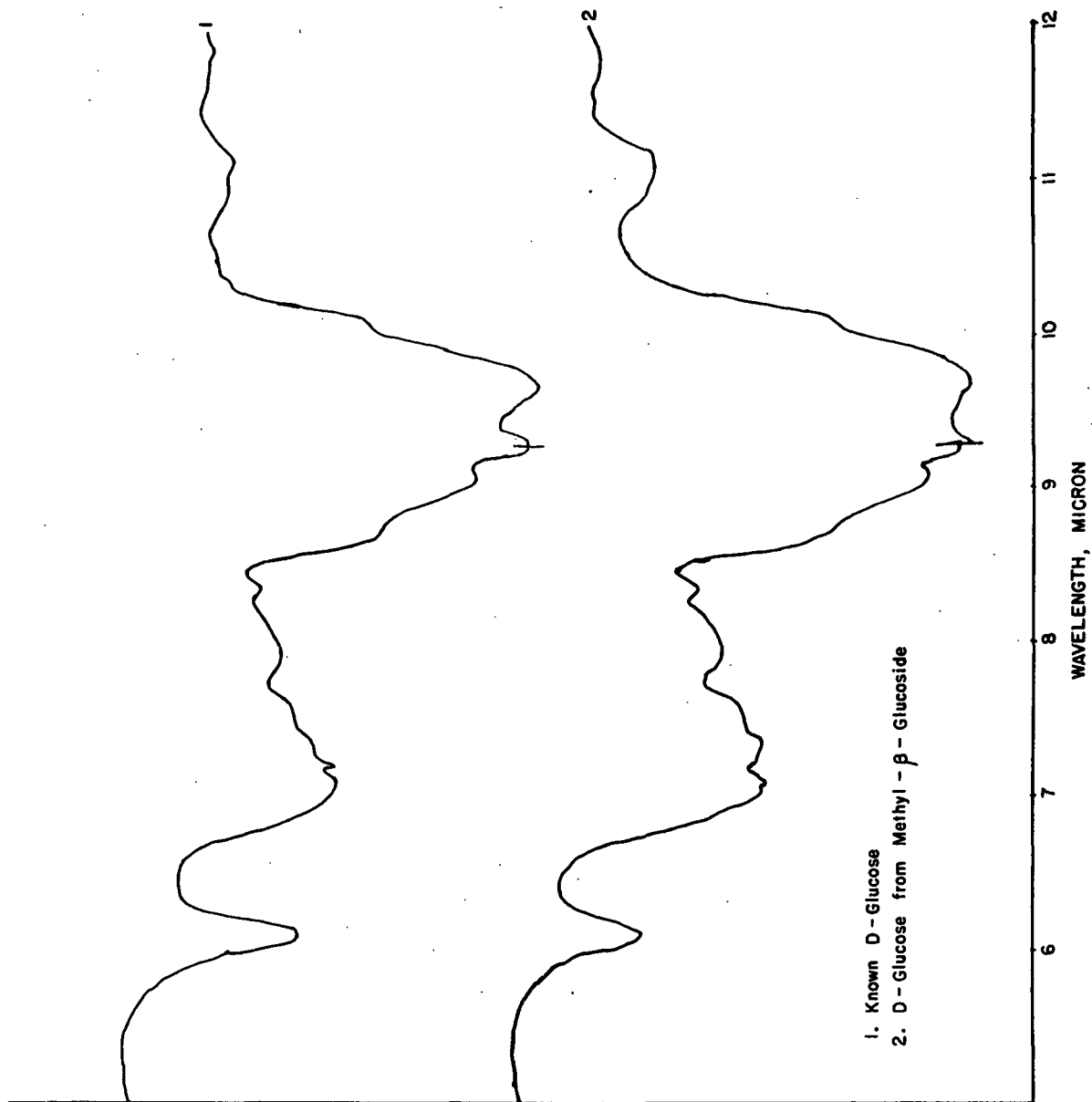


Figure 3. The Infrared Spectra Curves of Glucose and Arabinose

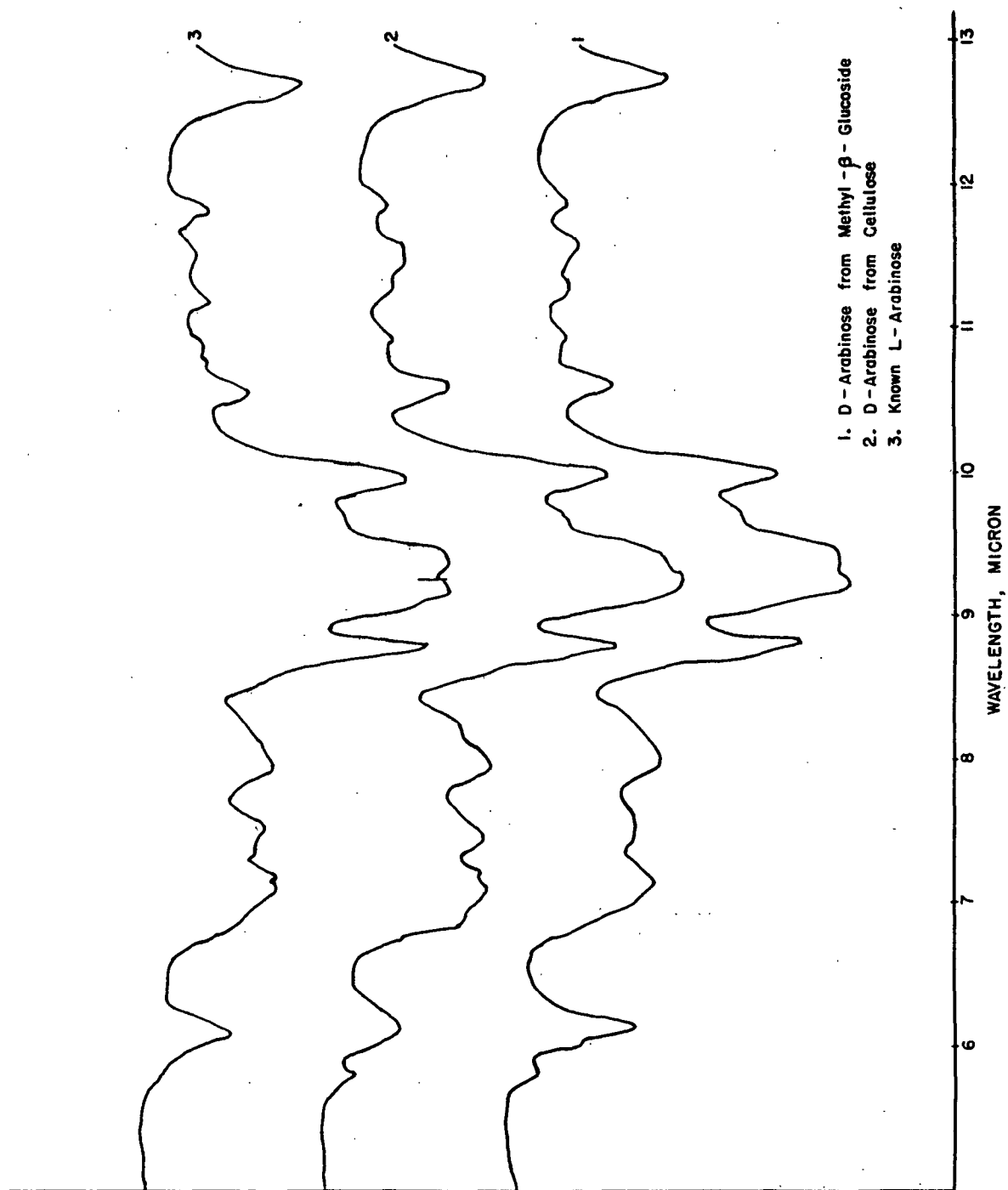


Figure 3. (continued) The Infrared Spectra Curves of Glucose and Arabinose

Part of the methyl- β -glucoside in the neutral fraction of the oxidation solution has been recovered by recrystallization. The yield was 30% and the final product had a melting point of 106°C. A quantitative measurement of the residual methyl- β -glucoside has been made by hydrolyzing it to D-glucose and determining the increase in D-glucose concentration, and then converting this figure to an equivalent amount of methyl- β -glucoside. The hydrolysis of 0.05 g. of methyl- β -glucoside 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 IR-120, and concentrated to an appropriate volume. The validity of this modification of Pridham's technique has been tested by analyzing purified methyl- β -glucoside and obtaining values of more than 99%.

