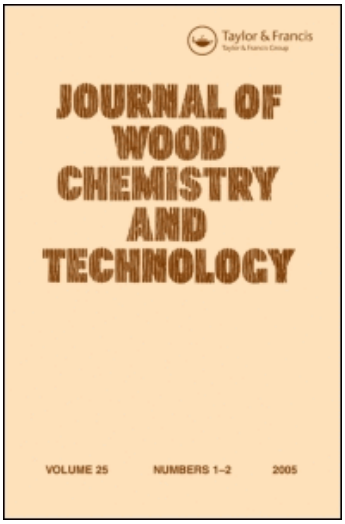

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MECHANISMS OF OXIDATIVE DEGRADATION OF CARBOHYDRATES DURING OXYGEN DELIGNIFICATION. II. REACTION OF PHOTOCHEMICALLY GENERATED HYDROXYL RADICALS WITH METHYL β-CELLOBIOSIDE

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ABSTRACT

Reactions involving methyl β-cellobioside and several oxygen species were used to investigate cleavage of glycosidic linkages in cellulose by reaction with photochemical hydroxyl radicals. The intent is not to reproduce delignification conditions, but rather to study the specific behavior of carbohydrate models toward hydroxyl radical. Experiments show that hydroxyl radicals are responsible for the degradation of glycosidic linkages in methyl β-cellobioside by substitution reactions displacing cellobiose, D-glucose, methyl β-D-glucoside, and methanol. Once the glycosidic linkages are broken, the reducing carbohydrates undergo a series of reactions forming

aldonic acids and lower order aldoses in the same manner as described previously.[1]

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 a 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.[2]

Previous research in which we studied the reactions of a monomeric carbohydrate model compound indicated that hydroxyl radicals are responsible for carbohydrate degradation during photolysis of hydrogen peroxide.[1] In the current work, a carbohydrate model compound, methyl β-cellobioside, was reacted with hydroxyl radicals produced by ultraviolet light irradiation of hydrogen peroxide to investigate the reaction products.

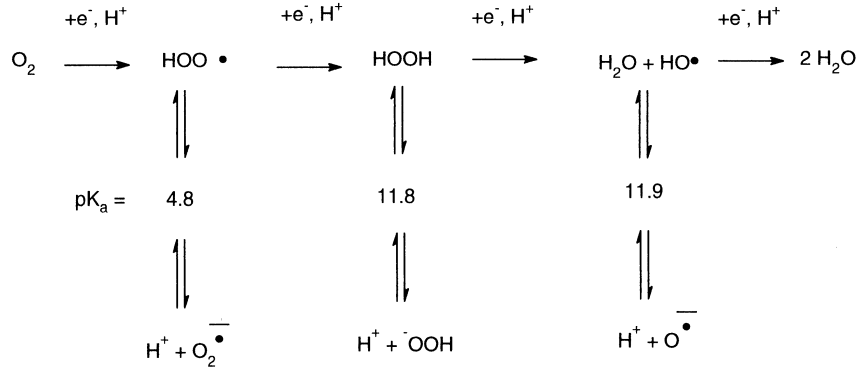

Figure 1. Oxygen chemistry in aqueous solution.[2]

EXPERIMENTAL

Materials

Methyl β-D-glucopyranoside hemihydrate, 30% hydrogen peroxide, 50% sodium hydroxide, β-D-glucose, D-arabinose, D-gluconic acid, L-focuse, pyridine, Sylon BTZ, sodium bicarbonate, isopropanol, β-cellobiose, β-cellobiose octaacetate, hydrobromic acid (33% in acetic acid), chloroform, silver nitrate, sodium sulfate, methanol, sodium thiosulfate, iodine, n-butylamine, and oxygen were purchased commercially and were best available reagent grade. Ultrapure water was from our own Barnstead Nanopur Still. Sodium bicarbonate buffer (pH 11) was prepared according to literature procedures.[3] Methyl β-cellobioside was synthesized according to the literature.[4] The purity of the synthesized methyl β-cellobioside was confirmed by gc-mass spec and ^{1}H NMR in d_6-DMSO. ^{1}H NMR ppm (#H); 3.00 (2H, multiplet), 3.18 (2H, multiplet), 3.29 (3H, multiplet), 3.39 (3H, singlet), 3.70 (3H, broad multiplet), 4.08 (1H, doublet, J = 7.5 Hz), 4.26 (1H, doublet, J = 7.5 Hz), 4.58 (1H, multiplet), 4.67 (1H, singlet), 5.01 (1H, doublet of doublets, J = 6.5 Hz), 5.18 (1H, doublet, J = 6.5 Hz), 5.24 (1H, doublet, J = 6.5 Hz). Not all OH absorptions could be detected owing to strong hydrogen bonding to the DMSO.

