

MECHANISMS OF OXIDATIVE DEGRADATION OF CARBOHYDRATES DURING OXYGEN DELIGNIFICATION. III. REACTION OF PHOTOCHEMICALLY GENERATED HYDROXYL RADICALS WITH 1,5-ANHYDROCELLOBITOL AND CELLULOSE

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ABSTRACT

Hydroxyl radicals, generated photochemically from hydrogen peroxide in aqueous base, have been shown to cleave directly glycosidic linkages in methyl β -D-glucoside and methyl β -cellobioside [1,2]. In this paper we show that hydroxyl radicals are responsible for the degradation of glycosidic linkages in 1,5-anhydrocellobitol and 2-methoxytetrahydropyran by substitution reactions displacing 1-deoxyglucose, D-glucose, tetrahydropyran-2-ol, and methanol. Once the glycosidic linkages are broken, reducing carbohydrates undergo a series of reactions forming aldonic acids and lower order aldoses in the same manner as described previously [1,2]. Under these same conditions, hydroxyl radicals degrade cellulose substantially as evidenced by viscosity loss.

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 yield, and possibly lesser pulp strength. 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 during oxygen delignification.

Several oxygen species are present under basic oxygen delignification conditions, including dioxygen, hydroxyl radical and superoxide anion radical. Literature reviews [3] as well as our previous research [1,2] suggest that hydroxyl radicals are the chief species responsible for carbohydrate degradation. In the present work, carbohydrate model compounds, 1,5-anhydrocellobitol, 2-methoxy tetrahydropyran, microcrystalline cellulose, and filter paper were reacted with hydroxyl radicals produced by ultraviolet light irradiation of hydrogen peroxide. The results strongly suggest that hydroxyl radicals can accomplish random cleavage of carbohydrate chains by attack at anomeric linkages, and that the commonly accepted mechanism for hydroxyl radical damage to cellulose is incorrect [1,2].

EXPERIMENTAL

Materials

2-Methoxytetrahydropyran, microcrystalline cellulose (MCC), Wheaton filter paper, 30% hydrogen peroxide, 50% sodium hydroxide, β -D-glucose, D-arabinose, D-gluconic acid, L-fucose, pyridine, Sylon BTZ (a commercial silylating agent that is a mixture of trimethylchlorosilane, N,O-bis(trimethylsilyl)acetamide, and trimethylsilylimidazole), β -cellobiose, methylene chloride, methanol, isopropanol, and oxygen were purchased commercially and were the best available reagent grades. Sodium bicarbonate buffer (pH 11) was prepared according to literature procedures [4]. 1,4-Anhydrocellobitol was graciously provided by Dr. Donald Dimmel of the Institute of Paper Science and Technology in Atlanta, Georgia.

Hydroxyl Radical Generation

Hydroxyl radicals were produced by ultraviolet light irradiation of hydrogen peroxide [5] at 254 nm, employing a Rayonet reactor with 16 lamps. 1,5-Anhydrocellobitol (100 mg) was dissolved in a buffer system (3.09 mL buffer), or alkaline aqueous system (0.100 mL NaOH 50%, 2.99 mL H₂O). The total volume before hydrogen peroxide addition was 3.09 mL. Hydrogen peroxide (0.240 mL 30%) was added for a total volume of 3.33 mL. The molar ratio of hydrogen peroxide to 1,5-anhydrocellobitol was 10:1. After the addition of hydrogen peroxide, each reaction mixture was placed immediately into the Rayonet reactor. Each reaction ran for 90 minutes 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.

2-Methoxytetrahydropyran (MTP)

2-Methoxytetrahydropyran (300 mg) was added to a buffer system (3.50 mL pH 10.8 buffer). Hydrogen peroxide (1.50 mL 30%) was added for a total volume of 5.00 mL. The molar ratio of hydrogen peroxide to MTP was 7.7 to 1. After the addition of hydrogen peroxide, each reaction mixture was placed immediately into the Rayonet photochemical reactor. Each reaction ran for 90 minutes at ambient temperature (~35 °C). After each reaction, the reaction mixture was extracted with dichloromethane (5 mL). The dichloromethane layer was injected directly into the GC/MS. The aqueous mixture was freeze-dried for silylation prior to GC/MS analysis.

Cellulose

Two forms of cellulose were used as model substrates for UV/H₂O₂ reactions, microcrystalline cellulose (MCC) and filter paper. MCC is readily available and was used directly in the UV/HOOH reactions. MCC (15 mg) was suspended in a buffer system (4.00 mL pH 10.8 buffer). Hydrogen peroxide (1.00 mL 30%) was added for a total volume of 5.00 mL. After the addition of hydrogen peroxide, each reaction mixture was placed immediately into the Rayonet reactor. Each reaction ran for 120 minutes at ambient temperature (~35 °C).

