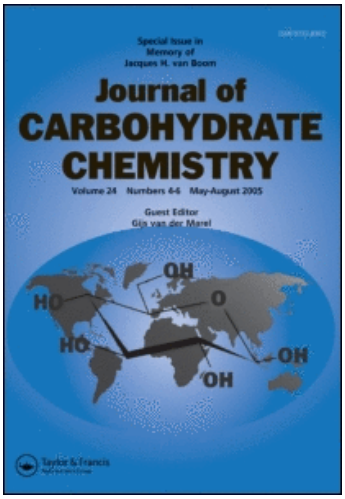

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Oxidation of Monosaccharides with Oxygen in Alkaline Solution. Separation, Identification and Estimation of the Aldonic Acids Produced by Liquid Chromatography[1,2]

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OXIDATION OF MONOSACCHARIDES WITH OXYGEN IN ALKALINE SOLUTION. SEPARATION, IDENTIFICATION AND ESTIMATION OF THE ALDONIC ACIDS PRODUCED BY LIQUID CHROMATOGRAPHY[1,2]

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ABSTRACT

A reliable liquid chromatographic method has been developed for the separation, identification and estimation of the products formed when saccharides are oxidized in alkaline solution with oxygen. The technique was then used to quantitate the acids produced when glucose is subjected to such an oxidation. The Bio-Rad cation exchange column HPX-87H$^+$, when eluted with 0.01 N H_2SO_4 and attached to a UV detector, was found to be satisfactory. Quantitative estimation of the products formed during the oxidation of glucose was achieved by withdrawing aliquots from the reaction mixture and injecting them directly into the chromatographic system. It was found that about 90% of the oxidation products formed were produced *via* a 1,2-enediol and less than 10% *via* a 2,3-enediol. The results confirmed the mechanism proposed by Isbell to account for the acids produced.

INTRODUCTION

In the ground state oxygen exists as a diradical[3] (·O-O·), whose reactivity is greatly enhanced by the presence of OH^- ions, or by catalysts such as Pd or Pt. In alkaline solution, oxygen degrades aldoses to aldonic acids having one carbon less than the starting

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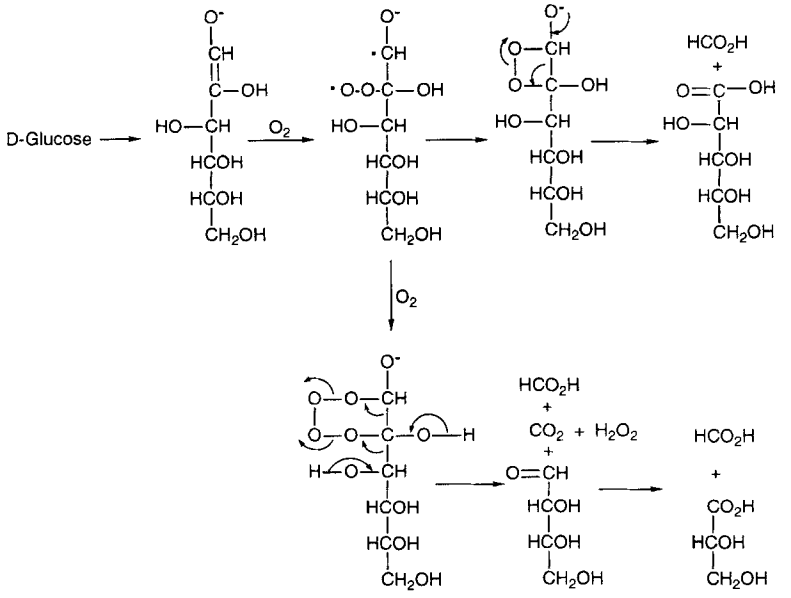
sugar.[4-7] Thus, D-glucose, when oxidized with oxygen (or air) in dilute KOH solution affords potassium D-arabinonate and potassium formate in relatively high yields. Careful examination of the products formed, shows that other acids such as D-erythronic, D-glyceric, and D-glycolic acids, as well as oxalic and carbonic acids are formed in minor proportions. Isbell[8] found that the reaction is initiated by the alkali, which converts an aldose to a 1,2-enediol. In the absence of oxygen this intermediate undergoes further rearrangement, by double bond migration, isomerization, degradation and polymerization to afford a dark colored reaction mixture.[9,10] In the presence of oxygen, such coloration does not occur, because the enediol quickly reacts with oxygen to afford a diradical having two unpaired electrons, whose spins are parallel (which prevents the two electrons from forming a bond). Addition of another oxygen diradical often occurs when high pressures are used, affording six membered tetroxane rings (scheme 1). Otherwise, spin reversal occurs and a bond is formed between oxygen and carbon, closing the dioxetane ring. In both cases a degradation of the saccharide chain follows to yield formic acid and the lower aldonic acid.[8]



Scheme 1

An estimation of the various acids produced during the oxidation of monosaccharides can be used to shed light on the mechanism of the reactions involved in their formation, and on the ratio of the different enediol intermediates that afforded them.

