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Reaction pathways of glucose oxidation by ozone under acidic conditions ☆

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ABSTRACT

The ozonation of D-glucose-1-^{13}C, 2-^{13}C, and 6-^{13}C was carried out at pH 2.5 in a semi-batch reactor at room temperature. The products present in the liquid phase were analyzed by GC–MS, HPAEC-PAD, and ^{13}C NMR spectroscopy. Common oxidation products of glucose have also been submitted to identical ozonation conditions. For the first time, a pentaric acid was identified and its formation quantitatively correlated to the loss of C-6 of glucose in the form of carbon dioxide. Potential mechanisms for the formation of this pentaric acid are discussed. The well-accepted pathway involving the anomeric position in glucose, gluconic acid, arabinose, and carbon dioxide is reinvestigated. The origin of small molecules such as tartaric, erythronic, and oxalic acids is clarified. Finally, new reaction pathways and tentative mechanisms consistent with the formation of ketoaldonic acids and smaller acids are proposed.

1. Introduction

While desired in many cases such as for water treatment,[1] carbohydrate oxidation with ozone is also often the undesired result of processes aimed at degrading or modifying other companion materials (e.g., delignification[2–5] of paper pulp, feedstock, or cotton stalk). Understanding the reaction mechanisms or identifying the reaction pathways is a key step toward a better control of the processes involved. Identification of stable intermediates and final products is therefore essential. Classically, techniques such as HPAEC-PAD, GC–MS, and more recently NMR spectroscopy have been used to identify products in the liquid phase. Site-specific isotopic labeling and molecular modeling are very useful tools also. Several studies on carbohydrate oxidation resorted to isotope-labeled substrates.[6] More recently, this approach was successfully applied to the study of the oxidative degradation of D-glucose in hot alkaline solution[7] and to the Maillard reaction.[8–10]

Our research focused on the action of ozone on cellulose and related compounds as part of an industrial collaboration on paper pulp bleaching. Numerous reports also studied the ozonation of mono-, oligo-, and polysaccharides often as part of research on ozone as a bleaching agent or oxidation agent for the removal of organic substances in water (for a selection of publications, see Table 1). Upon reviewing the literature, it became obvious that there was not a clear consensus in the literature as far as the relative reactivity of carbon atoms in hexoses or polysaccharides is concerned, we therefore set out to reinvestigate the oxidation of D-Glucose at various pH.

We previously published an analysis of the gas phase resulting from the ozonation of 1-^{13}C, 2-^{13}C, and 6-^{13}C labeled D-glucose molecules at pH 2.5 and 10.[6,11,12] To the best of our knowledge no other study of the ozonation of labeled carbohydrates has been reported. The equal contribution of carbon atoms C-1 and C-6 of the glucose molecule in the production of carbon dioxide at pH 2.5 raised anew the question of the reactivity of carbon atom C-6.[13–19]

Oxidation at C-1 is considered as the main pathway to lower products from glucose.[13,14,16,20–25] Because of the formation of both arabinose and carbon dioxide, observed in several cases, this aspect of the ozonation reaction has been compared[25,26] to the Ruff degradation[27] reaction.‡

Here, quantitative and isotopic analyses of the liquid phase and correlation with the analysis of the gas phase, allowed us to identify, for the first time, a 5-carbon atom molecule corresponding to the loss of C-6 from glucose.[12,28] We also confirmed the high reactivity of the anomeric position. The well-accepted pathway involving gluconic acid, arabinose and carbon dioxide is reinvestigated. The origin of small molecules such as tartaric, erythronic, and oxalic acids is also clarified. Finally, new reaction pathways and

☆ For a report on the analysis of the gas phase in ozonized ^{13}C-labeled D-glucose compounds, see Ref. 6.

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‡ In the presence of hydrogen peroxide and ferric ions, aldonic acids are oxidized to give CO_2 and an aldose with one less carbon atom.

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Table 1
Identification and quantitative and isotopic analyses of the oxidation products in the liquid phase (at 1.5 equiv of ozone consumed per mole of glucose)

Identified product	Number of carbon atoms	Mmol at 1.5 equiv of ozone consumed	Contains	Previously reported in Ref.
Gluconic acid	6	0.27	C-1, C-2, C-6	16,20–26,29,30,33,34,37,42–44
2-Ketogluconic acid	6	0.03	C-1, C-2, C-6	11,12,16,20,21,23
5-Ketogluconic acid	6	0.05	C-1, C-2, C-6	12,16,23,30
Hexodialdose	6	0.05	C-1, C-2, C-6	13
Glucuronic acid	6	0.06	C-1, C-2, C-6	From glucose, see: 13,16,18,20,23,30,43 and from methyl glucoside see: 13,15,23,26,31,37,45
Glucaric acid		0.01	C-1, C-2, C-6	16,23,24,29
Arabinose	5	0.017	C-2, C-6	13,20,22,29,33,34
Arabinonic acid		0.016	C-2, C-6	–
Xylaric acid		0.061	C-1, C-2	New
Tartaric acid -1	4	0.016	C-1, C-2	13,16,21,24,29,30,46
Tartaric acid -2		~0	C-2	
Erythronic acid		0.011	C-6	30. For erythrose, see Ref. 16
Glycaric acd	3	Traces	C-1, C-2	20
2-Ketoglycaric acid		Traces	C-6	30
Glyceric acid		0.009	C-6	–
Glycolic acid	2	0.129	C-6	16,20,23
Oxalic acid		0.031	C-1, C-2	

tentative mechanisms consistent with the formation of ketoaldonic acids and smaller acids are proposed. The variety of possible pathways led us to investigate the ozonation of commercially available oxidation products to support our discussions on mechanisms.[§] In this paper, 'C-1', 'C-2' and 'C-6' will refer to the carbon atoms in the parent glucose molecule.