Hydroxyl Radical Generation

Hydroxyl radicals were produced by ultraviolet light irradiation of hydrogen peroxide. Methyl β-cellobioside (100 mg) was dissolved in a buffer system (3.11 mL buffer), or alkaline aqueous system (0.100 mL NaOH 50%, 3.01 mL H_2O). The total volume before hydrogen peroxide addition was 3.11 mL. Hydrogen peroxide (0.220 mL 30%) was added for a total volume of 3.33 mL. The molar ratio of hydrogen peroxide to methyl β-cellobioside was 10:1. After the addition of hydrogen peroxide, each reaction mixture was placed immediately into a Rayonet photochemical reactor with 16 lamps emitting UV light at 254 nm. Each reaction ran for 90 min. at ambient temperature (~35°C). Experiments were conducted at initial pHs of approximately 10 and 12. The pH 10 reactions were held alkaline throughout the reaction by the use of sodium bicarbonate buffer. After each reaction, the aqueous mixture was analyzed by HPLC and freeze dried for GC/MS analysis.

Control Reaction

A reaction was run using a pressure vessel. Methyl β-cellobioside (100 mg) was dissolved in a buffer system (3.11 mL buffer). Hydrogen peroxide (0.220 mL 30%) was added for a total volume of 3.33 mL. The molar ratio of hydrogen peroxide to methyl β-cellobioside was 10:1. 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 m x 250 mm) with one slight modification.[5] Chemical quantitations were conducted using a calibration curve for each compound with L-fucose as an internal standard. All analyses were carried out in duplicate, and in triplicate if the quantity of material permitted.

GC/MS Analysis

GC/MS electron impact 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. The procedure followed was that supplied with the reagent by the manufacturer: 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 into 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. 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, to 300°C at a rate of 10°C/min, and 300°C for 3 min, for a total run time of 62 min. All analyses were carried out in duplicate, and in triplicate if the quantity of material permitted.

A Hewlett Packard 5890 series gas chromatograph with a flame ionization detector was used for methanol analysis. Separations were performed on a DB wax column with a length of 20. 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. Analyses were run in duplicate.

^{1}H NMR

Proton NMR analysis was conducted using a Varian 300 MHz spectrometer. Dimethyl sulfoxide-d_6 was used as the solvent and internal standard.

Theoretical Calculations

Theoretical calculations were conducted 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.

$$\Delta H = (\Sigma Ep + \Sigma Zp) - (\Sigma Er + \Sigma Zr) \quad (1)$$

ΣEp and ΣEr are the sums of the total energies for the products and reactants, respectively, in kcal/mol. ΣZp and ΣZr are the sums of the zero point energies for the products and reactants, respectively, in kcal/mol. Carbohydrate dimer structure calculations were run using GAUSSIAN 94 (3-21G* basis set) on the Cray YMP1 supercomputer at Auburn University.

RESULTS AND DISCUSSION

Previous work suggests tht hydroxyl radicals are most responsible for the degradation of carbohydrates during oxgyen delignification.[1,6] The mechanism proposed by other researchers suggests that hydroxyl radicals degrade carbohydrates by an attack at the C-2 position in the polysaccharide chains by the pathway depicted in Figure 2. The information of a ketone in the polysaccharide chain allows for the cleavage of the glycosidic linkage by β-elimination.

Examining the proposed mechanism using computational methods, we found that the third step involving elimination of superoxide is energetically unfavorable. The objective of this work, therefore, was to investigate potential alternative mechanisms that are consistent with both experimental and computational evidence. The focus of this work was to identify products formed by the degradation of methyl β-cellobioside by hydroxyl radicals.

