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Origin and significance of the production of carbon dioxide during the ozonization of ^{13}C-labeled D-glucose at different pH values ☆

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Abstract

[1-^{13}C], [2-^{13}C] and [6-^{13}C] D-glucose were, respectively, ozonized in a semi-batch reactor in acidic and basic conditions. The composition of the gas phase was evaluated by on-line mass spectrometry measurements. The quantitative and isotopic analyses of the carbon dioxide formed during ozonization are presented and discussed. The data, correlated with previous literature results, clearly show that at pH 2.5 the production of carbon dioxide from C-6 and C-1 carbon atoms is nearly equivalent. Conversely, at higher pH values, CO_2 is released with a greater selectivity from the reducing end. The importance of the decarboxylation reaction in the formation of by-products with fewer than six carbon atoms is also demonstrated. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The production of carbon dioxide during the oxidation of carbohydrates is a well-known phenomenon which has been demonstrated in many cases. A famous example is the Ruff degradation[2,3] which leads to arabinose by oxidative decarboxylation of calcium gluconate in the presence of hydrogen peroxide and Fe^{3+} cations (Scheme 1).

The formation of carbon dioxide is also observed during the oxidation of carbohydrates with hydrogen peroxide under many other conditions.[4] The oxidation with oxygen in alkaline media has been extensively studied and carbon dioxide formation is also reported, but as a secondary by-product.[5,6]

Several studies have shown and often measured the production of carbon dioxide during the ozonization of sugars, starch, cellulose, cellulose model compounds or monosaccharides.[7–15] Over the past few years, our laboratory tried to improve understanding of the ozonization reaction pathways of lignin model compounds[16] and simple carbohydrates (D-glucose, methyl-D-glucopyranose and cellobiose)[7,8] and evidenced in every case, the importance of carbon dioxide formation under acidic conditions. At neutral pH, Defaye and coworkers[10] demonstrated the key-role of the decarboxylation process in mass loss during cellulose ozonization.

☆ For preliminary communications on production of carbon dioxide in acidic conditions, see Ref. 1.

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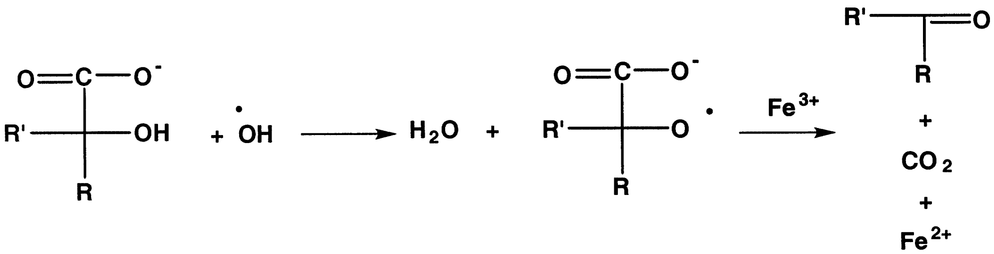


Scheme 1. Mechanism of the Ruff degradation.[2,3]

The origin of carbon dioxide formed during ozonization of sugars and especially glucose or cellobiose has not been clearly established and no convergence could be found in the literature. Gluconic acid is generally observed as the main degradation product[8,11–14,17,18] and Van Nifterik and coworkers[14] explained the formation of carbon dioxide and D-arabinose during the ozonization of D-glucose and cellobiose by a Ruff type of degradation of gluconic acid. A similar pathway was also proposed by Katai and coworkers[12] for the formation of arabinose. More recently, a Ruff type degradation was also considered by Holen and coworkers[11,18] but they concluded that this pathway was probably not the main source of carbon dioxide. From γ-radiolysis experiments, Schuchman and Von Sonntag[15] postulated that carbon dioxide as well as formic acid were formed directly from glucose at low pH via the decomposition of an hydrotrioxide intermediate. In the latter references, the anomeric position of glucose or cellobiose is considered as the most reactive and carbon dioxide is assumed to originate from this position.