2. Results

For convenience, we summarize in Table 1 qualitative, quantitative and isotopic analyses of products found in the liquid phase at pH 2.5. We also indicated in the last column relevant literature references where the same product was also reported. The third column gives the amount of product present after consumption of 1.5 equiv of ozone per mole of glucose. The fourth column indicates the presence of the original carbon atoms C-1, C-2, or C-6 in the product. The tracking of glucose carbon atoms in the oxidation products is based on the isotopic analyses conducted during the respective ozonation of 1-^{13}C, 2-^{13}C and 6-^{13}C labeled glucose molecules.

The products identified are in agreement with reports by other groups. As expected, products corresponding to an oxidation at C-1 represent the majority of the 6-carbon atom compounds. Hexodialdose was reported once before under batch and free pH conditions[13] but never under semi-batch conditions. Its oxidation product, glucuronic acid is classically identified. Glucaric acid is known to form at high concentrations of ozone.[16,24,29] Ketoaldonic acids have been reported during the oxidation of glucose or alkyl glucosides.[16,20,21,23,30–32] As expected, the absence of C-1 carbon atom is evidenced in the products identified as arabinose and arabinonic acid. Arabinose, identified by HPAEC and GC–MS, is observed in a rather low concentration in agreement with literature data.[13,20,22,23,29,33,34] It is noteworthy that the ozonation of arabinose leads to the formation of a ketopentonic acid identified as D-*arabino*-pent-4-ulosonic acid by mass spectrometry (molecular ion at 452 Da for the silylated derivative),[11,12] congruent with the results of Parthasarathy and Peterson on xylose.[35] However this product is not observed during the ozonation of glucose described here. Rather, a pentaric acid was identified by comparison of its mass spectrum (Fig. 1) with that of persilylated arabinaric acid reported by Peterson.[36] Erythrose, the precursor of erythronic acid, has been detected by HPAEC under similar experimental conditions.[16,23] Two peaks were identified by GC–MS as tartaric acid. Considering concentration (see Table 1), we focused on the main peak in the rest of this article. This main peak contains mainly both C-1 and C-2 atoms. Three acids with 3 carbon atoms were identified: glyceric, glycaric (hydroxypropanedioic), and 2-ketoglycaric acids. The latter was identified as its trimethylsilylketal. The products containing 3 carbon atoms are obviously formed by different pathways as indicated by the presence of C-1 in the glycaric acid and by the presence of C-6 in 2-ketoglycaric and glyceric acid. An increasing concentration of glycolic and oxalic acids (compounds containing 2 carbon atoms) was observed as already reported by our group[16,23] and Holen[20] during ozonation of cellulose model compounds. Oxalic acid appears to contain C-1 and C-2 atoms but not C-6 according to the analysis by ^{13}C NMR.[¶] No formaldehyde has been identified and the production of formic acid remains below carbon dioxide production.[6,11]

NMR monitoring showed that, for up to 2 equiv of ozone consumed per mole of glucose, the limited amount of formic acid produced is not formed from the C-2 carbon atom and that the concentration of HC(1)OOH is 4 times as large as that of HC(6)OOH. To the contrary, the amount of $C(1)O_2$ is similar to that of $C(6)O_2$ indicating that these two one carbon atom products are produced via different mechanisms.

2.1. Mass balances

To assess the reliability of our quantitative analyses, we computed mass balances for each of the labeled positions (C-1, C-2, and C-6). For that purpose, we used the quantitative and isotopic analysis summarized in Table 1 for the liquid phase, and the quantitative and isotopic analysis of the gas phase given in our

[§] We conducted direct ozonation of commercially available samples of D-glucuronic acid, D-gluconic acid, D-glucurono-1,4-lactone, 2- and 5-*keto*-D-gluconic acids, L-arabinose, and polygalacturonic acid (a summary of the gas chromatography data is given as Supplementary data). Results of these reactions are mentioned when needed throughout the text.

[¶] However other combinations (C-2/C-3, C-3/C-4, C-4/C-5) cannot be excluded. Indeed, under the conditions applied for ^{13}C NMR analysis, only labeled carbon atoms were detectable. Nevertheless, by integrating the ^{13}C NMR signal, we could determine that oxalic acid bearing glucose C-1 and C-2 carbon atoms represents a constant ratio in the overall oxalic acid produced.