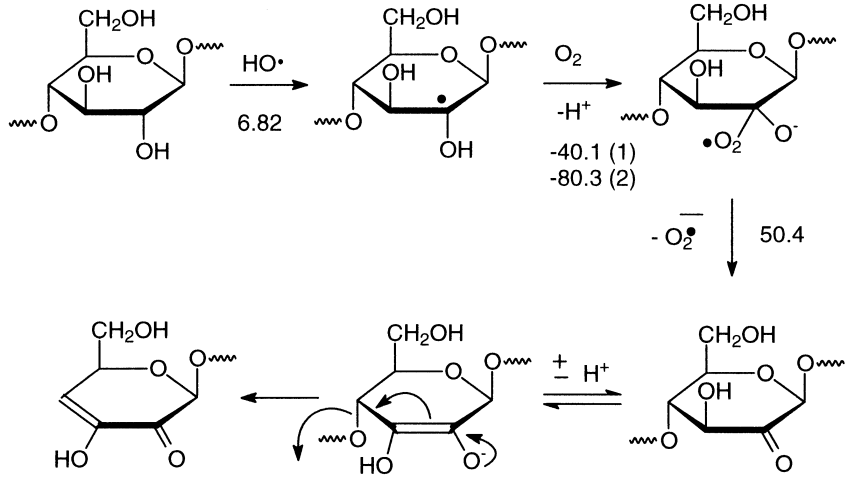

Figure 2. Literature mechanism for oxidative cleavage of carbohydrates by hydroxyl radicals.[6] (Numbers are computed heats of reaction in kcal/mol)

UV/Hydrogen Peroxide Hydroxyl Radical Generation

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

$$H_2O_2 + h\nu \rightarrow 2\,HO^{\cdot} \tag{2}$$

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

$$HO^{\cdot} + H_2O_2 \rightarrow H_2O + HO_2^{\cdot} \tag{3}$$

$$HO^{\cdot} + HO_2^- \rightarrow H_2O + {}^{\cdot}O_2^- \tag{4}$$

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.

Control Reaction Results

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-cellobioside and GC/MS analysis suggested no product formation. The control reactions suggest that molecular hydrogen, hydroxide ions, hydrogen peroxide, and hydroperoxy anions are not capable of degrading carbohydrates without a radical initiator, such as lignin or metal ions, present.

Hydroxyl Radical Reactions

Quantitation

The control experiments yielded a 100% recovery of the starting material as measured by HPLC analysis. However, UV/hydrogen peroxide reactions severely degraded the methyl β-cellobioside. The extent of the degradation is pH dependent. Table 1 shows the HPLC analytical data for UV/HOOH reactions with methyl β-cellobioside at two different pH levels.

Methyl β-cellobioside is less reactive to UV/hydrogen peroxide treatments as pH increases. While the extent of the degradation decreases at high pH, the overall chemistry is unchanged. That is, the same products form, but at a slower rate as evidenced by less D-glucose and D-arabinose forming at higher pH than at lower pH. Methyl β-D-glucoside is also less reactive at higher pH,[1] so more methyl β-D-glucoside is present at higher 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 prevalent because hydrogen peroxide is present as its con-

Table 1. Yields of Principal Products from Reaction of Hydroxyl Radical with Methyl β-Cellobioside

pH		% Me β-cello -bioside recovered	% Me β-D glucoside formed	% D-glucose formed	% D-arabinose formed
Start	End				
10.3[a]	9.1	14.9 ± 0.2	7.3 ± 0.1	7.11 ± 0.2	1.1 ± 0.1
12.2	12.7	27.5 ± 1.8	8.4 ± 0.6	2.0 ± 0.1	0.60 ± 0.1

[a] Buffered.

jugate base. This increases the amount of superoxide anions present. Since superoxide anions do not degrade carbohydrates, methyl β-cellobioside is degraded less at high pH. When the pH is lowered, Equation 3 is favored as the hydroperoxy anion is not present. However, as mentioned previously, 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-cellobioside occurs. A similar dependence on pH was observed in our earlier work with the monomeric carbohydrate model.[1]

Reaction Products

Reactions at different pH did not affect the overall chemistry. The reaction products formed at pH 10 are the same as those formed at pH 12. Products, which were identified as their silylated derivatives using GC/MS, are listed in Table 2. From the GC/MS peak areas, methyl β-D-glucoside, cellobiose, cellobionic acid, 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-cellobioside. Methyl β-D-glucoside and D-glucose are the predominant products formed with a yield of about 7-8%, as measured by HPLC (Table 1), depending on pH. In previous studies using carbohydrate