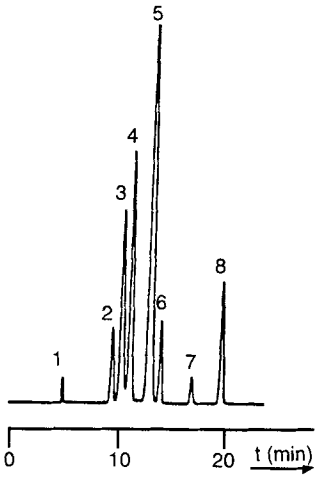


Figure 1. Chromatographic separation of oxalic acid, 1; D-glucose, 2; K^+ arabinonate, 3; Ca^{2+} erythronate, 4; D-arabinonolactone, 5; Ca^{2+} glycerate, 6; Na^+ glycolate, 7; K^+ formate, 8, on an Aminex cation exchange HPX-87 H^+ column at 20 °C and a flow rate of 0.6 mL/min.

This is because glucose yields arabinonic acid from a 1,2-enediol and erythronic acid from both 1,2- and 2,3-enediols, although mainly from the latter. The aim of the present work was to develop a reliable chromatographic method capable of estimating most of the oxidation products formed when monosaccharides are treated with oxygen in alkaline media, and to use the technique to follow the formation of the acids produced from glucose in order to understand better the mechanism of the reaction.

RESULTS AND DISCUSSION

The acids formed when sugars are oxidized with diradical oxygen in alkaline solution have been estimated gravimeterically by wet chemical methods.[11,12] These include precipitation of formic and oxalic acid as salts, and the higher acids as derivatives. It is evident that such gravimetric methods are time consuming and have a higher margin of error because of the extensive handling required. It was therefore desirable to develop a fast and accurate method that would quantitate the products in one measurement.

High performance liquid chromatography (HPLC) was chosen as the method of analysis because of its proven ability to separate and estimate complex mixtures of saccharides, and sugar acids.[13,14] It was possible to detect and estimate most of the minor constituents produced when glucose was oxidized with oxygen in base (see Fig. 1). The

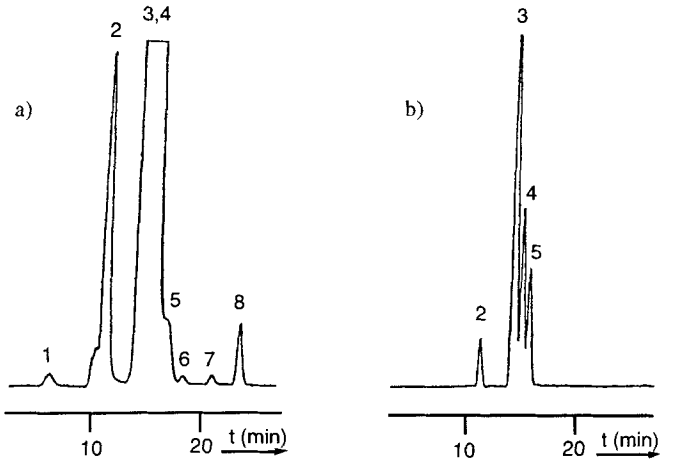


Figure 2. Resolution of the oxidation products of glucose after two hours on an Aminex cation exchange HPX-87 H^+ column at 20 °C and a flow rate of 0.4 mL/min, a) before dilution and b) after dilution. Peaks: 1, K^+ oxalate; 2, D-glucose; 3, K^+ arabinonate; 4, K^+ erythronate; 5, D-arabinonolactone; 6, K^+ glycerate; 7, K^+ glycolate; 8, K^+ formate.

analysis could be repeated at short time-intervals. The components of the mixture were separated at 20 °C and a flow rate of 0.6 mL/min with 0.01 N H_2SO_4 as the mobile phase. Unfortunately, decreasing the flow rate resulted in low resolution because of lactonization, so the acids present in the original sample appeared as two peaks, one for the acid and one for its lactone. For example, at a flow rate of less than 0.5 mL/min, potassium arabinonate appears as two bands, one with a retention time of 14.75 min for arabinonic acid, and the other with a retention time of 15.88 min for arabinonic acid lactone (see Fig. 2).