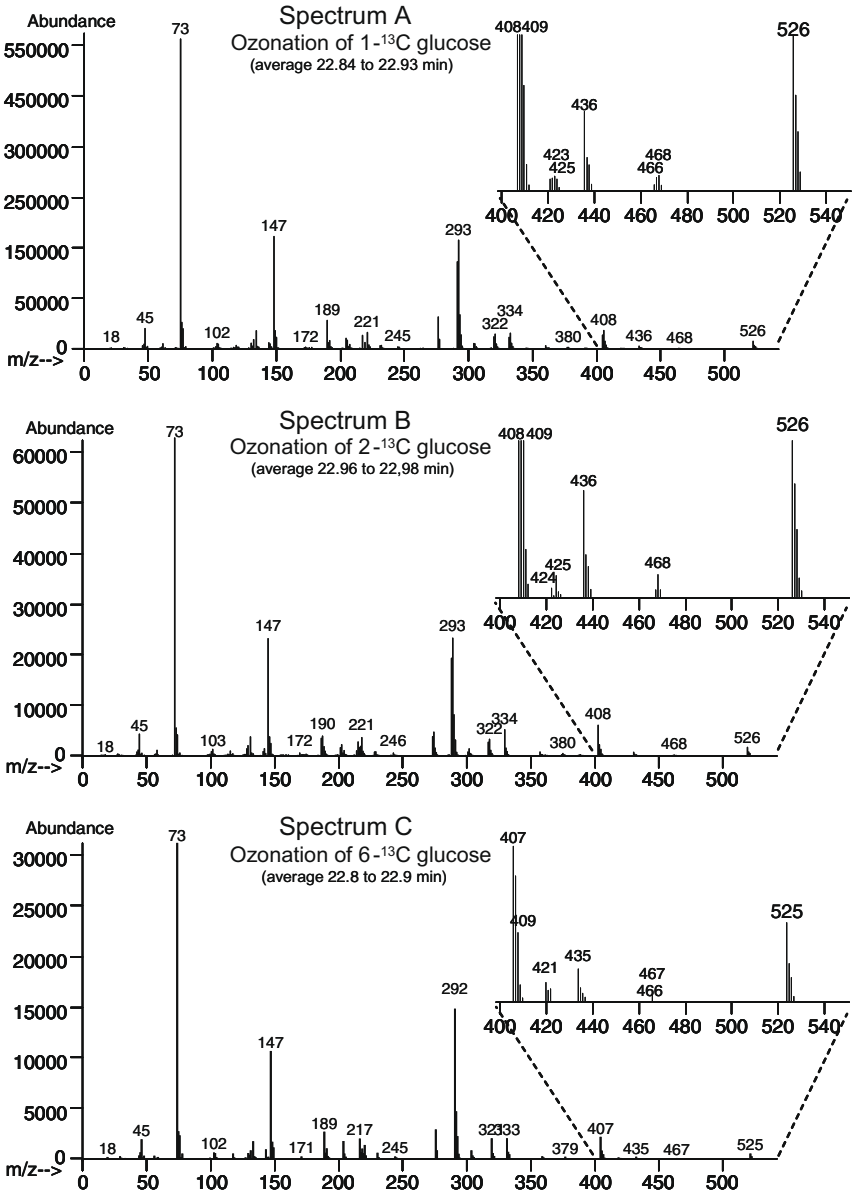


Figure 1. Typical mass spectra of xylaric acid sampled during the ozonation of 1-^{13}C glucose (spectrum A, averaging between 22.84 and 22.93 min), 2-^{13}C glucose (spectrum B, averaging between 22.96 and 22.98 min), and 6-^{13}C glucose (spectrum C, averaging between 22.80 and 22.90 min).

previous report.[6] Based on 8 points between 0.4 and 3 equiv of ozone consumed, the recoveries were 106% (% CV = 5.1) for C-1, 109% (% CV = 5.0) for C-6, and 103% (% CV = 3.9) for C-2. Hence, the present analysis can be considered reliable and the quantitative analysis as representative of the actual content of the samples.

2.2. Identification of xylaric acid

Figure 1 features the mass spectra of the silylated pentaric acid sampled during the ozonation of 1-^{13}C D-glucose (Fig. 1A), 2-^{13}C D-glucose (Fig. 1B), and 6-^{13}C D-glucose (Fig. 1C), respectively. The absence of ^{13}C-labeled fragments in the acid (Mw 540 g/mol^{-1}) formed during ozonation of D-glucose-*6*-^{13}C is obvious. One can consider especially peaks at *m/z* 525 $[M–CH_3]^{+\cdot}$, 435 $[M–CH_3–Me_3SiOH]^{+\cdot}$, 408, and 292 (Fig. 1C) which appear respectively at *m/z* 526, 436, 409, and 293 for the acid formed by ozonation of 1-^{13}C D-glucose (Fig. 1A) and 2-^{13}C D-glucose (Fig. 1B). Identification of this acid as xylaric acid is therefore established.