Table 2. Other Reaction Products Identified

Product	Match	Product	Match
Glycolic Acid	a	D-glucose[c]	a
D-glyceric Acid	a	Methyl Glucuronic Acid	b
3,4-Dihydroxybutanoic Acid	b	Malic Acid	a
D-erythronic Acid	a	Gluconolactone	b
D-arabinose[c]	a	D-glucuronic Acid[c]	b
D-arabinolactone	a	D-gluconic Acid	a
2,3-Dihydroxysuccinic Acid	b	D-gluconic Acid	a
D-arabinose[c]	a	D-glucaric Acid	b
D-arabinonic Acid	a	β-Cellobiose[c]	a
Arabinaric Acid[c]	b	Cellobionic Acid	a

a. Matched with authentic sample. b. Matched with library spectrum. c. Multiple diastereomers

model compounds and varying radical generation systems, we and other researchers have identified several of the products listed in Table 2 as significant degradation products.[1,10–12]

Proposed Reaction Mechanisms

The reaction products suggest two main degradation pathways of methyl β-cellobioside by hydroxyl radicals. The first pathway is a hydroxyl radical substitution reaction at the anomeric carbon forming cellobiose and methanol. The second degradative pathway is a hydroxyl radical substitution reaction at the glycosidic linkage between the two pyranose rings forming methyl β-D-glucoside and D-glucose. The other major reaction products are the result of secondary reactions with D-glucose, methyl β-D-glucoside, and cellobiose. As shown previously,[1] those products also are obtained when D-glucose and methyl β-D-glucoside are used as initial substrates.

A UV/hydrogen peroxide reaction was performed on cellobiose under the same conditions. All of the major products formed during the reaction with methyl β-cellobioside were formed with cellobiose as well, except methyl β-D-glucoside which, of course, would not be expected.

The formation of cellobiose from methyl β-cellobioside is proposed to occur through a two step process. Breaking the carbon/oxygen bond in the methoxy substituent at the 1-position forms cellobiose. 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 methoxyl group forming cellobiose and a methoxyl radical. The hydroxyl radical attacks the anomeric carbon displacing the methoxyl radical, or attacks the methyl displacing a cellobioxy radical. The methoxyl radical then can abstract a hydrogen from hydrogen peroxide or another hydrogen donor forming methanol and a hydroperoxyl radical; the cellobioxy radical can behave similarly. The heats of reaction shown for each step in Figure 3 are theoretical calculated energies; the reactions can be seen to be energetically favorable. Additional experimental evidence also supports this mechanism.

For this mechanism to be correct, methanol must be formed in fairly large yields. GC analysis showed a $45 \pm 3.7\%$ yield of methanol from the UV/hydrogen peroxide oxidations of methyl β-cellobioside at pH 10.3. The substantial amount of methanol produced in the reaction strongly supports the mechanism depicted in Figure 3.

To help establish the mechanism of this unusual cleavage, we undertook further computational work. The model chosen was 2-methoxytetrahydropyran, a simple six-membered ring containing a methoxyl group

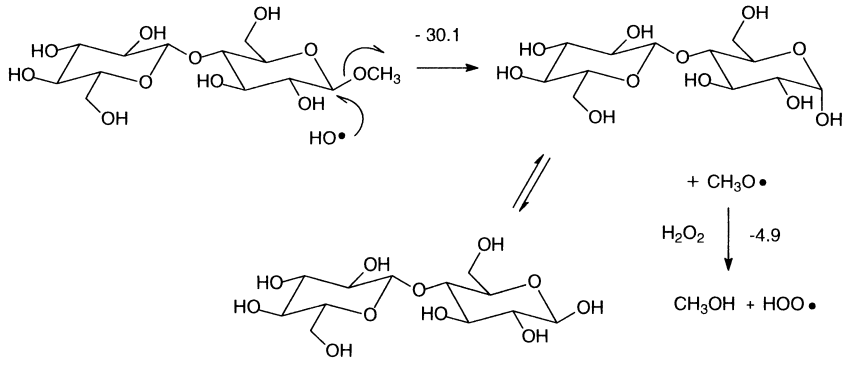

Figure 3. Proposed mechanism for cellobiose formation. (Numbers are computed heats of reaction in kcal/mol)

attached to an anomeric center. To further simplify calculations, we chose the UHF/AM1 semi-empirical Hamiltonian. We were able to locate three transition states, shown in Figure 4, that lead to cleavage of the methoxyl by atack of a hydroxyl radical. All three are characterized as transition states by the presence of a single imaginary frequency in their computed infrared spectra.

