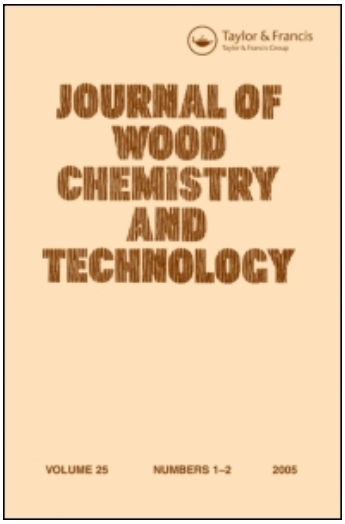

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Mechanisms of Oxidative Degradation of Carbohydrates During Oxygen Delignktcation. I. Reaction of Methyl β-D-Glucopyranoside with Photochemically Generated Hydroxyl Radicals

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MECHANISMS OF OXIDATIVE DEGRADATION OF CARBOHYDRATES DURING OXYGEN DELIGNIFICATION. I. REACTION OF METHYL β-D-GLUCOPYRANOSIDE WITH PHOTOCHEMICALLY GENERATED HYDROXYL RADICALS

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ABSTRACT

Hydroxyl radical is considered to be the major species causing degradation of carbohydrates during oxygen delignification. In this study, reactions involving a carbohydrate model compound and either photochemically generated hydroxyl radical or superoxide from potassium superoxide were carried out to investigate the cleavage of glycosidic linkages. Experiments show that hydroxyl radicals are responsible for the degradation of glycosidic linkages in methyl β-D-glucopyranoside by a substitution reaction displacing D-glucose. Once the glycosidic linkage is broken, reducing carbohydrates undergo a series of reactions forming aldonic acids and lower order aldoses. Control experiments established that no reaction occurs in the absence of UV light under otherwise identical conditions.

INTRODUCTION

Environmental concerns have heightened interest in chlorine-free bleaching sequences. Oxygen-alkali systems are of particular interest since the by-products are environmentally benign. Unfortunately, the use of oxygen as a bleaching chemical degrades carbohydrates as well as lignin, resulting in lower

pulp strength and yield. Obtaining a better knowledge of the reaction mechanisms involved in oxygen delignification will help us achieve the long term goal of this project, which is to promote lignin degradation while preserving carbohydrates. This paper describes a fundamental study of the degradation of carbohydrates by hydroxyl radicals.

Several oxygen species are present under oxygen delignification conditions. These species are shown in Figure 1. This complexity makes it difficult to decipher the specific reactions occurring during oxygen delignification.

In an effort to examine the effects of individual oxygen species, we have developed experiments that allow us to maximize one oxygen species at a time. A carbohydrate model compound, methyl β-D-glucopyranoside, was reacted with photochemically generated hydroxyl radical in an attempt to determine what chemistry can reasonably be attributed to this species when it is generated during oxygen delignification. We likewise have conducted several experiments with superoxide ion, generated from potassium superoxide, and a number of control reactions. While the reaction conditions used here differ from those of commercial oxygen delignification, the mechanistic information gained will be valuable for the development of more selective oxygen delignification systems.

EXPERIMENTAL

Materials

Methyl β-D-glucopyranoside hemihydrate, potassium superoxide, D-erythrose, 30% hydrogen peroxide, 50% sodium hydroxide, β-D-glucose, D-arabinose, D-gluconic acid, L-fucose, pyridine, Sylon BTZ, sodium bicarbonate, isopropanol, 2,4-dinitrophenylhydrazine, oxygen, and nitroblue tetrazolium were purchased commercially and were the best available reagent grade. Fresh

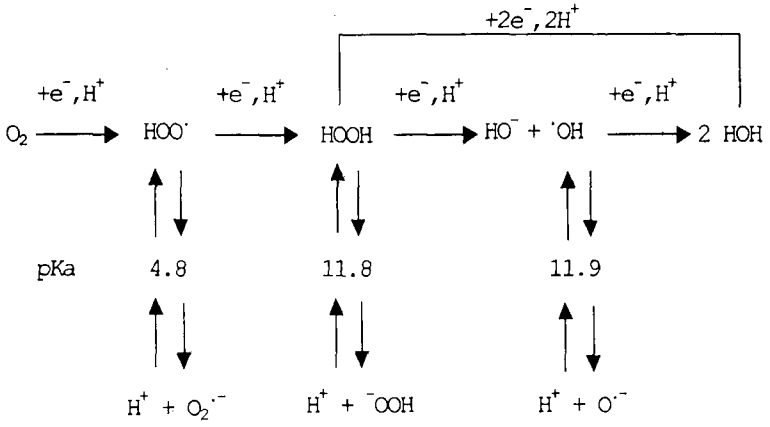

Figure 1. The reduction of oxygen during oxygen delignification.[1]

hydrogen peroxide was purchased frequently to assure nearly constant concentration. Sodium bicarbonate buffers (pH 10 and 11) were prepared according to literature procedures.[2] Water was ultrapure quality from a Barnstead still.