Filter paper was processed before being used in the reactions. A 15 mg/mL suspension was prepared by blending 750 mg of filter paper in 50 mL of water for 5 minutes. The filter paper stock suspension (1.0 mL) was added to a buffer system (3.00 mL pH 10.8 buffer). Hydrogen peroxide (1.00 mL 30%) was added for a total volume of 5.00 mL. After the addition of hydrogen peroxide, each reaction was placed immediately into the Rayonet reactor. Each reaction ran for 120 minutes at ambient temperature (~35 °C).

Twelve separate reactions were run for each cellulose substrate. Upon completion of the reaction period, the solution was filtered and washed with acetone. The reacted cellulose was then placed into an oven at 80°C for 1 hour. The collected cellulose fractions were combined to form three 50 mg samples for viscosity analysis.

Control Reaction

1,5-Anhydrocellobitol (100 mg) was dissolved in a buffer system (3.09 mL buffer). Hydrogen peroxide (0.24 mL 30%) was added for a total volume of 3.33 mL. The molar ratio of hydrogen peroxide to 1,5-anhydrocellobitol was 10:1. After the addition of hydrogen peroxide, the reaction mixture was placed immediately into a pressure vessel. Total reaction time was 90 minutes at 90 °C, with 60 psig O₂ pressure, initial pH was 10.2. 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 [6]. Chemical quantitations were conducted using a calibration curve for each compound with L-fucose as an internal standard.

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, following the procedure supplied with the reagent. Pyridine (2 mL) and Sylon BTZ (1 mL) were added to the freeze-dried residue and allowed to react for 5 minutes 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 μ m, 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, to 300 °C at a rate of 10 °C/min, and 300 °C for 3 min, for a total run time of 62 minutes.

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 20.0 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.

Viscosity Analysis

Cellulose samples (50 mg oven-dried) were weighed out into separate vials. Water (5 mL) was added and the solution was stirred until well dispersed. While the cellulose and water were stirring, the viscometer was placed into a water bath (25°C) with the top bubble submerged. When the cellulose was well dispersed (no fiber bundles), cupriethylenediamine (5 mL) was added while continuously purging with nitrogen gas. The vial was closed tightly and stirred for 15 minutes. Upon completion, 7 mL of the solution was pipetted into a Cannon-Fenske viscometer and allowed to come to temperature. Cannon-Fenske Models 50 and 150 viscometers were used. Viscosity in centipoise (cps) was calculated by Equation 1.

$$\text{Viscosity} = K \times 1.052 \times T \quad (1)$$

K is the constant for the viscometer which is recorded before analysis. T is the time required for the solution to flow between the two lines, in seconds. The other constant (1.052) is the density of the cupriethylenediamine cellulose solution.

Molecular Orbital Calculations

Molecular orbital calculations were carried out using a Silicon Graphics INDY II workstation. All energies were calculated using the Spartan V5.0 [7] *ab initio* program and the 3-21G* basis set. Calculated heats of reaction discussed in this paper are based on equation 2.

$$\Delta H = (\sum E_p + \sum Z_p) - (\sum E_r + \sum Z_r) \quad (2)$$

$\sum E_p$ and $\sum E_r$ are the sums of the total energies for the products and reactants, respectively, in kcal/mol. $\sum Z_p$ and $\sum Z_r$ 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 [8] (3-21G* basis set) on the Cray SV1 supercomputer at Auburn University.

Transition states were calculated using the B3LYP/6-31G(*) basis set in Gaussian 94 for *ab initio* calculations. Transition states were characterized by the presence of a single imaginary frequency in their computed infrared spectra.

RESULTS AND DISCUSSION

Since previous work suggests that hydroxyl radicals are most responsible for the degradation of carbohydrates during oxygen delignification [1,2,9], the focus of this work was to identify products formed by the reaction of 1,4-anhydrocellobitol and 2-methoxy tetrahydropyran with hydroxyl radicals, and to determine if microcrystalline cellulose and filter paper are degraded by hydroxyl radicals. The conditions used are not those of oxygen delignification, and are not intended to be. They are chosen to maximize the formation of hydroxyl radical.

Because of limited substrate only one control experiment was conducted with 1,4-anhydrocellobitol and hydrogen peroxide at pH 10 under oxygen pressure (60 psig; 4.1×10^5 Pa) at 90 °C, without UV irradiation. In the control reaction, HPLC analysis indicated a 100% recovery of 1,4-anhydrocellobitol and GC/MS analysis suggested no product formation. The control reaction indicates that molecular oxygen, hydroxide ions, hydrogen peroxide, and hydroperoxy anions are not capable of degrading 1,4-anhydrocellobitol without a radical initiator, such as light, lignin, or metal ions, present.