Axial attack on the anomeric carbon bearing an equatorial methoxyl leads to the first structure, which has an enthalpy of formation of -63.3 kcal/mol; equatorial attack on an axial methoxyl gives a transition state of $\Delta H_f = -63.9$ kcal/mol. The similarity is not unexpected, since the anomeric

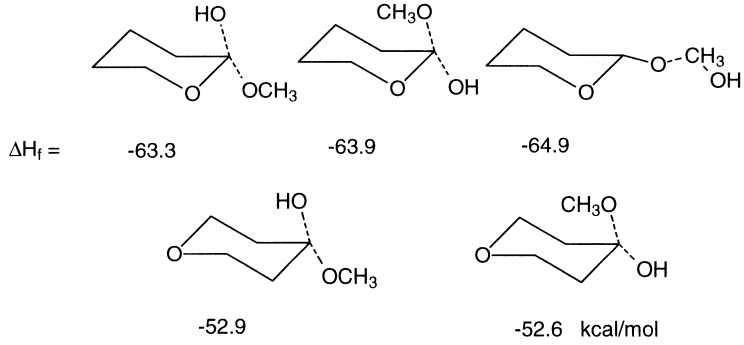

Figure 4. Transition states for methoxyl cleavage from UHF/AM1 molecular orbital calculations.

stabilization of axial OH and OCH_3 groups is well known to be similar. In the case of our experiments, we have only equatorial methoxyl present because making other ring substitutents axial creates a substantial barrier to ring inversion. The calculations suggest, however, that in simpler systems, both types of substituents should be cleaved readily.

The third transition state for methoxyl cleavage involves attack on the methyl carbon, with cleavage of the CH_3 - O bond. This transition state is of comparable stability to the other two (ΔH_f = -64.9 kcal/mol), and this mechanism may well compete with attack on the anomeric carbon. Although lacking in anomeric stabilization, this transition state is sterically far more accessible than the others.

To establish further the anomeric effect as the basis for the unusual cleavage, we also examined the remaining two structures in Figure 4, derived by axial and equatorial attack on 4-methoxytetrahydropyran, in which an anomeric effect cannot occur. These two transition states have calculated ΔH_f of -52.9 and -52.6 kcal/mol, respectively, suggesting a value of over 11 kcal/mol for the anomeric stabilization of the transition state for the observed cleavage.

Given the limitations of semi-empirical molecular orbital calculations, we consider these results as indicative rather than definitive. Higher level calculations are underway.

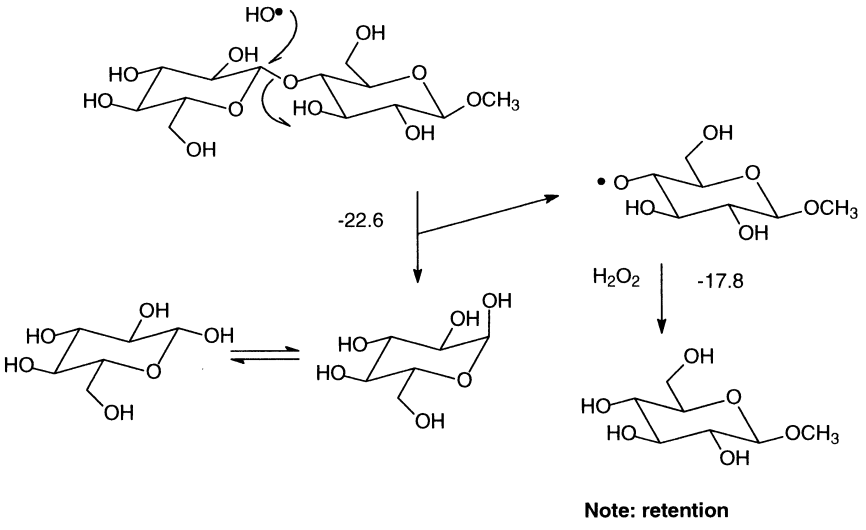

Figure 5. Proposed mechanism for formation of methyl β-D-glucoside and D-glucose. (Numbers are comuted heats of reaction in kcal/mole)