For other authors[7,8,10,19] the reactivity at C-6 (primary alcohol) is not negligible compared to that of the anomeric position of mono- or disaccharides. Rey and coworkers[20] even concluded that C-6 was more reactive. Moreover, some reports on polysaccharides showed the importance of the oxidation at the C-6 position[9,10,21].

The importance of the production of carbon dioxide in such oxidation reactions,[7] and our interest in discovering its source have led us to investigate the ozonization of specifically labeled glucose molecules. A few reports on oxidation reactions of labeled carbohydrates with hydrogen peroxide, oxygen, chlorine, or periodate[3,6,22] have appeared in the literature. The aim of such studies was, in most cases, to get a better understanding of complicated oxidation reactions and to deal mostly with the origin of carbon dioxide production. However, to our knowledge, no ozonization reaction of labeled sugars has ever been carried out. Therefore, we performed a detailed study at pH 2.5 in the presence of sulfuric acid or acetic acid and at pH 10 (sodium hydroxide). During these reactions, the gas phase was monitored with an online mass spectrometer to provide an isotopic and qualitative analysis of this phase. In order to assess the early production of carbon dioxide and also to be able to detect in real time very low levels of this by-product, a suitable procedure was developed and is described in Section 2.

2. Experimental

[1-^{13}C], [2-^{13}C] and [6-^{13}C] D-glucose were purchased from Omicron Biochemical Inc. (USA). Ozone was generated from pure oxygen (‘C’ quality from Air Liquide Co.) or a standard mixture of CO_2 (0.5 ppm) in pure oxygen (Air Liquide Co.). Solutions of sugars were prepared with ultrapure water (18 MΩ Elga). The O_3–O_2 mixture was generated with a cold-plasma ozonizer (Labo-Lox Trailigaz) and the concentration of ozone before and after the semi-batch reactor was monitored using UV-analyzers (BMT 961) and the flow rate controlled with a mass flow-meter (Alphagaz).

Typical ozonization procedure in a semi-batch reactor.—The substrate was dissolved (250 mg) in 100 mL of pure water, 1 h before the reaction, then the tubing and the solution were purged with the CO_2–O_2 mixture. The pH was set with concd H_2SO_4, glacial AcOH or 250 mM NaOH solution, 2 min prior to the introduction of the ozone mixture into the reactor. Before bubbling into the solution, the O_3–O_2 mixture (55–60 mg/L, generated from the standard mixture of CO_2 in O_2), was passed through the tubing and over the void volume above the solution surface at a flow rate of 120 mL/min. Once the concentrations of ozone before and after the reaction flask were equal, the flow rate was brought to 60 mL/min and the oxidizing mixture was bubbled into the solution under magnetic stirring. The pH was regulated throughout the reactions by automatic titration with a 250 mM NaOH solution.

The various purges carried out in this procedure are of major importance. Indeed, the purge of the connection tubing with the CO_2–O_2 enables zero of the analytical instruments to be set and any exogenous CO_2 to be removed from the solution. The purge at high-flow rate with the oxidizing mixture before starting the reaction was performed in order for the zero of ozone consumption to be set.

Quantitative analysis of the gas phase.—The gas phase was continuously analyzed using a mass-spectrometer (GasLab–Fisons; electron impact mode) plugged on line at the outlet of the reactor. The reliability of the measurements was assessed by analyzing ambient air and the standard mixture (CO_2 in O_2). The data acquisition was started before the purge of the tubing with the oxidizing mixture, so that the amount of CO_2 or CO produced by the ozonizer, or by reaction of ozone with the tubing was measured. An average level of carbon dioxide production during this purge was computed and subtracted from the values acquired during the ozonization reaction. In each case, data corresponding to the molecular ion $M^{+\bullet}$ (for instance *m*/*z* 44 for $^{12}CO_2$) were added to data corresponding to the ion of mass $M+1$ (for instance *m*/*z* 45 for $^{13}CO_2$) in order to determine the overall production of a component of the gas phase. An example of the recorded curves is given in Fig. 1. The production of CO_2 (% vol) is plotted against reaction time and three zones can clearly be defined corresponding, respectively, to the purge with the standard gas, to the purge with the oxidizing mixture and to the beginning of the ozonization reaction.