However, UV/hydrogen peroxide reactions severely degraded the 1,4-anhydrocellobitol. The extent of the degradation is pH dependent. Table I shows the major products for UV/HOOH reactions with 1,4-anhydrocellobitol at two different pH levels. Anhydrocellobitol becomes less reactive to UV/hydrogen peroxide as pH increases. While the extent of the degradation decreases at high pH, the overall chemistry is unchanged; that is, the same products are formed. The dependence of reactivity on pH is attributed to conversion of hydroxyl radical to superoxide by reaction with the conjugate base of hydrogen peroxide, as described previously [1,2, 10]. Because 1-deoxyglucose does not contain an anomeric carbon, it is less reactive to hydroxyl radicals than all previous carbohydrates studied, and thus its yield is similar at both high and low pH.

TABLE I. Effect of pH on Products from Reaction of Anhydrocellobitol

<i>pH</i>		% <i>Anhydrocellobitol Recovered</i>	% <i>Deoxyglucose Formed</i>	% <i>D-Glucose Formed</i>	% <i>D-Arabinose Formed</i>
<i>Start</i>	<i>End</i>				
10.19*	8.97	8.9	7.0	9.0	1.2
11.67	12.45	34.7	8.7	2.8	0.8

* Buffered

Reactions at different pH did not affect the overall chemistry; that is, the reaction products formed at pH 10 are the same as those formed at pH 12. Products identified as their silylated derivatives using GC/MS are listed in Table II. The predominant products are 1-deoxyglucose and D-glucose, formed in yields of up to 8%, as measured by HPLC (Table I), depending on pH. Other significant products are D-arabinose, D-arabinonic acid, D-erythronic acid, D-glyceric acid, D-sorbose, and glycolic acid.

TABLE II. Products Identified from Reaction of Anhydrocellobitol with Hydroxyl Radical

<i>Product</i>	<i>Match*</i>	<i>Product</i>	<i>Match*</i>
Glycolic Acid	A	D-Xylose [#]	A
D-Glyceric Acid	A	D-Xylonic Acid	A
3,4-Dihydroxybutanoic Acid	L	D-Arabinonic Acid	A
D-Erythronic Acid	A	1-Deoxyglucose	A
2,3,4-Trihydroxybutanoic Acid	L	D-Sorbose	A
D-Arabinose [#]	A	D-Glucose [#]	A
D-Arabinonic Acid Lactone	A	D-Gluconic Acid	A
2,3-Dihydroxysuccinic Acid	L	D-Cellobiose	A

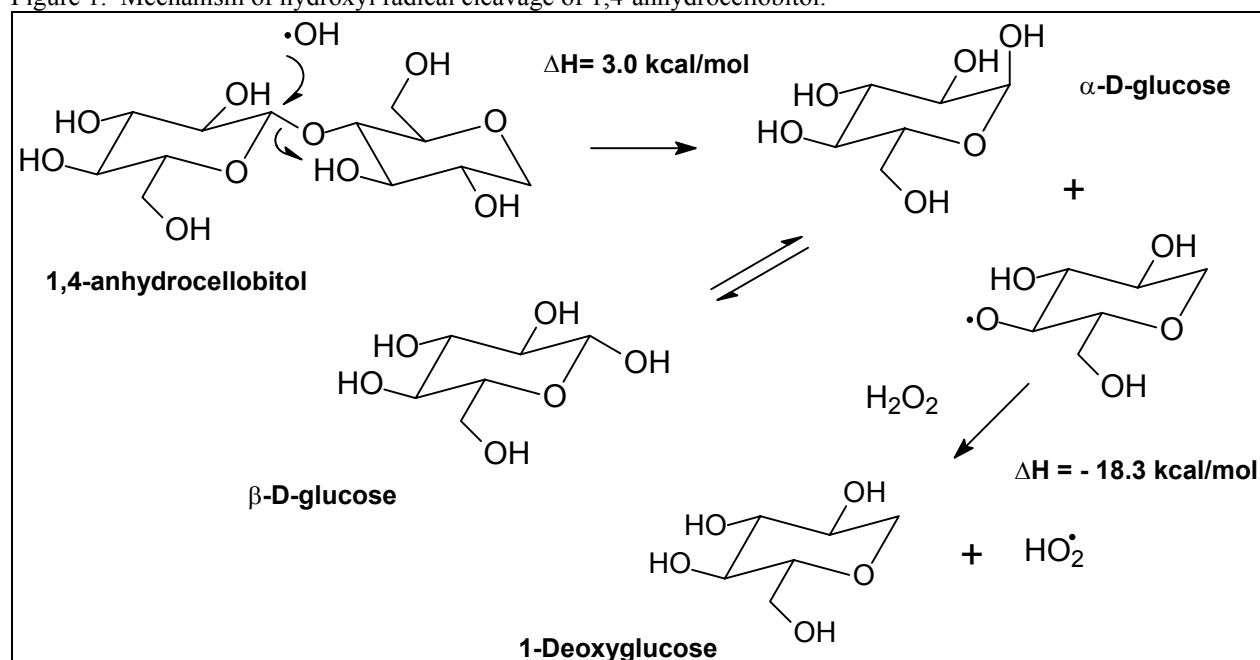
* A = authentic sample; L = library; # = multiple diastereomers