2.3. Isotopic content over time

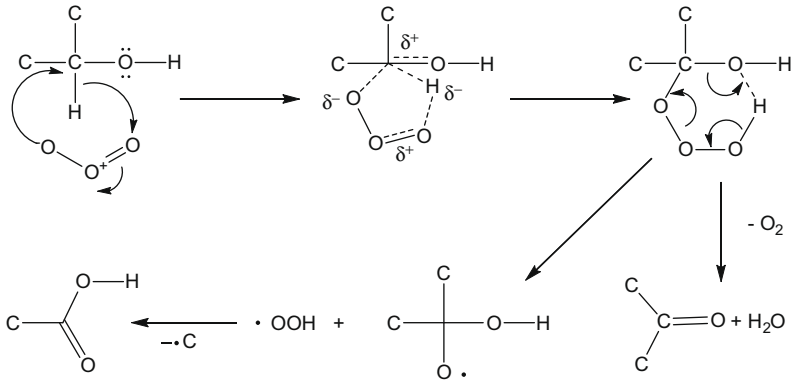
The isotopic enrichment of xylaric but also tartaric acids was calculated[‖] and the results are consistent with constant pathways for their formation. Xylaric was found to contain between 73% and 80% of C-6 and C-2 depending on the peak studied (292 or 321 Da) and tartaric acid was found to contain 72% of C-1 and 99% of C-2. The higher C-2 content in tartaric acid is explained by a high ratio of the C-1 through C-4 segment and a small proportion of the C-2 through C-5 segment in this acid.

[‖] Available as Supplementary data.