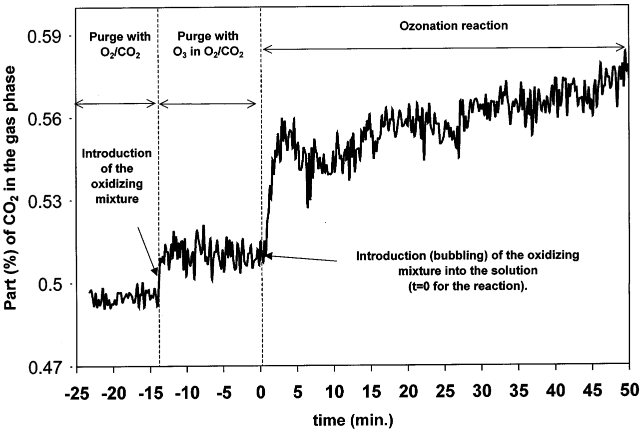


Fig. 1. Variation of the volumic percentage of CO_2 in the gas phase during purge phases and at the beginning of the ozonization reaction of glucose (pH 2.5, H_2SO_4).

Isotopic analysis of the gas phase.—The experiment was carried out at atmospheric pressure. The mass-spectrometer was used in the M.I.M. mode (multiple ion monitoring) and the data were recorded as partial pressures. The *m*/*z* values of the recorded ions were equal to 16, 18, 28, 29, 30, 32, 44, 45 and 48, corresponding, respectively, to O, H_2O, N_2 and/or ^{12}CO, ^{13}CO and/or $H^{12}CO$, $H^{13}CO$, O_2, $^{12}CO_2$, $^{13}CO_2$ and O_3. In these experiments, we determined the contribution of the labeled carbon atoms in the production of compounds in the gas phase, especially CO_2. Therefore, for CO_2, we expressed the percentage of CO_2, noted $\%{*}CO_2$, containing the labeled carbon atom from the partial pressures (%) of $^{13}CO_2$ (i.e., *m*/*z* 45) and of $^{12}CO_2$ (i.e., *m*/*z* 44). The value corresponding to $^{13}CO_2$ was corrected for the contribution of $^{13}CO_2$ derived from 'natural' CO_2. The same calculation was done during the ozonization of all the glucose labeled isomers to provide values noted $\%{*}C^1O_2$, $\%{*}C^2O_2$ and $\%{*}C^6O_2$, corresponding, respectively, to the percentage of CO_2 originating from C-1, C-2 and C-6. The reproducibility of CO_2 formation from one experiment to another was checked to allow comparisons between the values $\%{*}CO_2$.

Analysis of the liquid phase.—The concentration of glucose in the liquid phase was analyzed either by gas chromatography–mass

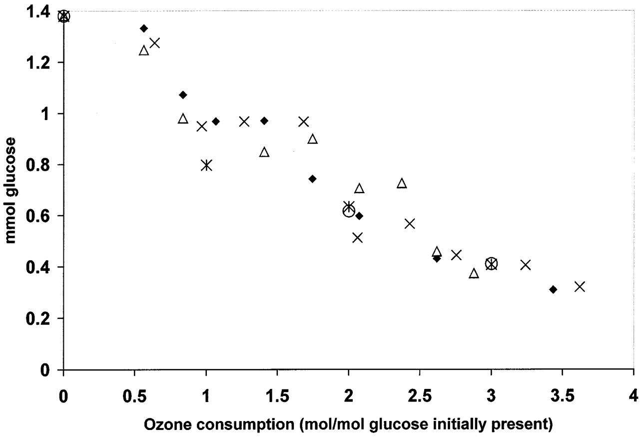


Fig. 2. Reproducibility of the ozonization reaction with regards to the consumption of starting material. (◆) [1-^{13}C] D-glucose (NMR/Experiment 1); (○) [1-^{13}C] D-glucose (GCMS/Experiment 2); (△) [2-^{13}C] D-glucose (HPLC/Experiment 1); (*) [2-^{13}C] D-glucose (GCMS/Experiment 2); (×) [6-^{13}C] D-glucose (NMR/Experiment 1).