The chromatograms were recorded at high settings of detector sensitivity. This was necessary to detect the small amounts of glyceric, oxalic and glycolic acids produced at the beginning of the oxidation, but it prevented the resolution of glucose, arabinonic and erythronic acids. To overcome this difficulty, these solutions were diluted and reinjected (see Fig. 2). After twelve hours, the concentration of arabinonic acid increased more rapidly than that of erythronic acid, causing a poor resolution of these two acids (the peak of arabinonic acid was followed by a shoulder of erythronic acid). Another difficulty emerged towards the end of the reaction, when the amount of glucose remaining in the solution decreased and could not be accurately estimated. Note that if the flow rate was decreased, lactonization of arabinonic acid occurred (see Fig. 2). It should be mentioned that the amount of carbonic acid could not be determined because the addition of 0.01 N H_2SO_4 resulted in the evolution of carbon dioxide.

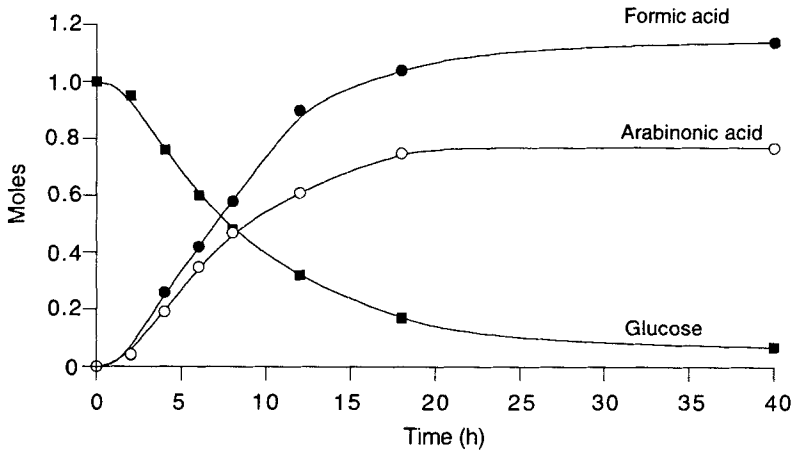


Figure 3. Comparison between the rates of consumption of glucose and formation of arabinonic and formic acids.

Figure 3 shows the consumption of glucose and the formation of arabinonic and formic acids. Moles of products formed per mole of glucose are given in Table 1. It can be seen that 93% of the glucose was oxidized after 24 hours, and that less than one mole of arabinonic acid and more than one mole of formic acid were produced. The production of less than one mole of arabinonic acid may be attributed to incomplete oxidation and to the conversion of some glucose into other sugar acids, saccharinic acids, and products of the Nef reactions.[4] From the graphs it can be seen that the consumption of glucose and the formation of arabinonic acid increase rapidly and then level off. The rate of consumption of glucose exceeds the rate of formation of arabinonic acid, which would indicate that glucose was producing other acids. The amount of formic acid exceeded the amount of arabinonic acid (0.9 mol vs. 0.6 mol after 12 h), which suggests that formic acid is produced by several pathways including ones that do not afford arabinonic acid. For example, Scheme 1 shows how two moles of formic acid are produced when glyceric acid is formed from a 1,2-enediol. However, the amount of glyceric acid formed (< 0.1 mol) cannot account for all the excess formic acid observed. Another reaction that could account for the excess formic acid is the stepwise degradative oxidation of carbohydrates to formic acid, which occurs in basic media in the presence of hydrogen peroxide. The reactive species in this reaction was found to be the hydroxyl radical, produced by the decomposition of hydrogen peroxide.[15] In the present reaction, hydrogen peroxide is produced as a by-product (for example in Scheme 1), and when diradical oxygen reacts with water. Since the degradative oxidation affords six moles of formic acid per mole

Table 1. Oxidation of Glucose with Oxygen in Alkaline Solution

Time (h)	Moles of glucose consumed	Moles of products formed per mole of glucose					
		Arabinonic acid	Formic acid	Erythronic acid	Glycolic acid	Glyceric acid	Oxalic acid
2	0.05	0.043	0.042	0.027	0.01	0.001	0.001
4	0.24	0.192	0.26	0.038	0.02	0.004	0.003
6	0.40	0.35	0.42	0.043	0.025	0.004	0.004
8	0.52	0.47	0.58	0.044	0.032	0.005	0.004
12	0.68	0.61	0.90	——[a]	0.05	0.006	0.005
20	0.83	0.75	1.04	——[a]	0.11	0.018	0.005
40	0.93	0.77	1.14	——[a]	0.14	0.097	0.006

a. Amount not detected because of low resolution.