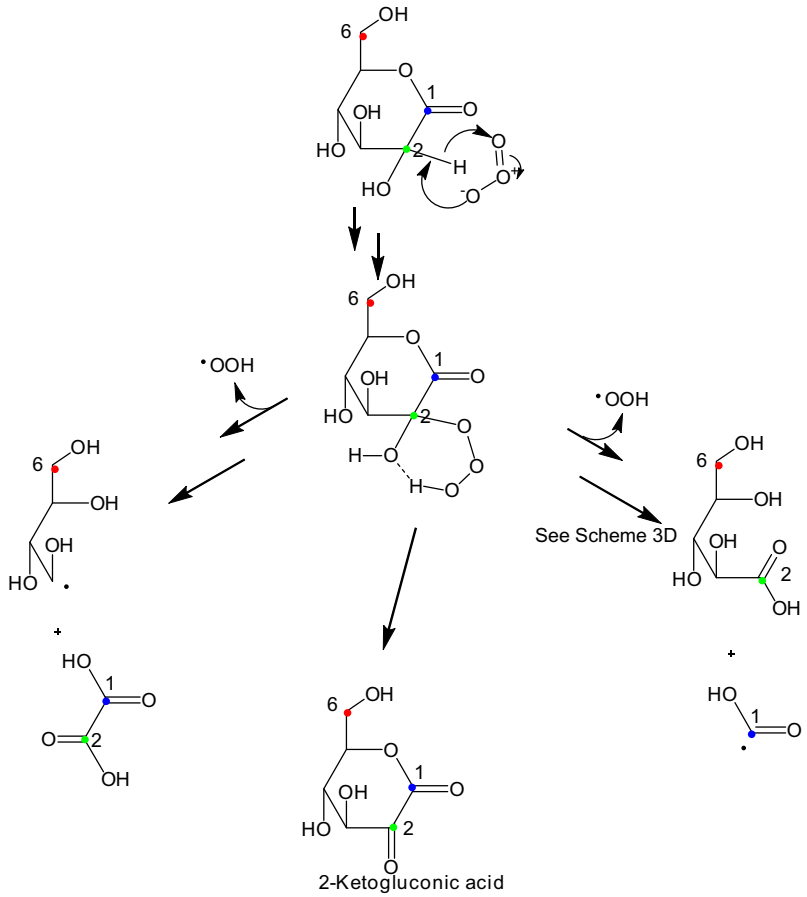
3. Discussion

3.1. Oxidation at position C-1, subsequent oxidation at C-2 and corresponding products

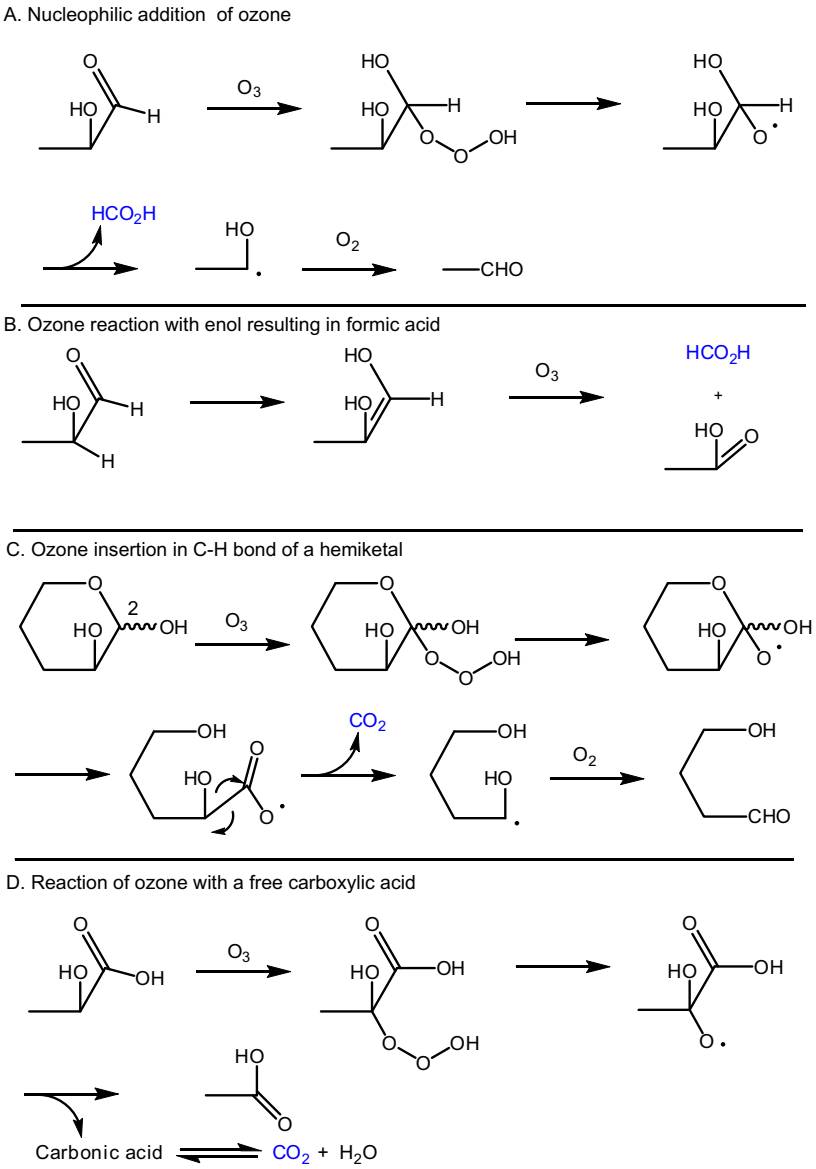
The high concentration of gluconic acid in the reaction mixture provides, here again, a clear evidence of the high reactivity of the C-1 position (hemiacetal) as often reported before.[20,25,26,29,30,33,34,37] To evaluate the significance of oxidation at C-1 in the formation of other oxidation products, we need to consider potential oxidation products of gluconic acids. For instance, formation of arabinose[13,14,16,20,22,23,25,29] has been explained in several cases as the result of the loss of C-1 in form of carbon dioxide.[29,34] Our report on carbon dioxide origin[6] actually gave a direct evidence for the early participation of the C-1 carbon atom in the production of CO_2 and formic acid. As expected, arabinose and arabinonic acid do not contain the C-1 atom (see Table 1). Arabinonic acid can simply result from further oxidation of arabinose as we observed during the ozonation of arabinose. The formation of arabinonic acid has also been attributed to the decarboxylation of D-*arabino*-hex-2-ulosonic acid (2-ketogluconic acid).[13,14,16,23] However, ozonation of the commercially available hemicalcium salt of 2-ketogluconic acid did not lead to the expected arabinonic acid. This suggests that 2-ketogluconic acid might be a minor or relatively stable product, rather than the precursor to arabinose or arabinonic acid at pH 2.5. Based on a mechanism (Scheme 1)



Scheme 1. Formation of acetone and ethyl acetate during ozonation of diethyl ether.[38]



Scheme 2. Proposed mechanism leading to both 2-keto derivatives and lower aldonic acids.



Scheme 3. Proposed mechanisms leading to carbon dioxide or formic acid formation from aldehyde, hemiketal, or carboxylic acid intermediates.

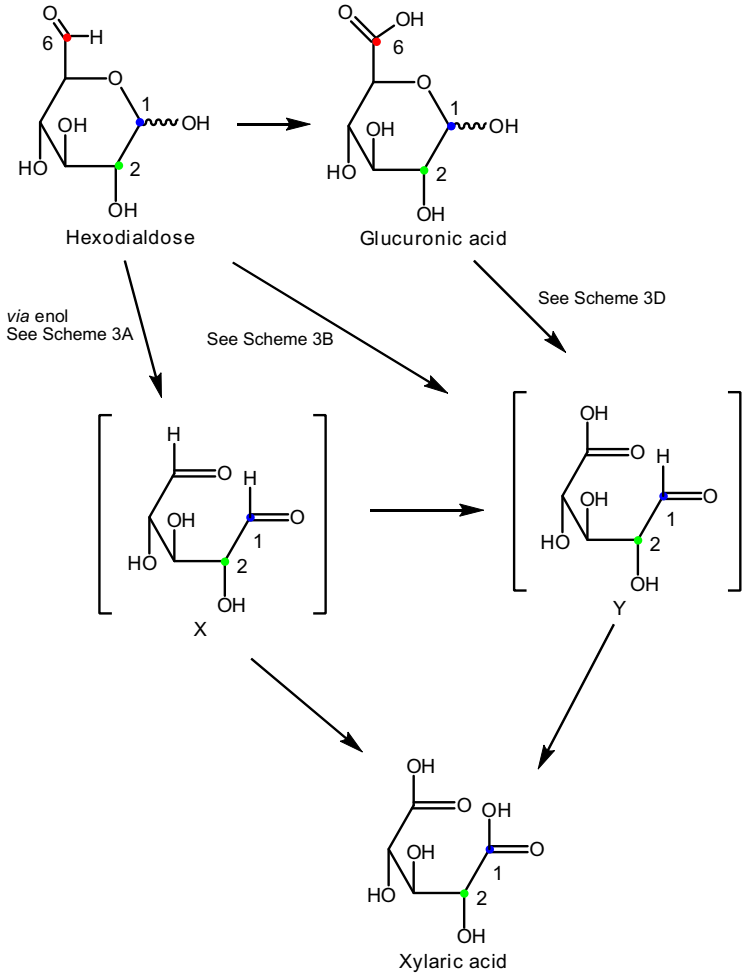
proposed to explain the formation of acetone and ethyl acetate during ozonation of diethyl ether,[38] we now suggest that under acidic conditions, an unstable intermediate leading to 2-ketogluconic acid (from gluconic acid), can also decompose into arabinonic acid and carbonic acid (or carbon dioxide). This is summarized in Scheme 2.[††]