Hydroxyl Radical Generation

Hydroxyl radicals were produced by ultraviolet light irradiation of hydrogen peroxide. Methyl β-D-glucopyranoside (300 mg) was dissolved in a buffer system (8.85 mL buffer), or alkaline aqueous system (0.300 mL NaOH 50%, 8.55 mL H_2O). The total volume before hydrogen peroxide addition was 8.85 mL. Hydrogen peroxide (1.15 mL, 30%) was added for a total volume of 10.0 mL. The molar ratio of hydrogen peroxide to methyl β-D-glucopyranoside was always 10:1. After the addition of hydrogen peroxide, each reaction was placed immediately into a Rayonet photochemical reactor with 16 lamps emitting UV light at 254 nm. Each reaction ran for 90 min at room temperature (~35°C). Experiments were conducted at initial pHs of approximately 10, 11, and 12. The

pH 10 and 11 reactions were held alkaline throughout the reaction by the use of buffers. After each reaction, the aqueous mixture was analyzed using HPLC and freeze dried for GC/MS analysis.

Superoxide Anion Generation

Superoxide anions are formed when potassium superoxide is dissolved in water. Methyl β-D-glucopyranoside (300 mg) was dissolved in 9.805 mL of water. Sodium hydroxide (50%, 0.195 mL) was added to raise the pH to approximately 11. Potassium superoxide (solid; 1.05 g) was added slowly over 5 min. The molar ratio of potassium superoxide: sodium hydroxide: methyl β-D-glucopyranoside was 10:2.5:1. The reaction was continued another 85 min at room temperature. The reaction mixture was analyzed using HPLC and freeze dried for GC/MS analysis.

Hydrogen Peroxide and Hydroperoxy Anion Generation

Adding hydrogen peroxide to aqueous alkali generates hydroperoxy anions. The molar ratio of hydrogen peroxide: sodium hydroxide: methyl β-D-glucopyranoside was 10:2.5:1. Methyl β-D-glucopyranoside (300 mg) was dissolved in 8.0 mL of water and 1.15 mL hydrogen peroxide (30%). Sodium hydroxide (50%) was added to raise the pH to approximately 10 or 11. The total volume was then adjusted to 10.0 mL with water. The total reaction time was 90 min at room temperature at initial pH of 10 and 11. The mixture was analyzed using HPLC and freeze dried for GC/MS analysis.

General Oxygen Species

Reactions were also carried out with sodium hydroxide and oxygen, or ultraviolet light as blank reactions. All molar ratios, reaction times, volumes and temperatures were consistent with previous experiments. Molecular oxygen was

added by bubbling through the reaction mixture at a constant rate for the entire reaction time. Upon completion of reaction, samples were analyzed using HPLC and freeze dried for GC/MS analysis.

Pressure Vessel Reactions

A series of reactions was run using a pressure vessel. All molar ratios were maintained equivalent to previous experiments. Reactions were conducted with and without hydrogen peroxide at pH 10 and 12. Total reaction time was 90 min at 90°C, with 60 psig O_2 pressure. The reaction mixture was analyzed by HPLC and freeze dried for GC/MS analysis.

HPLC Analysis

HPLC analysis was carried out using Hewlett Packard 1100 series pumps, and a Hewlett Packard 1049A electrochemical detector. Separations were done according to the literature using a Dionex Carbopac PA1 column (4 x 250 mm) with one slight modification.[3] Potassium carbonate can be used to shorten run times. However, run times for our experiments were not long, and the use of potassium carbonate was unnecessary. Chemical quantitations were conducted using a calibration curve for each compound with L-fucose as an internal standard.