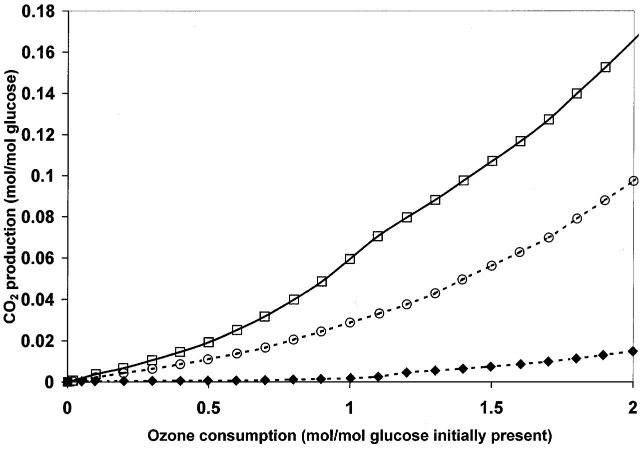


Fig. 3. Influence of the reaction conditions on the production of carbon dioxide during the ozonization of D-glucose. (□) pH 2.5 (H_2SO_4); (--○--) pH 2.5 (AcOH); (--◆--) pH 10 (NaOH).

spectrometry (Hewlett–Packard 5890/5972 MSD) after silylation using a high performance capillary column HP-1-MS, or by high pressure anionic exchange chromatography coupled with conductimetric detection (HPAEC/CD, Dionex Inc.) using a AS11 column (Dionex Inc.). On the graph, drawn in Fig. 2, the consumption of glucose is plotted against the consumption of O_3 expressed as equivalents of the initial substrate. The consumption of O_3 is systematically expressed that way in the other graphs presented in this article. The curves displayed in Fig. 2 follow almost exactly the same trend. These data indicate a good reproducibility between all the experiments and the reliability of the analytical techniques.

^{13}C NMR spectroscopy was used to follow the consumption of glucose as can be seen in Fig. 2. Samples (400 μL) were added to a solution (200 μL) of $NaH^{13}CO_3$ used as an internal standard (5 mg/mL). ^{1}H decoupled ^{13}C NMR experiments with a 30° pulse and 900 accumulations were performed for quantification purposes on a Bruker DRX Avance 500 MHz spectrometer. Such experiments also allowed the estimation of the concentration of labeled formic acid formed during the ozonization of labeled glucose. The signal of formic acid was observed at 171.4 ppm (C=O resonance).[11]

3. Results and discussion

Ozonization reaction at pH 2.5.—Our first interest was to confirm the importance of the production of carbon dioxide at pH 2.5 adjusted with concentrated sulfuric acid. As shown in Fig. 3, this result is clearly evidenced.

Moreover, by using the specific experimental conditions used here (i.e., purges and use of a standard mixture) and continuous monitoring of the carbon dioxide production, we observed (Fig. 1) that the production of carbon dioxide starts almost immediately after the introduction of the oxidizing mixture in the solution. This indicates that carbon dioxide is not a final product of the oxidation process but one of the first in the conditions described here.

It has to be noted that the formation of CO and further oxidation to CO_2 in the presence of ozone is unlikely to happen under the experimental conditions used.[23] At room temperature and atmospheric pressure, the oxidation of CO to CO_2 is indeed very slow. No carbon monoxide has been observed by mass spectrometry (*m*/*z* 28, CO and 29, ^{13}CO) during the reaction at pH 2.5 either with sulfuric or acetic acid (see Section 3.2). Moreover, in all cases, neither a signal at *m*/*z* 30 (parent peak $[H^{13}CO]^+$ of $H^{13}CHO$) in the gas phase, nor HCHO in solution were detected. The reproducibility of our experiments at pH 2.5 (with H_2SO_4) allowed us to use NMR as an analytical tool to determine the quantity of formic acid formed. By cumulating the amounts of formic acid measured by this method during the ozonization of the three labeled glucose molecules, we could obtain an estimation of the overall amount of formic acid formed from these positions. The amount of carbon dioxide formed throughout the reaction is larger than the amount of formic acid (approx 0.075 mmol and 0.05 mmol, respectively, after 1 equiv of ozone consumed, and 0.22 mmol and 0.06 mmol after 2 equiv of ozone consumed). Carbon dioxide appears as the only gaseous by-product and as the main 1-carbon atom compound formed at pH 2.5.