The formation of both erythronic acid and oxalic acid (containing C-1 and C-2 carbon atoms) could also be explained by further oxidation of gluconic acid by oxidative cleavage of the C-2/C-3 bond. Such a carbon–carbon bond cleavage has been previously suggested.[39] As shown in Scheme 2, we propose that the same intermediate leading to either 2-ketogluconic acid or arabinose and carbon dioxide can decompose into a C-1–C-2 fragment (oxalic acid) and a C-3 through C-6 fragment (erythrose or erythronic acid). Erythronic acid (trihydroxybutyric acid) can obviously be also the result of oxidation of erythrose (observed under similar conditions[23]).

[††] While radical processes cannot be excluded, the mechanisms proposed in this article (Schemes 2 and 3) focus on molecular ozone and are consistent with the absence of detectable amounts of hydroxyl radicals at this pH. (see Ref. 16).

3.2. Oxidation at position C-6 and subsequent decarboxylation

While highlighted in a few studies,[11,13–15,18,19,23,28,31] oxidation at C-6 has never been as well established as oxidation at C-1. As others, we do observe a relatively low concentration of 6 carbon atom products oxidized at the C-6 position (namely, glucuronic acid and hexodialdose), compared to the concentration of gluconic acid. Conversely, we found a substantial involvement of the C-6 carbon atom in the production of carbon dioxide.[6] We monitored the production of carbon dioxide during ozonation of gluconic acid, gluconic acid-1,5-lactone, glucuronic acid, and glucaric acid-1,4-lactone. The decarboxylation of gluconic acid appears to be limited in its lactone form, predominant under acidic conditions. We were able to conclude to an enhanced sensitivity of uronic acid groups toward ozone under acidic conditions.[11,12,28,40] Their tendency to decarboxylate in the presence of ozone certainly contributes to both the presence of carbon atom C-6 in the carbon dioxide formed and the low concentration of products oxidized at C(6). However, these data alone are not sufficient to allow to conclude that the newly identified xylaric acid is the result of the decarboxylation of glucuronic acid.



Scheme 4. Reaction pathway to xylaric acid after oxidation at C-6.

To establish a correlation, we calculated the ratio of carbon dioxide formed from C-6 to the amount of xylaric acid formed. We found that this ratio remains close to 0.9 up to 2.5 equiv of ozone consumed. The same ratio determined for the C-1 position based both on the production of carbon dioxide and on the production of arabinose, arabinonic, and tartaric acids gave only an average value of 0.76. Carbon dioxide formation from C-6 is therefore quantitatively correlated to the formation of xylaric acid whereas that of carbon dioxide form C-1 is the result of multiple reactions.

If xylaric acid is the result of a decarboxylation, what is the mechanism and is the decarboxylation actually occurring on glucuronic acid? It is noteworthy that no aldehyde precursor of xylaric acid is identified whereas arabinose, the potential aldehyde precursor to arabinonic acid is detected. Still, xylaric acid could be formed by the fast oxidation of an aldehyde precursor not stabilized as a hemiacetal. By analogy with suggestions given by others for gluconic acid,[22,25,29,34] a dialdehyde intermediate X (Scheme 4) could be derived from glucuronic acid by decarboxylation or from hexodialdose by deformylation of carbon 6. Mechanisms given in Scheme 3A and B could account for that.