Formaldehyde analysis was conducted on a Hewlett Packard 1090 series HPLC with a UV detector. After derivatization with 2,4-dinitrophenylhydrazine (DNPH), the absorbance of the formaldehyde/DNPH derivative was measured at a wavelength of 370 nm.[4]

GC/MS Analysis

GC/MS electron impact and ammonia chemical ionization analyses were conducted using a Hewlett Packard 6890 series gas chromatograph and mass

spectrometer. All products were silylated before injection using Sylon BTZ (Supelco) silylating reagent. Pyridine (2 mL) and Sylon BTZ (1 mL) were added to the freeze-dried residue and allowed to react for 5 min at room temperature. The silylated mixture was injected directly onto the GC/MS. Separations were done on a HP-5 crosslinked phenyl methylsiloxane column (ID 0.25 mm, film thickness 0.25 μm, length 30.0 m). The following temperature gradient was used for product elution: 70°C for 6 min, to 175°C at a rate of 5°C/min, to 240°C at a rate of 2.5°C/min for a total run time of 53 min.

A Hewlett Packard 5890 series gas chromatograph with a flame ionization detector was used for methanol analysis. Separations were performed on a J&W DB-wax column with a length of 20.0 m, ID of 0.18 mm, and film thickness of 0.3 microns. A constant temperature of 45°C was held throughout each run. Isopropanol (0.5 mg/mL) was used as an internal standard.

Molecular Orbital Calculations

Molecular orbital calculations were made using a Silicon Graphics INDY II workstation. All energies were calculated using the Spartan V5.0 *ab initio* program and the 3-21G* basis set. Calculated heats of reaction discussed in this paper are based on equation 1.

$$\Delta H = (\Sigma E_p + \Sigma Z_p) - (\Sigma E_r + \Sigma Z_r) \quad (1)$$

ΣE_p and ΣE_r are the sums of the total energies for the products and reactants, respectively, in kcal/mol. ΣZ_p and ΣZ_r are the sums of the zero point energies (residual vibrational energy at 0°K) for the products and reactants, respectively, in kcal/mol. Solvation energies, when appropriate, were calculated using the (C-T) solvation model for AM1 semi-empirical calculations in Spartan.[5] The solvation energies were then added to the energy values in Equation 1 for each structure.

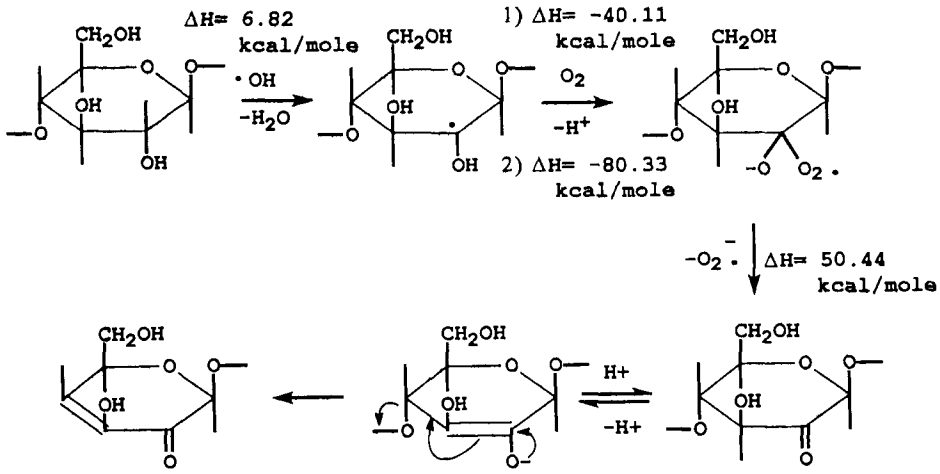

Figure 2. Oxidative cleavage of carbohydrates by hydroxyl radicals as proposed by Gierer.[6] Heats of reaction were calculated in the present study.

RESULTS AND DISCUSSION

Much previous work in the area of oxygen delignification suggests that hydroxyl radicals are most responsible for the degradation of carbohydrates during oxygen delignification. The mechanism proposed by Gierer and coworkers suggests that hydroxyl radicals degrade carbohydrates by an attack at the C-2 position of the anhydroglycose units in the polysaccharide chains by the pathway depicted in Figure 2.[6] The formation of a ketone in the glycose unit allows for the cleavage of the glycosidic linkage in the polysaccharide by β-elimination.