The D-glucose, D-arabinose, and methyl- β -glucoside in the neutral fraction of the oxidation solution have the same chromatographic properties as do authentic samples.

The chromatograms have shown a faint spot that may be erythrose. The reference erythrose sample was so impure that it was of little or no value, and the quantity of material on the chromatograms was so small that it could not be worked with.

The acidic fraction of the oxidation solution seemed to contain very little material, but when this fraction was concentrated to a thick sirup in a rotating vacuum concentrator at 30°C., 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 solution with calcium carbonate the sirup gave a white precipitate. The precipitate was then filtered off and washed. The solid gave a yellow solution when treated with sulfuric acid and resorcinol; the color changed to blue upon standing. After neutralization with ammonium hydroxide the solution had a faint pink color. Another sample of the solid was treated with sulfuric acid and pyrogallol, and a dirty green color resulted, which then 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 (27). 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 IR-120 to remove the calcium ions, concentrated, and chromatographed. There was a spot which corresponds 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-diketogluconic acid was available. The only previous report of this compound has been by Katznelson,

Tanenbaum, and Tatum (28). The R_g value reported for this compound in developer C was 0.59. The values observed in this work have ranged from 0.57 to 0.61. The pale yellow color which resulted when the chromatograms were sprayed with p-anisidine hydrochloride was characteristic.

The yields of D-glucose, D-arabinose, residual methyl- β -glucoside, carbon dioxide, and calcium oxalate have been tabulated in Table IV.

TABLE IV

THE OXIDATION OF METHYL- β -GLUCOSIDE

No.	Methyl- β -glucoside,		Oxidation Products				
	initial	residual ¹	Glucose, g.	Arabi- nose, g.	CO ₂ , g.	CaC ₂ O ₄ · H ₂ O, g.	Yield, %
15	5.0	2.5	-----	-----	-----	-----	50.0
16	0.2	-----	-----	-----	-----	-----	-----
17	8.713	5.0	0.104	0.208	-----	-----	85.8
18	2.0	1.6	0.024	0.071	-----	-----	85.9
19	1.0	0.73	0.004	0.008	0.202	-----	91.7
20	0.35	0.25	0.004	0.010	0.065	-----	89.1
21	0.97	-----	-----	-----	-----	-----	-----
22	0.94	0.666	0.014	0.033	-----	-----	77.0
23	0.23	0.149	0.003	0.008	0.045	0.025	87.5

¹ The values for methyl- β -glucoside in No. 15 and 17 were determined by recrystallizing the unreacted material and weighing. The values in No. 18 through 23 were determined by the modification of Pridham's technique (26) which was described in the text.

² The yields are based upon the carbon content of the products and recovered starting material.

OXIDATION OF CELLULOSE

The oxidation of 0.75 g. of cotton cellulose was performed with

50 ml. of 1N sodium hypochlorite in a system buffered at pH 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 a vacuum desiccator.

The filtrate was deionized in the manner used for methyl- β -glucoside oxidation solutions, and the neutral fraction chromatographed. The neutral fraction was 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 IR-120. The solution was concentrated in vacuo and chromatographed. Glucose, arabinose, and 2-ketogluconic acid were identified chromatographically, but there were three other products which 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 yellow spot had an R_g value of 3.9 in developer C. No further attempt was made to identify these compounds which are probably lower sugars.

Another oxidation of cellulose was made with 5 g. of cellulose and

140 ml. of 1N sodium hypochlorite at pH 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 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 to dryness in vacuo. 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 examined by Pridham's technique (26). The extraction with methanol may not have removed all of the arabinose from the solid but it probably removed the bulk of it.

Two seven by twenty-four 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 the glucose and arabinose bands had been determined, the arabinose area was cut out and eluted with water. The solution was diluted to 3 ml. and the rotation determined. A rotation value, $[\alpha]_D^{23} = -105^{\circ} \pm 5$ (c 1.7, water) was obtained.

The rotation solution was dried and put into a potassium bromide pellet for infrared analysis. The spectra was the same as that of l-arabinose. This spectra curve is shown in Figure 3.