Proposed Reaction Mechanisms

The reaction products suggest one main degradation pathway of 1,4-anhydrocellobitol by hydroxyl radicals. Hydroxyl radicals undergo a substitution reaction with 1,4-anhydrocellobitol at the anomeric carbon forming D-glucose and 1-deoxyglucose. Hydroxylation at the C-1 position yielding D-cellobiose is a minor reaction pathway. The other major reaction products are the result of secondary reactions of hydroxyl and hydrogen peroxide with D-glucose and 1-deoxyglucose. D-sorbose is a secondary product from 1-deoxyglucose. As shown previously, the other products are obtained when D-glucose is used as the initial substrate [1,2].

The formation of D-glucose and 1-deoxyglucose from 1,4-anhydrocellobitol is proposed to occur through a two step process (Figure 1). Breaking the anomeric bond between the two pyranose rings forms D-glucose and 1-deoxyglucosy radical. The 1-deoxyglucosy radical then abstracts a hydrogen from hydrogen peroxide or some other substrate forming 1-deoxyglucose. The calculated (UHF/3-21G*) reaction energies are not as favorable as for our other model compounds, but are not unreasonable.

Figure 1. Mechanism of hydroxyl radical cleavage of 1,4-anhydrocellobitol.

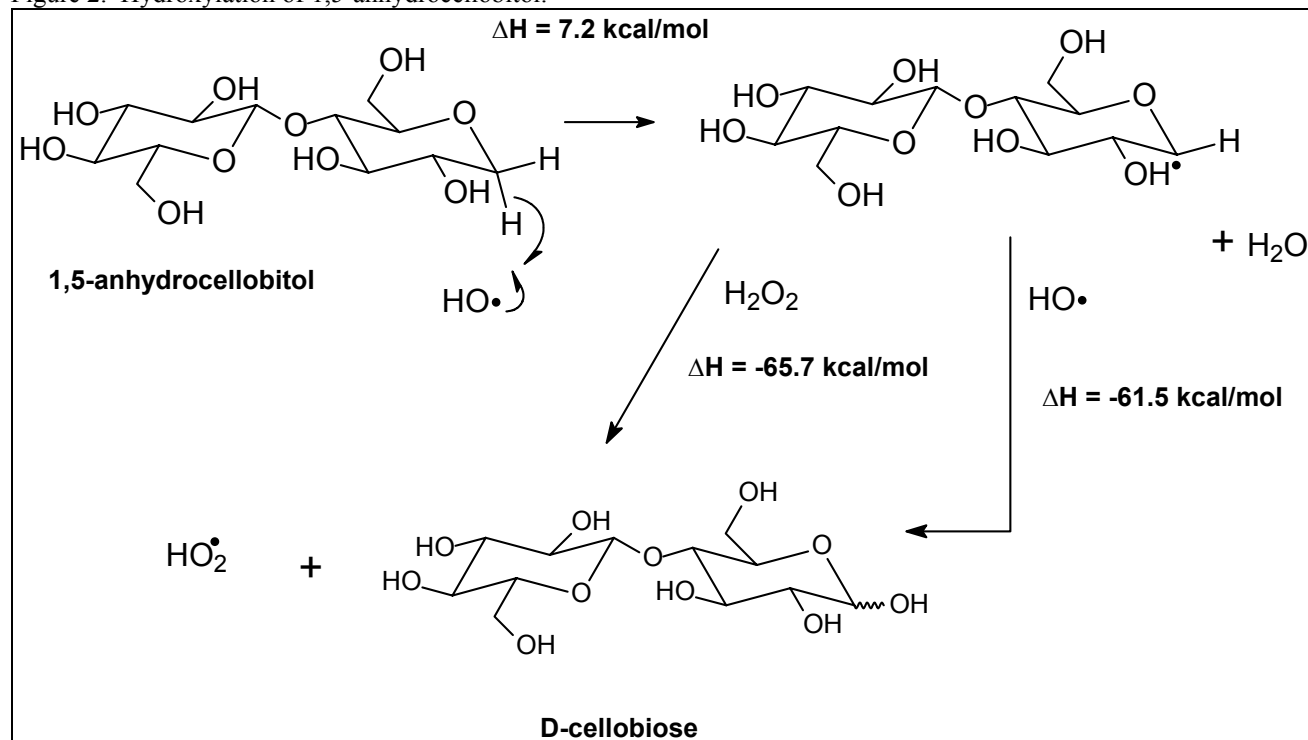


The hydroxyl radical attack must occur at the anomeric carbon because the formation of 1-deoxyglucose is stereospecific. If the attack took place at the opposite carbon of the glycosidic linkage, 1-deoxygalactose would be expected, but no 1-deoxygalactose is detected.

The mechanism in Figure 1 supports the notion that hydroxyl radicals can simply cleave any randomly encountered glycosidic linkage in cellulose. All anomeric linkages, whether between two rings or a ring and methoxy group, are susceptible to cleavage.