We examined the proposed mechanism using computational methods. The computational results are shown in Figure 2 together with the proposed mechanism. We found that the third step involving elimination of superoxide is energetically unfavorable. The objective of our work, therefore, was to investigate potential alternative mechanisms which are consistent with both

experimental and computational evidence. We chose to use photochemically generated hydroxyl radicals to ensure that the maximum concentration of this species was available to react with the methyl β-D-glucopyranoside.

UV/Hydrogen Peroxide Hydroxyl Radical Generation

Ultraviolet light dissociates hydrogen peroxide, producing two hydroxyl radicals as shown in equation 2.[7]

$$H_2O_2 + h\nu \rightarrow 2\,HO^{\cdot} \quad (2)$$

The hydroxyl radical is a very reactive oxidizing species capable of reacting with most organic compounds, typically by hydrogen abstraction.[7] However, the presence of hydrogen peroxide and hydroperoxy anions complicates the system. Hydroxyl radicals can react with both hydrogen peroxide and hydroperoxy anions through equations 3 and 4, producing hydroperoxy radicals and superoxide anions respectively.[8]

$$HO^{\cdot} + H_2O_2 \rightarrow H_2O + HO_2^{\cdot} \quad (3)$$

$$HO^{\cdot} + HO_2^{-} \rightarrow H_2O + {}^{\cdot}O_2^{-} \quad (4)$$

The reaction depicted by equation 4 is faster than the reaction in equation 3.[9] At a pH of approximately 11.8, where roughly half of the hydrogen peroxide is present as the conjugate base, the formation of superoxide anions should be more significant. At a lower pH, however, more hydroxyl radicals will be present to react with the carbohydrate model compound.

Superoxide Generation

Most research with potassium superoxide is done in organic solvents with crown ethers. Unfortunately, because of limited solubility of carbohydrates in

organic solvents, this method is unsuitable for our investigations. Therefore, the potassium superoxide must be added directly to water. Superoxide anions are formed by the dissociation of potassium superoxide in water according to equation 5.

$$KO_2 \rightarrow K^+ + \cdot O_2^- \quad (5)$$

To test for formation of superoxide anions, a probe reaction was conducted. Nitroblue tetrazolium was used as a probe reagent. When superoxide anions donate an electron to nitroblue tetrazolium, it turns from yellow to blue.[10] The addition of potassium superoxide to an aqueous solution of nitroblue tetrazolium changed the color of the solution from yellow to blue, indicating the presence of superoxide anions.

Control Reaction Results

Several blank reactions were conducted to provide proof that any reactions occurring in the potassium superoxide or UV/hydrogen peroxide system did not originate from species already present. Experiments were conducted with ultraviolet light, hydroxide anions, molecular oxygen, hydrogen peroxide and combinations of these species. In each case, HPLC analysis indicated a nearly quantitative recovery of methyl β-D-glucopyranoside, and GC/MS revealed no products.

Control reactions were also run in a pressure vessel to better simulate typical oxygen delignification conditions. Experiments were conducted with and without hydrogen peroxide at pH 10 and 12 under oxygen pressure (60 psig) at 90°C. In each reaction, HPLC analysis indicated a 100% recovery of methyl β-D-glucopyranoside and GC/MS analysis suggested no product formation. The control reactions and pressure vessel reactions indicate that molecular oxygen, hydroxide, hydrogen peroxide, and hydroperoxy anions are not capable of

degrading carbohydrates without a radical initiator, such as lignin or metals, present.

Superoxide Reactions

HPLC analysis of the reaction with superoxide anions showed a nearly quantitative recovery of methyl β-D-glucopyranoside. However, the more sensitive GC/MS analyses indicated the presence of reaction products. Very small levels of D-glucose, D-arabinose, D-gluconic acid, and D-erythronic acid were detected. The formation of these products is attributed to the presence of a small concentration of hydroxyl radicals. One possible pathway for the formation of hydroxyl radicals is through a reaction between superoxide anions and water (Eq. 6).

$$\cdot O_2^- + H_2O \rightarrow HOO^- + HO\cdot \quad (6)$$

Hydroxyl Radical Reactions

Quantitation

All of the control and superoxide experiments yielded approximately 100% recovery of the starting material as measured by HPLC analysis. However, UV/hydrogen peroxide reactions severely degraded the methyl β-D-glucopyranoside. The extent of the degradation is highly pH dependent. Table 1 shows the HPLC quantitation data for UV/HOOH reactions with methyl β-D-glucopyranoside at three different pH levels. We show also two major products to show the parallelism between methyl β-D-glucopyranoside recovery and product formation.