The second degradative pathway is very similar to the first, except the cleavage is between two pyranose rings instead of a ring and a methoxy group. A hydroxyl radical attacks the anomeric carbon displacing D-glucose and methyl β-D-glucoside oxy radical at C-4. The methyl β-glucoside radical then abstracts a hydrogen from hydrogen peroxide or some other hydrogen donor, forming methyl β-D-glucoside. The mechanism and calculated heats of reaction are depicted in Figure 5.

The heats of reaction shown for each step in Figure 5 are theoretical calculated energies. The calculations suggest that this mechanism is energetically favorable. There is also experimental evidence to support this mechanism. The hydroxyl radical attack must occur at the anomeric carbon because the formation of methyl β-D-glucoside is stereospecific. If the attack took place at the opposite carbon of the glycosidic linkage, methyl β-D-galactoside would be an expected product, but no methyl β-D-galactoside is detected as a product. This mechanism is very significant to oxygen delignification, because it suggests that hydroxyl radicals can simply cleave any glycosidic linkage in cellulose by the mechanism in Figure 5. Such random attacks would allow even a few hydroxyl radicals to greatly reduce cellulose chain length, and properties dependent upon it, such as viscosity.

CONCLUSIONS

The experiments conducted in this research support the view that hydroxyl radicals are responsible for the degradation of carbohydrates during oxygen delignification.[2] Molecular oxygen, hydrogen peroxide, and hydroperoxy anions do not appear to degrade carbohydrates directly. Also, previous studies suggest that superoxide anions do not degrade carbohydrates.[1] The products identified thus far suggest two substitution reactions between methyl β-cellobioside and hydroxyl radicals as the mechanism for cleaving the glycosidic linkages. Experimental identification of methanol, cellobiose, D-glucose, and methyl β-D-glucoside, and molecular orbital calculations support the proposed mechanisms. Once reducing carbohydrates form, they undergo a series of reactions producing aldonic acids and lower order aldoses similar to those seen in previous research.[1] 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 the previous research, which used pulse radiolysis to generate hydroxyl radicals. Evidence has recently been published suggesting that cellulose degradation during pulse radiolysis arises from direct ionization of the fibers rather than from hydroxyl radicals.[13]

ACKNOWLEDGEMENTS

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REFERENCES

1. D. F. Guay, B. J. W. Cole, R. C. Fort, Jr., J. M. Genco, and M. C. Hausman, J. Wood Chem. Technol., *20*(3), (2000).
2. J. S. Gratzl, Papier, *10A*, V1 (1992).
3. D. R. Lide, ed., *CRC Handbook of Chemistry and Physics*, 74th ed., pp. 8-42; CRC Press, Boca Raton, (1993).
4. M. L. Wolfrom, and S. Haq, Tappi J., *47*(4), 183, (1964).
5. P. J. Wright, and A. F. A. Wallis, Holzforschung, *50*, 518, (1996).
6. J. Gierer, Holzforschung, *51*, 34, (1997).
7. O. Legrini, E. Oliveros, and A. M. Braun, Chem. Rev., *93*(2), 671, (1993).
8. Y. Sun, A. F. A. Wallis, and and K. L. Nguyen, J. Wood Chem. Technol., *17*(1&2), 163, (1997).
9. G. V. Buxton, C. L. Greenstock, W. P. Helman, and A. B. Ross, J. Phys. Chem. Ref. Data, *17*, 513, (1988).
10. A. N. de Belder, B. Lindberg, and O. Theander, Acta Chem. Scand., *17*, 1012, (1963).
11. M. N. Schuchmann and C. von Sonntag, J. C. S. Perkins II, 1958, (1977).
12. J. Tronchet, A. Cier, M. Ravier, and M. C. Nofre, Academie Des Sciences, 2433, (1963).
13. J. Lind, G. Merenyi, and N. O. Nilvebrant, J. Wood Chem. Technol., *17*(1), 111 (1997).