Hydroxylation yielding D-cellobiose is a minor reaction pathway that proceeds via two steps. The proposed reaction mechanism is depicted in Figure 2. The first step involves a hydrogen abstraction at the C-1 position in 1,4-anhydrocellobitol by a hydroxyl radical. The second step is either a radical coupling reaction between the newly formed alkyl radical and another hydroxyl radical or a reaction between the alkyl radical and hydrogen peroxide. The reaction between the alkyl radical and hydrogen peroxide should be favored since the very low concentrations of radical species make radical couplings unlikely [11]. The H-abstraction step is slightly endothermic, but the reaction with hydrogen peroxide is predicted to be very exothermic.

Figure 2. Hydroxylation of 1,5-anhydrocellobitol.



2-Methoxytetrahydropyran was used as a model compound to investigate whether an anomeric linkage could be broken without all of the hydroxyl groups present on carbons 2,3,4, & 6. No control reactions were run with 2-methoxy-tetrahydropyran because no chemistry was observed in control reactions with the previous compounds [1,2]. Indeed, 2-methoxytetrahydropyran was degraded during UV/HOOH experiments.

Table 3 lists the products identified in two separate fractions analyzed by GC/MS, a methylene chloride extraction layer, and the aqueous layer silylated after freeze-drying. A major difference between 2-methoxytetrahydropyran and the other carbohydrate model compounds used in this study is the formation of hydroxylated products. Several mono- and dihydroxylated 2-methoxytetra-hydropyran products were identified. The most abundant products are 5-hydroxypentanoic acid and its oxidation product, pentanedioic acid.

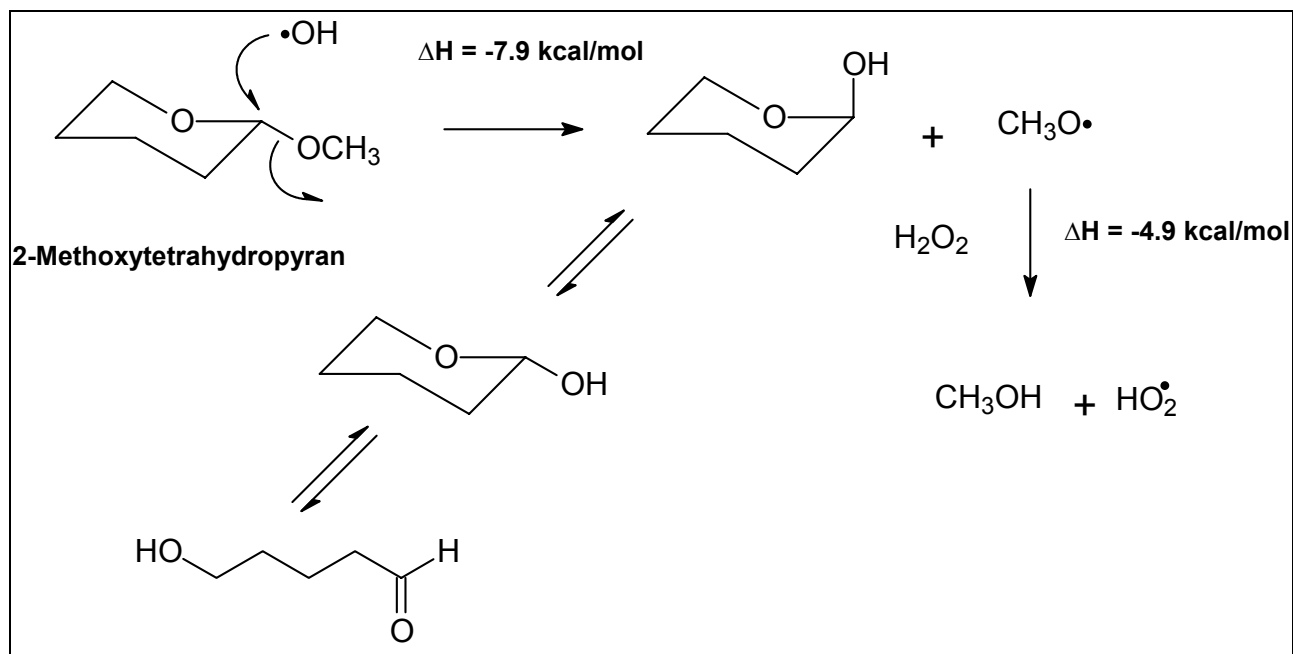
TABLE III. Reaction Products from Methoxytetrahydropyran with UV/HOOH