Methyl β-D-glucopyranoside is less reactive to UV/hydrogen peroxide treatments as pH increases. Even though the extent of the degradation decreases

TABLE 1

HPLC Quantitation of Glucose Recovery

pH		% Me β-D-gluco-	% D-Glucose	% D-Arabinose
Start	End	side recovered	Formed	Formed
9.5[a]	7.6	26.2	5.0	1.3
10.2[a]	9.9	45.5	4.6	1.1
11.8	12.4	69.1	1.1	0.2

a. Buffered, as described in the Experimental section.

at high pH, the overall chemistry is unchanged. The same products form, but at lower conversion as evidenced by less D-glucose and D-arabinose forming at higher pH than at lower pH. Reaction products and mechanisms will be discussed later in the paper.

The dependence of reactivity on pH is attributed to a difference in the distribution of the oxygen species present. As pH increases, Equation 4 should become more prevalent because hydrogen peroxide is present as its conjugate base. This increases the amount of superoxide anions present. Since superoxide anions do not degrade carbohydrates, methyl β-D-glucopyranoside is degraded less at high pH. When the pH is lowered, equation 3 is favored since the hydroperoxy anion is not present. However, as previously mentioned, equation 3 is slower than equation 4.[9] Thus at lower pH, fewer hydroxyl radicals are lost by reaction with hydrogen peroxide than with hydroperoxy anions at a higher pH. With more hydroxyl radicals present at a lower pH, more degradation of methyl β-D-glucopyranoside occurs.

Reaction Products

While varying pH did affect the amounts of products formed, it did not affect the nature of the products. The reaction products formed at pH 10 are the

TABLE 2

Products from Methyl β-D-Glucopyranoside and Hydroxyl Radical (GC/MS)

Glycolic Acid	Methyl β-D-Xylopyranoside
D-Glyceric Acid	D-Arabinonic Acid
2-Hydroxypropanedioic Acid	Arabinaric Acid[a]
D-Erythrose	D-Glucose[a]
D-Erythronic Acid Lactone	Methyl Glucuronic Acid
Malic Acid	Gluconic Acid Lactone
D-Erythronic Acid	D-Glucuronic Acid[a]
D-Arabinose[a]	D-Gluconic Acid
D-Arabinonic Acid Lactone	D-Glucaric Acid
2,3-Dihydroxysuccinic Acid	

a. Multiple diastereomers.

same as those formed at pH 12. Products, which were identified as their silylated derivatives using GC/MS, are listed in Table 2. All compounds listed were matched against reference mass spectra or authentic samples. Although a complete quantitation is not practical, the data indicate that D-glucose, D-gluconic acid, D-arabinose, D-arabinonic acid, D-erythronic acid, D-glyceric acid, and glycolic acid are the major products formed during the UV/HOOH treatment of methyl β-D-glucopyranoside. D-glucose is the single predominant product formed with a yield of up to 5%, as measured by HPLC (Table 1), depending on pH. Previous studies using carbohydrate model compounds and varying radical generation systems have identified several of the products listed in Table 2 as significant degradation products.[11-13]

PROPOSED REACTION MECHANISMS

The major products formed suggest four reaction mechanisms. The first step is the formation of D-glucose. It appears that the other major products are secondary products originating from D-glucose by aldonic acid formation and subsequent Ruff degradation reactions. A minor reaction pathway is the formation of uronic acids.

A UV/hydrogen peroxide reaction was performed on D-glucose under the same conditions. All of the major products formed during the reaction with methyl-β-D-glucopyranoside were formed with D-glucose as well. The other major products can be accounted for by oxidation of D-glucose to the corresponding aldonic acids and Ruff degradation leading to smaller carbohydrate fragments.

The formation of D-glucose from methyl-β-D-glucopyranoside is proposed to occur through a two step process. Breaking the carbon/oxygen bond in the methoxy substituent at the 1-position forms D-glucose. Hydroxyl radicals could initiate this according to the mechanism in Figure 3. The first step is a substitution reaction between a hydroxyl radical and the methoxy group forming D-glucose and a methoxyl radical. The hydroxyl radical attacks the anomeric carbon displacing the methoxyl radical. The methoxyl radical can then abstract a hydrogen from hydrogen peroxide or another hydrogen donor forming methanol and a hydroperoxy radical. The heats of reaction shown for each step in Figure 3 are obtained from MO calculations as described in the Experimental section. The calculations suggest that this mechanism is energetically viable. The first step in the mechanism is favored by over 6.5 kcal/mol over the abstraction of any aliphatic hydrogen from methyl β-D-glucopyranoside by hydroxyl radicals. There is also experimental evidence to support this mechanism, or the alternative attack of hydroxyl radical on the methyl carbon, displacing glucoxyl radical.