The isotopic compositions of CO_2 produced from [1-^{13}C], [2-^{13}C] and [6-^{13}C] D-glucose, especially at the beginning of the reactions, were determined to establish whether it was the result of random degradations or of processes resulting in the loss of C-1, C-2 or C-6 specifically. The percentage of carbon dioxide derived from each labeled position was computed as described in Section 2. Values of $\%C^1O_2$ and $\%C^6O_2$ (averages of ten measurements) are plotted in Fig. 4 as a function of ozone consumption.

This figure clearly shows that decarboxylation reactions at C-1 and C-6 positions can account for at least 80% of the carbon dioxide formed during the ozonization of glucose at pH 2.5 (H_2SO_4). It has also to be emphasized that the contributions of C-1 and C-6 carbon atoms to the overall production of carbon dioxide are nearly equal. Even a larger part of the carbon dioxide may originate from C-6 (47%) rather than from C-1 (36%) carbon atom between 0.25 and 1 equiv of ozone consumed. [2-^{13}C] D-glucose has been also ozonized in sulfuric acid medium. The average values of $\%{}^*CO_2$ between 1 and 3 equiv of ozone consumed for the ozonization of glucose labeled in C-1, C-2 and C-6 positions are, respectively, 39, 20 and 37.5%. These values are only averages but the linear shape of the curves indicates that the decarboxylation rate is fairly constant after 0.5 equiv of ozone consumed which may imply a reproducible reaction mechanism throughout the reaction. It appears also that CO_2 originates almost exclusively from carbon atoms in C-1, C-2 and C-6 positions (more than 90–95% of the overall production of carbon dioxide) of the starting glucose molecule.

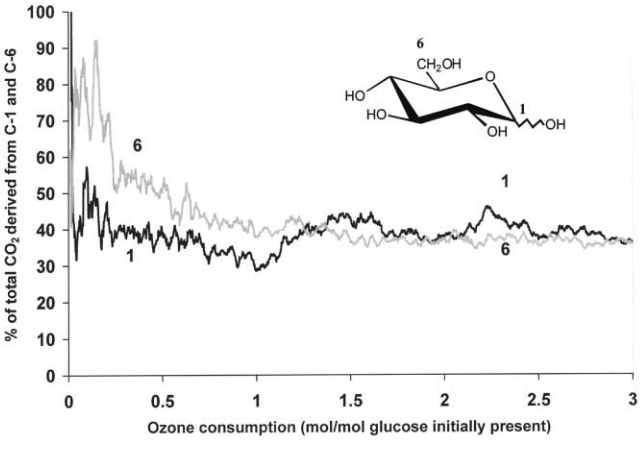


Fig. 4. Evolution of the percentages of carbon dioxide containing, respectively, C-1 ($^*C^1O_2$) and C-6 ($^*C^6O_2$) carbon atoms during the ozonization of glucose at pH 2.5 (H_2SO_4).

The two extremities of the glucose molecule (1 and 6) account for the major part of the carbon dioxide production which starts quickly after the beginning of the reaction. We can hence conclude that the C-6 carbon atom is affected by the presence of ozone as fast as the reducing end of the glucose molecule, at least as far as decarboxylation is concerned in the presence of sulfuric acid.

Some reports on ozonization of carbohydrates have focussed on the use of acetic acid as a solvent or to set the pH.[11,24] For example, acetic acid has been used as a solvent by Mbachu[25] in bleaching reactions with ozone. Holen[11] has recently investigated the effect of this acid used to set the pH. These authors demonstrated that acetic acid has a protective effect on a cellulose model compound (cellobiose) during ozonization. Holen and coworkers[11,18] also observed an enhanced production of gluconic acid during ozonization of glucose in the presence of acetic acid. It was of interest for us to investigate the influence of acetic acid on the production of carbon dioxide and to compare the results with those obtained in the presence of sulfuric acid. We ozonized [1-^{13}C] and [6-^{13}C] D-glucose in acetic acid, and as Holen and coworkers[11,18] had found, no significant difference was noted be-