<i>Product</i>	<i>Match*</i>	<i>Product</i>	<i>Match*</i>
2-Methoxy-5,6-dihydropyran	A	5-Hydroxypentanal	A
Hydroxymethoxytetrahydro-pyran [#]	L	4-Hydroxybutanoic Acid	L
5-Hydroxypentanoic Acid	A	Butanedioic Acid	A
Pentanedioic Acid	A	Dihydroxypentanoic Acid ^{\$}	L
Dihydroxymethoxytetra-hydropyran ^{&}	L		

A = authentic sample; L = library; # = all 10 regio- and stereoisomers; \$ = two regioisomers; & = five regio- and stereoisomers

The demethoxylation mechanism (Figure 3) is the same mechanism proposed for the formation of D-glucose from methyl- β -D-glucoside [1]. The first step is a substitution reaction between a hydroxyl radical and the methoxy group forming tetrahydropyran-2-ol and a methoxyl radical. The displaced 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 derived from UHF/3-21G* *ab initio* calculations.

Figure 3. Mechanism of hydroxyl radical cleavage of 2-methoxytetrahydropyran.

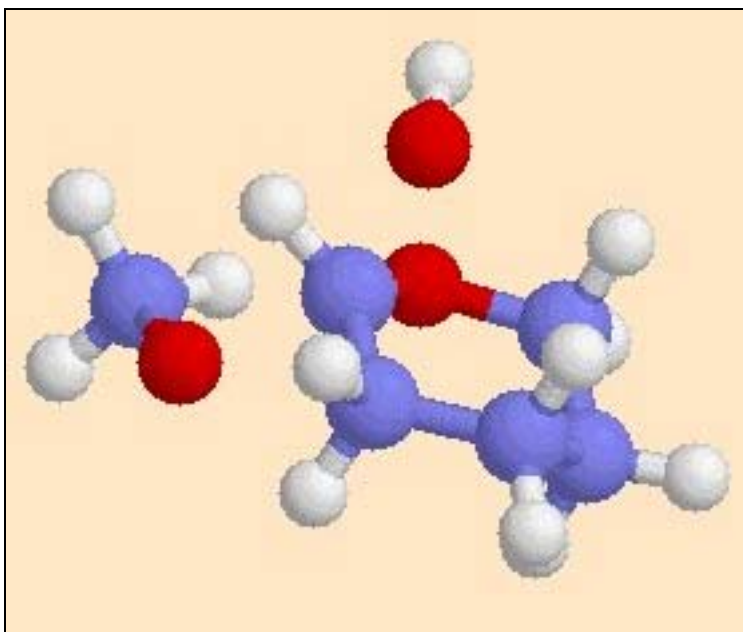


The calculations suggest that this mechanism is energetically viable. The first step in the mechanism is computed to be favored by over 11 kcal/mol compared to the abstraction of any aliphatic hydrogen from 2-methoxytetrahydropyran by hydroxyl radicals.

If this mechanism is correct, methanol should be present in fairly large yields. GC analysis showed a 18% yield of methanol from the UV/hydrogen peroxide oxidations of 2-methoxytetrahydropyran. The significant amount of methanol produced in the reaction strongly supports the mechanism depicted in Figure 3.

Previously, transition states for the demethoxylation of 2-methoxytetrahydropyran were calculated using the semi-empirical Hamiltonian [2]. The semi-empirical Hamiltonian is a rather low level of theory for computational experiments. Therefore *ab initio* level computational experiments using the B3LYP/6-31G(*) basis set were run to locate the transition state for the demethoxylation of 2-methoxytetrahydropyran. The transition state found (Figure 4) is characterized by the presence of a single imaginary frequency in its computed infrared spectrum.