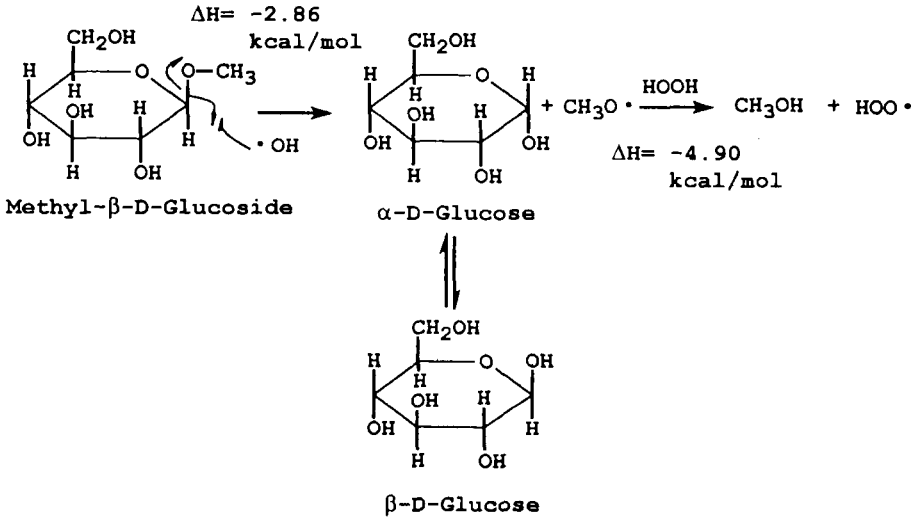

Figure 3. D-glucose formation.

For either mechanism to be correct, methanol must be produced in fairly large yields. GC analysis showed a 40% yield of methanol from the UV/hydrogen peroxide oxidation of methyl-β-D-glucopyranoside at pH 10.2. The substantial amount of methanol produced in the reaction strongly supports the mechanism depicted in Figure 3. Methanol, however, is also degraded by hydroxyl radicals.

Methanol in the presence of hydroxyl radicals and molecular oxygen forms formaldehyde.[14] The presence of formaldehyde also would indicate the formation of methanol during the reaction. HPLC with an ultraviolet detector easily detects formaldehyde after derivatization with DNPH. HPLC analysis showed a substantial amount of formaldehyde present after UV/hydrogen peroxide treatment of methyl-β-D-glucopyranoside. The detected formaldehyde also supports the predicted reaction mechanism in Figure 3.