OXIDATION OF POSSIBLE INTERMEDIATE COMPOUNDS

The oxidation of 1.6 g. of methanol with 50 ml. of 1N sodium

hypochlorite at pH 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 is believed to have been 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 more formic acid could be detected. The only possible compound which 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 -glucoside. The neutral fraction contained 50% of the initial glucose and about 1% arabinose. These were detected by Pridham's technique (26). Traces of 2-ketogluconic acid were found in the acidic portion of the oxidation solution. Chlorate ion was also found in this acidic fraction.

The oxidation of gluconic acid seems to be rather simple. The major product seems to be 2-ketogluconic acid which was obtained as the calcium salt. This salt had a m.p. of 153°C. (decompn.), known 153°C. (decompn.). The free acid from the calcium salt had the same chromatographic properties as authentic 2-ketogluconic 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-ketogluconic 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 -glucoside oxidations. The neutral and acidic fractions were concentrated and chromatographed. The chromatograms indicated that the acidic fraction contained about 80% of the original 2-ketogluconic 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-ketogluconic acid was 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 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.

LITERATURE CITED

1. Clifford, P. H., and Fargher, R. G., J. Textile Inst. 13:T189(1922).
2. Birtwell, C., Clibbens, D. A., and Ridge, B. P., J. Textile Inst. 16:T13(1935).
3. Davidson, G. F., J. Textile Inst. 32:T132(1941).
4. Rutherford, H. A., Minor, F. W., Martin, A. R., and Harris, M., J. Research Natl. Bur Standards 29:131(1942).
5. Clibbens, D. A., and Ridge, B. P., J. Textile Inst. 18:T135(1927).
6. Kaverzneva, E. D. Commun., 13th Intern. Congr. Pure and Appl. Chem., Stockholm, 1953:328-50.
7. Meller, A., Revs. Pure and Appl. Chem. (Australia), 6, no. 1:40 (1956).
8. Haskins, J. F., and Hogsed, M. J., J. Org. Chem. 15, no. 6: 1264-74(Nov., 1950).
9. Blair, M. G., and Reeves, R. E., J. Am. Chem. Soc. 74:2622(1952).
10. Lindberg, B., Dyfverman, A., and Wood, D., Acta. Chem. Scand. 5:253(1951).
11. Dyfverman, A., Acta. Chem. Scand. 7:280-4(1953).
12. Whistler, R. L., Linke, E. G., and Kazeniac, S., J. Am. Chem. Soc. 78:4704-9(1956).
13. Shilov, E. A., and Yasnikov, A. A., Ukrain. Khim. Zhur. 18:595, 611(1952).
14. Ruff, O., Ber. 31:1573(1898).
15. Hlasiwetz, H., and Habermann, J., Ann. 155:120(1870).
16. Honig, N., and Tempus, F., Ber. 57:787(1924).
17. Reichstein, T., and Neracher, O., Helv. Chim. Acta. 18:892(1935).
18. Ruzicka, W., Z. Zuckerind. Českoslovak. Rep. 64:219(1941); C.A. 38:2014.
19. Perlin, A. S., J. Am. Chem. Soc. 76:2595(1954).
20. Huffman, G. W., Lewis, B. A., Smith, F., Spriestersbach, D. R., J. Am. Chem. Soc. 77:4346(1955).

21. Dorée, C. The methods of cellulose chemistry. p. 118. London, Chapman and Hall Ltd., 1947.
22. Raymond, A. L., and Schroeder, E. E., J. Am. Chem. Soc. 70:2789(1948).
23. White, J. F., Am. Dyestuff Repr. 31:485(1942).
24. Smith, W. T., and Shriner, R. L. The examination of new organic compounds. p. 85-88. New York, John Wiley and Sons, Inc., 1956.
25. Strole, U., Makromol. Chem. 20, no. 1:19(1956).
26. Pridham, J., Anal. Chem. 28, no. 12:1803(1956).
27. Allen's Commerical Organic Analysis. Fifth ed. Vol. I. p. 641. Philadelphia, P. Blakiston's Son and Company, 1923.
28. Katznelson, H., Tanenbaum, S. W., and Tatum, E. L., J. Biol. Chem. 2-4:43(1953).
29. Jackson, E. L., and Hudson, C. S., J. Am. Chem. Soc. 59:994(1927).