Figure 4. B3LYP/6-31G* transition state for hydroxyl radical cleavage of 2-methoxytetrahydropyran.



Intrinsic reaction coordinate (IRC) calculations at the same level of theory were also conducted on this transition state, taking five steps toward product and five back toward reactants, to establish that the transition state found actually lies on the reaction coordinate for the cleavage reaction.

Hydroxylation of 2-methoxytetrahydropyran during UV/HOOH reactions is proposed to occur by the same mechanism as with 1,4-anhydrocellobitol. First an aliphatic hydrogen is abstracted by a hydroxyl radical. The abstraction of any aliphatic hydrogen from 2-methoxytetrahydropyran is computed to be slightly endothermic and the limiting step in hydroxylation.

Once the alkyl radical forms, it either couples with another hydroxyl radical, or, more likely, reacts with hydrogen peroxide forming the corresponding alcohol and a hydroxyl radical. Both reaction pathways are expected to be rather exothermic as calculated in the hydroxylation of 1,4-anhydrocellobitol.

Finally, we sought to establish that the cleavage process described has some relation to the degradation of cellulose occurring during oxygen delignification. Two types of cellulose therefore were used as model compounds to investigate whether hydroxyl radicals could break longer insoluble cellulose chains. Microcrystalline cellulose (MCC) and filter paper were chosen as cellulose model compounds to simulate pulp during oxygen delignification.

The large size of the MCC and filter paper cellulose molecules means that analysis by GC/MS is not especially useful. Nonetheless, trace amounts of D-glucose were identified after UV/HOOH reactions with both cellulose substrates. To get a better idea about how much cellulose degradation occurred during the UV/HOOH treatment of both models, pulp viscosity was measured before and after reaction. As fiber length decreases, pulp viscosity should decrease as well.

The results of viscosity measurements on untreated and UV/HOOH-treated cellulose are shown in Figures 5 and 6. The viscosity of the filter paper (Figure 5) is greatly reduced by the hydroxyl radicals produced during the UV/HOOH experiments. The MCC viscosity is also lowered (Figure 6), but the difference is small because the initial viscosity is very low.

Figure 5. Viscosity change of filter paper suspension upon reaction with UV/HOOH.

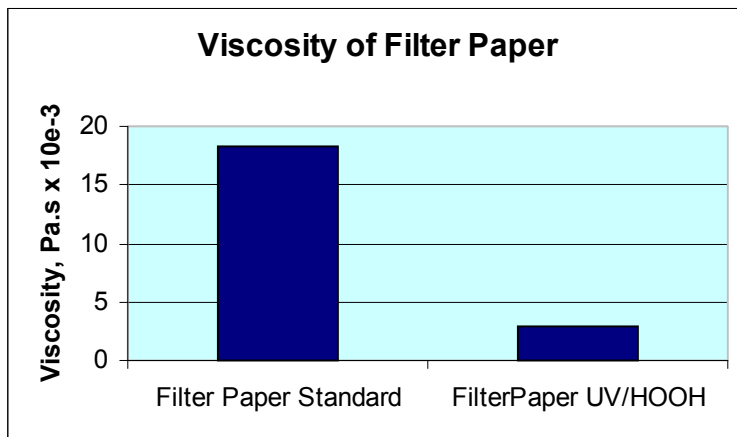
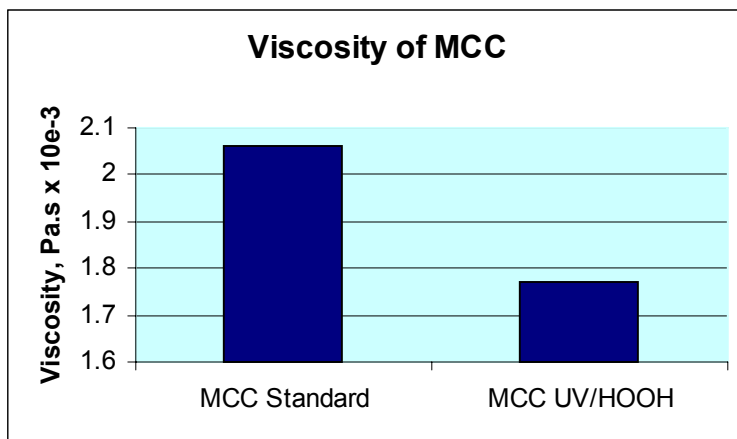


Figure 6. Viscosity change upon reaction of microcrystalline cellulose (MCC) with UV/HOOH



The results of these experiments are significant because they correlate the results from the carbohydrate model compounds to actual cellulose. Both the carbohydrate model compounds and cellulose are severely degraded by the hydroxyl radicals produced by UV/HOOH experiments.