The formation of xylaric acid could also be explained by a mechanism similar to that given in Scheme 2. This mechanism, applied to the carbon 5 of glucuronic acid, would lead either to xyluronic acid and carbon dioxide or to 5-ketoglucuronic acid. Neither xyluronic nor 5-ketoglucuronic acids have been observed in this study or elsewhere. The same mechanism, when applied to C-5 of gluconic acid, would actually give xylaric acid (observed) but also involves the formation of the least stable radical intermediate. 5-Ketogluconic acid (observed) however is likely to form via the mechanism leading to 2-ketogluconic acid by oxidation at position 2. Additionally, if ozonation of glucuronic acid does lead to xylaric acid, the ozonation of gluconic acid does not lead to any xylaric acid. Ozonation of 5-ketogluconic acid, gives no detectable amount of xylaric acid either. Therefore, we favor a mechanism such as that in Scheme 3D. We summarize this path in Scheme 4 with intermediate Y giving carbon dioxide from glucuronic acid.

Glucaric acid which can be formed from hexodialdose or oxidation of gluconic acid (directly or via guluronic acid), is not likely to be a key intermediate in the formation of xylaric acid. Further oxidation of such an aldaric acid would potentially lead to the formation of both xylaric and arabinaric acids but the latter was detected.

3.3. Formation of products with less than 5 carbon atoms

Tartaric acid unexpectedly contains mainly the C-1 through C-4 segment and to a lesser extent, the C-2 through C-5 segment[‡‡]

[‡‡] Tartaric acid containing the C-2/C-5 segment forms in a very limited amount according to the isotopic analysis. Such a diacid could be the result of a double decarboxylation of glucaric acid leading to the formation of both C^6O_2 and C^1O_2. Decarboxylation of xylaric acid can also explain the formation of both C-1 through C-4 or C-2 through C-5 tartaric acids. The slow decrease in concentration of xylaric acid after 2 equiv of ozone consumed could account for such a degradation.

throughout the reaction. In previous reports based on HPLC[16,23–25,29] or NMR spectroscopy[20,21] during the ozonation of unlabeled glucose, the formation of tartaric acid could not be attributed to the oxidation of a specific precursor. We believe that oxidation at position 5 of gluconic acid leads mainly to 5-ketogluconic acid. And, we propose that glycolic acid and tartaric acid would result from an oxidation at position 4 of gluconic acid or glucuronic acid. There is evidence of oxidation at the C-4 position.[15] The isotopic analysis of the products containing 3 carbon atoms also supports an oxidation at position 4. They are formed in very small amounts, but we were able to show that these compounds contain either the C-1–C-3 or C-4–C-6 segments implying a cleavage of the C-4–C-3 bond. By applying once more the mechanism given in Scheme 1 to carbon C-4, one could explain formation of 4-keto derivatives (not observed here) and formation of fragments with 3 carbon atoms in addition to fragments with 2 and 4 carbon atoms as mentioned earlier.

4. Conclusion

The use of ^{13}C-labeled D-glucose allowed for a better understanding of the origin of carbon dioxide and gave a significantly better insight in the formation of ozonation products of glucose.

By using isotopic and quantitative analyses, we were able to directly correlate the formation of a pentaric acid (xylaric acid) to the loss of carbon C-6. Presence or formation of carboxyl groups in lieu of exocyclic hydromethyl groups in polyhexoses could be of significance during treatment by ozone. Their fast decarboxylation would indeed lead to an 'open' residue in the structure. Such modification would directly affect the physical properties of the polysaccharide (critical in paper manufacturing for example) and could lead to subsequent decrease in molecular weight such as by β-elimination for example. We indirectly assessed some of these possibilities by treating pure polygalacturonic acid (pectin) with ozone at pH 2.5[§§] and observed the expected formation of carbon dioxide and the corresponding pentaric acid.[11,40]

In the present report, we establish for the first time a defined path for oxidation on C-6 (Scheme 4) while confirming the well-accepted formation of arabinose by loss of C-1 and the high reactivity of C-1. The paths given in Scheme 3A–D account for the observed formation of both carbon dioxide and formic acid from carbon C-1 (as aldehyde or hemiketal or carboxylic acid) and mainly carbon dioxide from C-6 (as uronic acid).

Finally, a set of mechanisms for glucose ozonation under acidic conditions is proposed that accounts for the majority of the products reported in the literature by explaining: (i) The formation of arabinose or arabinonic acid and carbon dioxide or formic acid from gluconic acid or glucose, of oxalic acid and erythronic acid from gluconic acid and of 2-ketogluconic acid from gluconic acid as shown in Scheme 2; (ii) The formation of 5-ketogluconic acid; and (iii) The formation of 4-keto derivatives of glycolic acid and tartaric acid or fragments with 3 carbon atoms.

5. Experimental

5.1. Materials and methods

5.1.1. Ozonation reactions

Reactions were carried at room temperature on 250 mg of D-glucose (1.38 mmol) in 100 mL of deionised water (UHQ water from an Elgastat apparatus). The soln was magnetically stirred and ozone introduced (55–60 mg/L and 60 mL/min produced from oxygen 'C' Air Liquide with a Lab-Lox Trailigaz ozonizer) into the solution through a glass bubbler. The initial pH was set with concd sulfuric acid and maintained by automatic titration with NaOH (250 mmol). For more details on experimental conditions and measurement of ozone concentration see Ref. 6. Ozone consumption was determined by the difference between the concentrations of ozone before and after the reactor. Throughout the article, ozone consumption is expressed in mole of ozone consumed per mole of glucose introduced (i.e., equivalent of ozone).