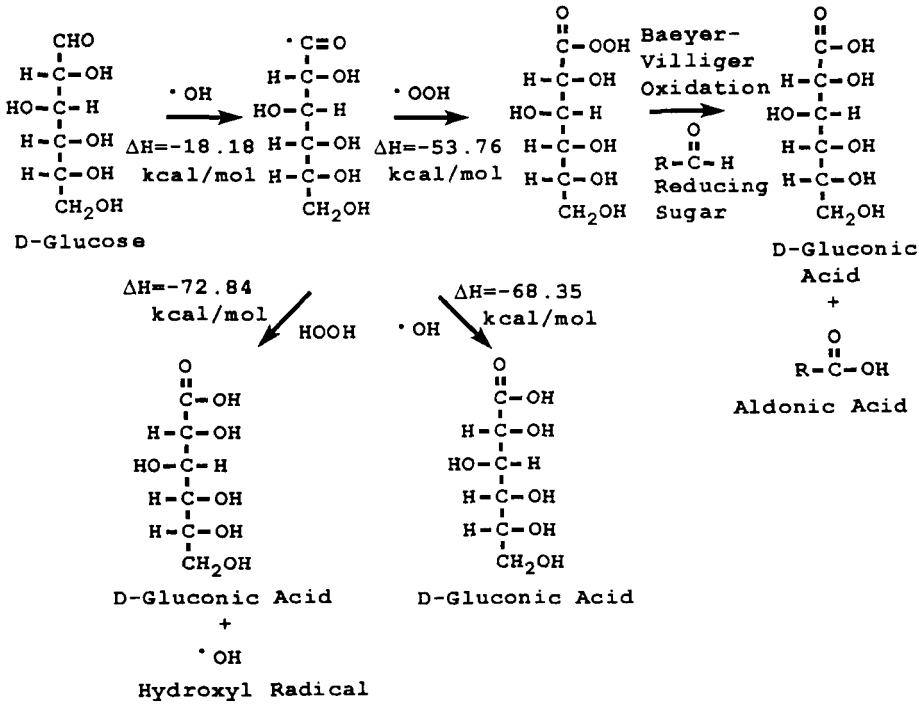

Figure 4. Aldonic acid formation.

Once D-glucose is formed, the other major products can be formed through oxidation to aldonic acids followed by Ruff degradation. Aldonic acids can form by either of two mechanisms depicted in Figure 4. Hydroxyl radicals can pull off hydrogen from the reducing carbon. Gluconic acid can then form by coupling with hydrogen peroxide, hydroxyl radical or through a reaction with a hydroperoxy radical and a subsequent Baeyer-Villiger reaction. The calculated reaction energies are all significantly exothermic supporting the mechanism depicted by Figure 4.

The Baeyer-Villiger oxidation is a reaction between a peroxy carboxylic acid and an aldehyde forming two carboxylic acids, and is shown in Figure 5.[15]

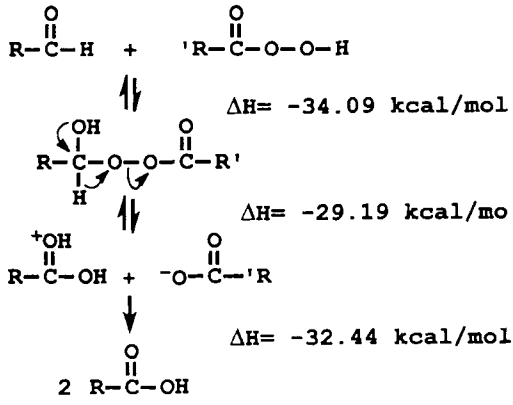

Figure 5. Baeyer-Villiger oxidation.

The calculated reaction energies listed in Figure 5 are for the reaction between D-glucose and the peroxy acid formed from gluconic acid. Because two charged species are forming after the second step, solvation is likely to strongly facilitate the reaction. Forming two charged species is unfavorable in the gas phase but favorable when solvation is taken into account (using the C-T model, as described in the Experimental section). The reaction energy for cleaving the structure in step two in Figure 5 is over 100 kcal/mol endothermic in the gas phase, but 29.19 kcal/mol exothermic when solvation energies are included in the calculation.

The formation of aldonic acids through the Baeyer-Villiger reaction facilitates the Ruff degradation. The Ruff degradation cleaves the C-1, C-2 bond forming carbon dioxide and the corresponding lower aldose. Although the mechanism is not completely understood, it is believed to be that depicted in Figure 6.[16]

The formation of D-glucose is the key to the formation of lower order aldoses and aldonic acids. Once D-glucose is present, it can undergo a series of

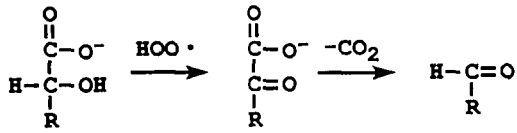

Figure 6. Ruff degradation.

oxidations and Ruff degradations to eventually form the lower order aldoses and aldonic acids identified by GC/MS.

Uronic acid formation is a minor reaction pathway in the UV/hydrogen peroxide degradation of methyl-β-D-glucopyranoside. Uronic acids form by oxidation of the primary alcohol at C6 by the mechanism in Figure 7. The calculated reaction energies that support the reaction mechanism depicted in Figure 7 are reasonable, but not as favorable as the other pathways discussed in this paper. The formation of acid groups at both C1 and C6 provides an explanation for the formation of the diacids, glucaric, arabinaric, and succinic acid as well as the uronic acid products identified experimentally.

Methyl β-D-glucopyranuronic acid is also capable of undergoing decarboxylation in a similar fashion to the aldonic acids, forming methyl β-D-xylopyranoside (Figure 8). However, very little of the methyl β-D-glucopyranuronic acid is converted to methyl β-D-xylopyranoside which is consistent with the calculated reaction energies for this mechanism. The abstraction of a hydrogen by a hydroxyl radical from the carboxyl group is not a very favorable step energetically.