CONCLUSIONS

The experiments conducted in this research support the view that hydroxyl radicals are responsible for the degradation of carbohydrates during oxygen delignification. Dioxygen, hydrogen peroxide, and the hydroperoxy anion do not appear to degrade carbohydrates directly under our conditions. The products produced during the UV/HOOH oxidations of 1,5-anhydrocellobitol and 2-methoxytetrahydropyran suggest a substitution reaction between hydroxyl radicals and the carbohydrate model compounds at the anomeric carbon. Experimental identification of D-glucose, 1-deoxyglucose, methanol and tetrahydropyran-2-ol, and molecular orbital calculations support the proposed mechanisms. The reaction mechanisms proposed in this research reinforce the mechanisms suggested in earlier work [1,2].

The experiments conducted with the cellulose compounds support the model compound results. The UV/HOOH system is capable of degrading both types of cellulose compounds as well as the carbohydrate model compounds. The results of this research show that hydroxyl radicals can simply cleave any glycosidic linkage in the carbohydrate chains. Limiting or preventing hydroxyl radical formation is essential to hinder carbohydrate degradation during oxygen delignification.

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REFERENCES

1. Guay, D. F.; Cole, B. J. W.; Fort, Jr., R. C.; Genco, J. M.; and Hausman, M. C., "Mechanisms of Oxidative Degradation of Carbohydrates During Oxygen Delignification. I. Reaction of Photochemically Generated Hydroxyl Radical with Methyl β -D-Glucoside", *J. Wood Chem. Technol.*, 20(3), 375 (2000).
2. Guay, D.F.; Cole, B. J. W.; Fort, Jr., R. C.; Genco, J. M.; Elder, T. J.; Hausman, M. C.; and Overly, K. R., "Mechanisms of Oxidative Degradation of Carbohydrates During Oxygen Delignification. II. Reaction of Photochemically Generated Hydroxyl Radical with Methyl- β -D-Cellobioside", *J. Wood Chem. Technol.*, 21(1), 67, (2001).
3. Gratzl, J. S., "The Chemical Basis of Pulp Bleaching with Oxygen, Hydrogen Peroxide and Ozone - A Short Review", *Papier*, 10A, V1 (1992).
4. Lide, D. R., ed., *CRC Handbook of Chemistry and Physics*, 74th ed., p. 8-42; CRC Press, Boca Raton, (1993).
5. Sun, Y.; Wallis, A. F. A.; and Nguyen, K. L., "Reactivity of Lignin and Lignin Models Towards UV-Assisted Peroxide", *J. Wood Chem. Technol.*, 17(1&2), 163, (1997).
6. Wright, P. J.; and Wallis, A. F. A., "Rapid Determination of Carbohydrates in Hardwoods by High Performance Anion Exchange Chromatography", *Holzforschung*, 50, 518, (1996).
7. SPARTAN version 5.0; Wavefunction, Inc., 18401 Von Karman Avenue, suite 370, Irvine, CA 92612.
8. *Gaussian 94* (Revision D.1), M. J. Frisch, G. W. Trucks, H. B. Schlegel, P. M. W. Gill, B. G. Johnson, M. A. Robb, J. R. Cheeseman, T. A. Keith, G. A. Petersson, J. A. Montgomery, K. Raghavachari, M. A. Al-Laham, V. G. Zakrzewski, J. V. Ortiz, M. B. Forsman, J. Cioslowski, B. B. Stefanov, A. Nanayakkara, M. Challacombe, C. Y. Peng, P. Y. Ayala, W. Chen, M. W. Wong, J. L. Andres, E. S. Replogle, R. Gomperts, R. L. Martin, D. J. Fox, J. S. Binkley, D. J. Defrees, J. Baker, J. P. Stewart, M. Head-Gordon, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Pittsburgh, PA, 1995.
9. Gierer, J., "Formation and Involvement of Superoxide ($O_2^{\cdot-}/HO_2^{\cdot}$) and Hydroxyl (OH^{\cdot}) Radicals in TCF Bleaching Processes: A Review", *Holzforschung*, 51, 34, (1997).
10. Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; and Ross, A. B., "Critical Review of Rate Constants for Reactions of Hydrated Electrons, Hydrogen Atoms and Hydroxyl Radicals (OH/O^{\cdot}) in Aqueous Solution", *J. Phys. Chem. Ref. Data*, 17, 513, (1988).
11. Jones, Jr., M. "Organic Chemistry", 2nd ed., Norton, New York, 2000; p. 434.