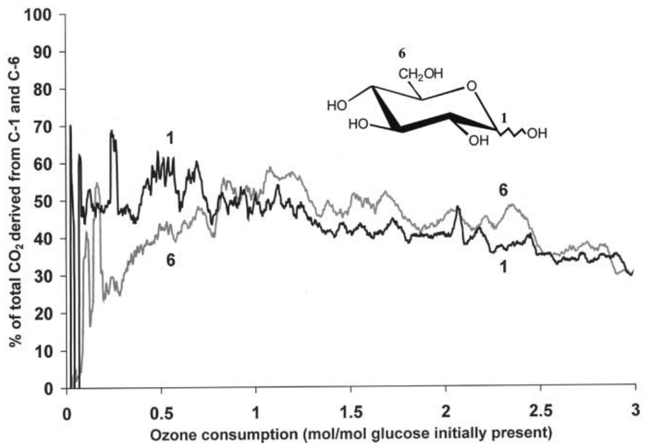


Fig. 5. Evolution of the percentages of carbon dioxide containing, respectively, C-1 ($^{13}C^1O_2$) and C-6 ($^{13}C^6O_2$) carbon atoms during the ozonization of glucose at pH 2.5 (AcOH).

tween the sulfuric acid and the acetic acid media with respect to the initial substrate (glucose) consumption, but the formation of byproducts with fewer than six carbon atoms was less important in the case of acetic acid.[26] We also observed that the consumption of ozone as a function of time followed the same trend in both acidic media.[26] However, as shown in Fig. 3, the production of carbon dioxide is much lower during ozonization in the presence of acetic acid (half the amount of carbon dioxide produced in the presence of sulfuric acid after 1 equiv of ozone consumed). The limitation of carbon dioxide formation is hence another aspect of the protecting effect of acetic acid on glucose. This is in good agreement with an enhanced stability of gluconic acid[11,18] if we assume that gluconic acid undergoes a Ruff type of reaction in this medium as well.

The plots of %*C^1O_2 and %*C^6O_2 values against consumption of ozone are given in Fig. 5. Carbon dioxide originating from C-6 is obviously observed and, it can therefore be concluded that C-6 is also oxidized by ozone in the presence of acetic acid.

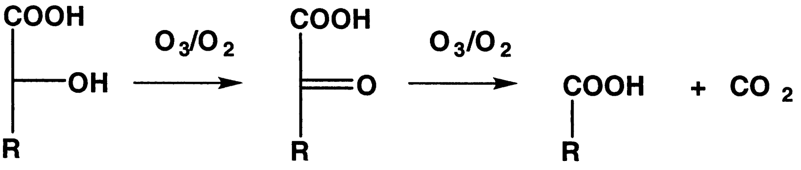
From the results obtained under acidic conditions, the decarboxylation reaction appears clearly as a key step in the glucose oxidation process after the oxidation of the reducing end. The occurrence of a reaction at the anomeric position followed by a reaction analog to the Ruff degradation is well supported by the analysis of the gas phase. Moreover, we can affirm that the same type of reaction must be considered for the oxidation of C-6 alcohol function.

Defaye et al.[9,10] proposed a stepwise process from experiments in neutral (pH 7) and slightly acidic conditions (pH 5). This process, depicted in Scheme 2, implies the formation of a carboxylic acid followed by oxidative decarboxylation via an α keto acid and occurs at both ends of the sugar molecule in such a way that aldaric and α-keto aldaric acids could be involved.

Our results clearly indicate that such a process is likely to happen even if a radical decomposition of an hydrotrioxyde at C-1 cannot be excluded.[8,15] In acidic conditions, this process would essentially affect C-1 and C-6 positions and can be extended to account for the liberation of C-2 as CO_2. The production of carbon dioxide containing the C-2 carbon atom can be explained by oxidation of arabinonic acid that can be formed by decarboxylation of D-*arabino*-hex-2-ulosonic acid (2-*keto*gluconic acid) as we suggested[8] in agreement with the general proposal of a stepwise mechanism[9,10] and by further oxidation of arabinose.