D-Glucose-1-^{13}C, D-glucose-2-^{13}C and D-glucose-6-^{13}C, 99% ^{13}C were purchased from Omicron Biochemical—USA.

Commercially available D-glucuronic acid, D-gluconic acid, D-glucono-1,4-lactone, D-glucaric acid, 5-keto-D-gluconic acid, 2-keto-D-gluconic acid hemicalcium salt, L-arabinose, and pectic acid (Aldrich cat # 37,474-1) were also submitted to the same oxidation conditions.

5.1.2. Analysis of neutral sugars by HPAEC (D-Glucose, L-arabinose)

HPAEC (High Performance Anion Exchange Chromatography) analyses[16,23,24,29,41] were performed with a Dionex system (DX-500) using a 250 × 4 mm anion exchange column (Carbopac PA1) fitted with a pre-column (50 × 4 mm) and a pulsed amperometric detector (PAD). A ternary eluent was used at a flow rate of 1 mL/min. Eluents A (water), B (NaOH 150 mMol), and C (500 mmol AcO-Na and 150 mmol NaOH) were mixed according to the following gradient. At *t* 0 s, the eluent was a mixture of A (70%) and B (30%). At 60 min, the eluent was a mixture of A (30%), B (40%), and C (30%).

Compounds identification was performed by comparison of their retention times with those of standard mixtures. Quantification was made using calibration curves established by analyzing standard solutions.

5.1.3. Quantification and identification by GC–MS

Analyses with a combined GC–MS apparatus were performed after silylation of the samples on a Hewlett Packard 5890/5972 MSD instrument. The separation was achieved using an apolar High Performance Capillary Column (HP1-MS, 30 m length and 0.25 mm of internal diameter). The helium flow rate was set at 1.6 mL/min. Injections were done in the split/splitless mode with an injector temperature of 100 °C. Solvent delay was set to 5 min. The temperature was kept at 60 °C for 1.5 min, then a linear temperature program was applied up to 31.5 min to reach 300 °C at 10 °C/min. Finally the temperature was maintained at 300 °C until 36 min.

5.1.4. Silylation procedure

The reaction sample (600 μL) was concentrated to dryness at room temperature and dissolved in 700 μL of dry pyridine. The mixture was placed for 2 min in an ultrasonic bath before addition of 500 μL of a 5% soln of trimethylsilyl chloride (Aldrich 99% purity) in bis(trimethylsilyl)trifluoroacetamide (Aldrich 99% purity). The sample was then stored 2 h in the dark prior to analysis.

Compounds identification was performed either by comparison of their mass spectra with those obtained from commercially available chemicals submitted to an identical silylation process or by comparison with spectra of compounds in the database of the National Bureau of Standards (NBS-USA) or relevant literature.

Before relying on GC integration as a quantitative tool, we took the additional step of determining a response coefficient of commercially available by-products by reference to the glucose signal (see Supplementary data).[11] The relevance of these coefficients was then assessed by comparing GC quantification with HPAEC-PAD (for neutral by-products) or HPAEC-PED (for acidic by-products using a Dionex AS11 column). For experimental samples, glucose, measured independently by HPAEC-PAD, was used as an internal GC standard and the respective GC response coefficients

[§§] This will be detailed in a forthcoming paper.

were applied as appropriate. For the pentaric acid identified as xylaric acid, a conservative coefficient was calculated by averaging the response coefficients of glucaric and tartaric acids.

Amounts of by-products are expressed in moles throughout the article.

5.1.5. Analysis of formic and oxalic acids

The presence of these acids was monitored by ^{13}C NMR analyses on a Bruker DRX 500 MHz Avance of the 'Centre de Spectrométrie Moléculaire de l'Université de Bourgogne'.

The signal of the carbon atoms of oxalic acid is observed at 164 ppm[20] and that of the carboxylic group of tartaric acid at 171.4 ppm.[6,20] ^{1}H decoupled ^{13}C NMR experiments were recorded on samples diluted in a soln of $NaH^{13}CO_3$ (reference/buffer) in D_2O.

5.1.6. Isotopic analysis

The almost complete replacement of peaks at mass M by a peak at mass M+1 on the mass spectra obtained by GC–MS was clearly indicative of a ^{13}C content close to 100% and this observation was considered as sufficient in most cases. A peak at *m/z* M, for key compounds, was still observed and therefore the enrichment ratio was calculated by comparison with the mass spectrum of the unlabeled compound (either from a commercial source or from reaction mixture sampled during ozonation of unlabeled glucose). For instance, for each compound produced by ozonation of unlabeled glucose, we selected a peak at mass M of the compound's spectrum and determined the natural ratio between the peak at mass M+1 and the peak at mass M. This ratio was used to calculate the true contribution of isotopic enrichment in the signal at M+1 and to calculate a percentage of enrichment. This method enabled us to check the evolution over time of the enrichment of xylaric or tartaric acids (for an example see Supplementary data).[11]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.05.012.

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