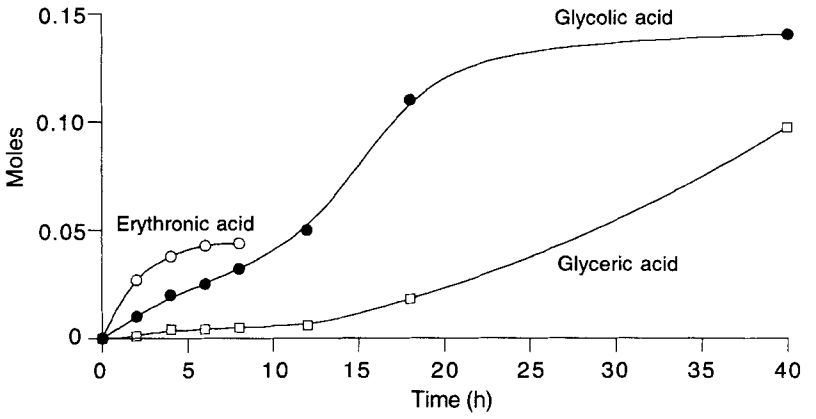


Figure 4. Rate of formation of erythronic, glycolic and glyceric acids per mole of glucose.

hexose, only a small percentage of the glucose used would produce the excess formic acid observed.

Figure 4 shows the rates of formation of glycolic acid, glyceric acid and erythronic acid. This is also represented in Table 1 which shows the number of moles of erythronic, glyceric and glycolic acid formed per mole of glucose. From the data obtained, it is apparent that the amount of erythronic acid, glyceric acid and glycolic acid formed per mole of glucose is quite small compared to the amount of arabinonic acid formed. Since erythronic acid is produced from both 1,2 enediols and from 2,3 enediols its amount should exceed the amount of the 2,3 enediol formed during the reaction. On the other hand, arabinonic acid is formed only from 1,2-enediols, so its amount will reflect more accurately the amount of 1,2-enediols formed. Table 1 shows that after eight hours, the amount of erythronic acid produced (0.044 mol) is less than 10% of that of arabinonic acid (ca. 0.47 mol). This indicates that the ratio of 2,3 enediols to that of 1,2 enediols formed must be less than 10%.

Table 1 shows also that the numbers of moles of glyceric and oxalic acid formed per mole of glucose are very low. It seems that after an initial increase, the rate of formation of glyceric acid remains nearly constant between two and eight hours of reaction, and then increases. This suggests that after most of the arabinonic acid is produced from 1,2-enediols, glyceric acid is formed from a small amount of glucose converted into saccharinic acids and the other products of Isbell's scheme.[8]

EXPERIMENTAL

High performance liquid chromatography was performed using a Waters model 501 pump, a Rheodyne fixed loop (20 μL) injector, a Waters Lambda-Max model 481 UV detector, and a Hewlett-Packard 3392 integrator. The column used was a Bio-Rad HPX-87 H^+ (30 cm x 7.8 mm), eluted at 20 °C with filtered (0.5 μm membrane type AH) 0.009 N sulfuric acid. To minimize separation problems and avoid damaging of the column, a Bio-Rad precolumn containing the same resin (H^+) was used. Figures 1 and 2 show the ability of the Aminex cation exchange HPX-87 H^+ column to separate samples containing mixtures of sugar acids, their salts and carbohydrates. The oxidation experiment was performed in a Parr model 3911 pressure reaction apparatus. The materials used throughout the course of this study, namely D-glucose, K^+ arabinonate, K^+ ribonate, Na^+ glycolate, Ca^{2+} erythronate, Ca^{2+} glycerate, D-arabinonolactone, D-ribonolactone and D-gluconolactone were obtained from Dr. H. S. Isbell's collection of pure sugars. Testing these compounds with HPLC confirmed that they were pure (none showed more than one peak). Oxalic acid and formic acid potassium salt were purchased from Aldrich chemical company.

The following procedure was employed for the oxidation of D-glucose.[16,17] A 0.0125 mole of the sugar was added to 50 mL of 0.85 N KOH solution, previously cooled to 0 °C, and saturated with oxygen. The solution was shaken with oxygen at a pressure of 20 PSI. Aliquots (1 mL each) were withdrawn at intervals, neutralized with a N solution of sulfuric acid and then diluted to 2 mL in volumetric flasks. The solutions were filtered and injected directly into the LC system and known weights of authentic acids, their salts and lactones were used as standards to identify the oxidation products and determine their concentrations. The results obtained are depicted in Figures 3 and 4. The plots were obtained using Kaleida Graph software on a Mac II computer.

CONCLUSION

In summary, HPLC was successfully used to separate and estimate the oxidation products formed when glucose is treated with oxygen in alkaline medium. The study also showed the ability of an Aminex HPX-87H^+ column to separate sugar acids. Monitoring the formation of the oxidation products of glucose with time showed that a mechanism previously proposed by H. S. Isbell is correct. The study showed that about 90% of the oxidation products formed were produced from 1,2 enediols and less than 10% via the 2,3 enediols. The amount of formic acid exceeds that of arabinonic acid suggesting that the former is produced by various pathways including ones that do not involve the formation of arabinonic acid.

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