CONCLUSIONS

The experiments conducted in this research support the notion that hydroxyl radicals are responsible for the degradation of carbohydrates during

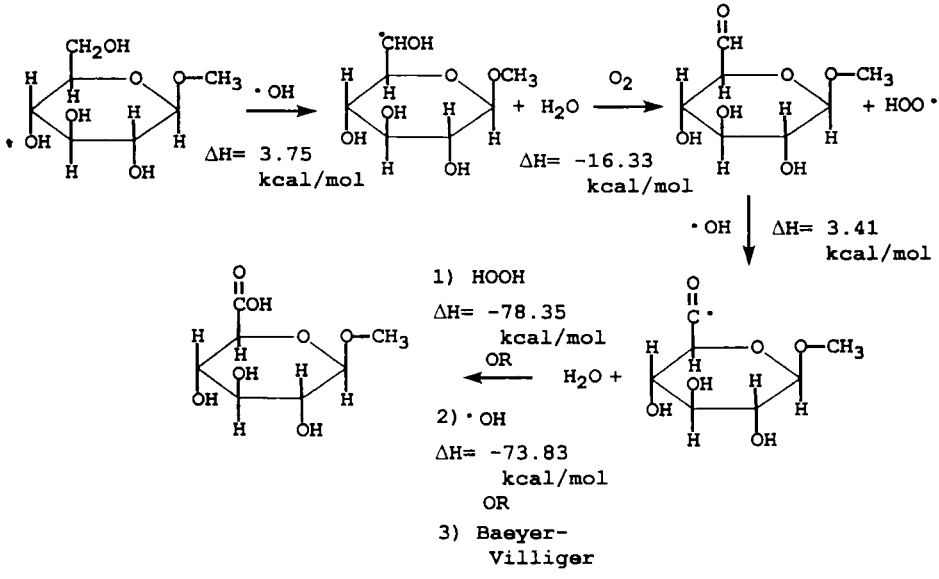

Figure 7. Uronic acid formation.

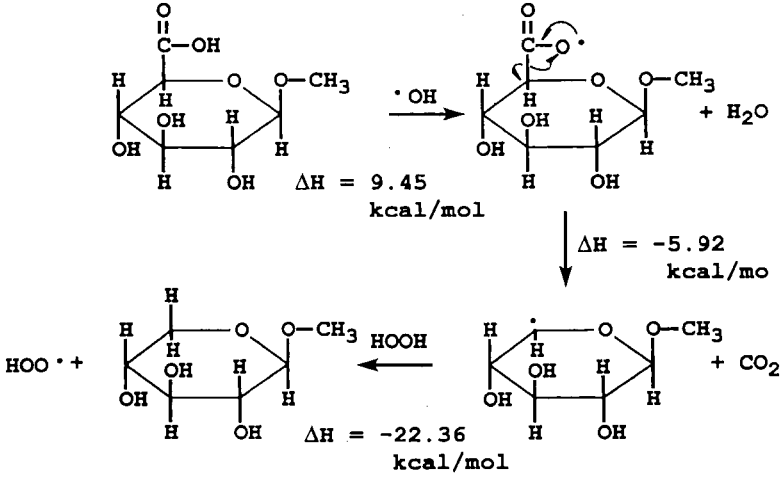

Figure 8. Decarboxylation.

oxygen delignification. However, molecular oxygen, superoxide anions, hydrogen peroxide, and hydroperoxy anions do not appear to degrade our model directly under the conditions of our experiments. The products identified thus far suggest a substitution reaction between methyl β-D-glucopyranoside and hydroxyl radicals as the mechanism for cleaving the glycosidic linkage. Experimental identification of methanol and theoretical calculations support the predicted mechanism. Once reducing carbohydrates form, they undergo a series of reactions producing aldonic acids and lower order aldoses. Uronic acid formation is also evident, but is not as significant as the other reaction pathways. No experimental evidence has been found to support the reaction mechanism depicted in Figure 2. This may be a result of the different experimental conditions in this work and previous research involving pulse radiolysis.

Work is in progress using carbohydrate dimer model compounds. Carbohydrate dimer compounds will provide a more realistic substrate on which to perform experiments. The goal of using the dimer compounds is to document the cleavage of a glycosidic linkage between two glucose units.

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