Ozonization reaction at pH 10.—The experimental conditions developed (see above) also enabled us to monitor the production of carbon dioxide at pH 10 using sodium hydroxide. The purge of the solution with the standard mixture of CO_2 in oxygen before the reaction ensured a pre-saturation of the solution so that once the reaction was started, at least a part of the carbon dioxide formed evolved fast enough from the solution to be analyzed by mass-spectrometry. The reaction at pH 10 was conducted only on [1-^{13}C] D-glucose and it should be noted that in these conditions again, neither carbon monoxide (determined by mass-spectrometry), nor labeled formic acid (determined by NMR) were observed. The production of carbon dioxide hence is low but not negligible as represented in Fig. 3.

The very limited production of compounds with only one carbon atom in alkaline conditions is in agreement with results reported by our laboratory.[7,8] However, we showed that some carbon dioxide is formed implying that decarboxylation is still involved in this oxida-



Scheme 2. Decarboxylation process via α-*keto*-carboxylic acids.

tion of glucose. The isotopic analysis of the evolved carbon dioxide again allowed a link to be made between CO_2 production and the formation of product derived by loss of C-1. The specificity of the ozonization in alkaline conditions (formation of carbonates) prevented us from determining the amount of carbon dioxide produced by the ozonizer as accurately as under low pH conditions. As a consequence, the production of carbon dioxide being low at the beginning of the reaction and the participation of C-1 carbon being very high, the calculated percentages $\%{*}C^1O_2$ were found to be even greater than 100%. Nevertheless the information displayed in Fig. 6 is consistent with more selective oxidation at the anomeric position and the formation of a carboxylic group at this position.[7,8]

The formation of carbon dioxide exclusively from the C-1 position at the beginning of the reaction under alkaline conditions strongly supports the mechanism proposed in Scheme 2. The decarboxylation of arabinonic acid to produce carbon dioxide containing the C-2 carbon atom could be related to the decrease of the percentage of carbon dioxide containing the C-1 carbon atom after 1 equiv of ozone consumed (Fig. 6).

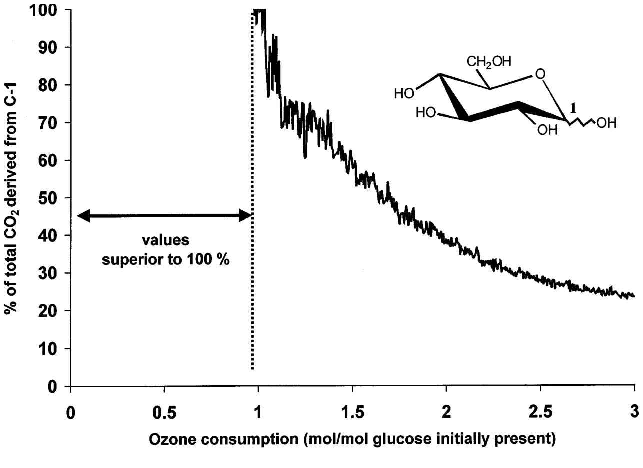


Fig. 6. Evolution of the percentage of carbon dioxide containing C-1 (${*}C^1O_2$) carbon atom during the ozonization of glucose at pH 10 (NaOH).

4. Conclusion

It is obvious that with glucose the decarboxylation is not a random reaction but is a key-step in the formation of by-products with fewer than six carbon atoms. The early formation of carbon dioxide indicates that this compound is not the result of the decomposition of secondary by-products. The detailed study carried out at pH 2.5 (sulfuric acid or acetic acid) leads to the conclusion that oxidations at C-6 and C-1 contribute similarly to the formation of products with fewer than six carbon atoms. Conversely, at high pH values, CO_2 is released with greater selectivity from the reducing end.

In all cases, correlations with literature and our results support some previous proposals and the limitation of the decarboxylation reaction has been illustrated to be another example of the 'protective effect' of acetic acid.

Our results clearly show that the formation of carbon dioxide should therefore be investigated in any application of ozone involving oxidation of carbohydrates. Reaction pathways will be further discussed along with the quantitative and isotopic analysis of the liquid phase in a forthcoming paper.[†]

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[†] For preliminary communications, see Ref. 26.

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