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Title	Studies on the Polysaccharide Reactions in Ozone Bleaching of Kraft Pulp
Author(s)	Kishimoto, Takao
Degree Grantor	Kyoto University
Degree Name	博士(農学)
Issue Date	1996-11-25
Doc URL	https://hdl.handle.net/2115/32904
Type	doctoral thesis
File Information	thesis.pdf



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Studies on the Polysaccharide Reactions in Ozone Bleaching of Kraft Pulp

1996

Takao Kishimoto

CONTENTS

Introduction 1

Chapter 1 Evaluation of Polysaccharide Reactions in Ozone Bleaching 4

1.1 Introduction 4

1.2 Basic idea for evaluating the reactions of polysaccharides in ozone bleaching 4

1.3 Evaluation of GC and OX reactions during ozone bleaching in acidified water and in methanol 10

1.4 Summary 21

Chapter 2 Preparation and Analyses of Methyl 4-*O*-Ethyl-β-D-glucopyranoside and Its Acetylated Carbonyl Sugars 22

2.1 Introduction 22

2.2 Synthesis of methyl 4-*O*-ethyl-β-D-glucopyranoside 23

2.3 Preparation of carbonyl sugars by oxidation of regio-specifically acetylated model compounds 25

2.3.1 Regioselective acetylation of the model compound 25

2.3.2 Preparation of carbonyl sugars 25

2.4 Preparation of *O*-methyloximes (15-17) of carbonyl sugars (2-4) and analyses by gas chromatography and mass spectrometry 29

2.5 Summary 36

Chapter 3 Quantitative Analyses of Reaction Products from Ozonation of Methyl 4-*O*-Ethyl-β-D-glucopyranoside ···· 37

3.1 Introduction 37

3.2 Analysis of reaction products by NMR spectroscopy 39

3.3 Analysis of reaction products by gas chromatography 39

3.4 Reactions at C1-C6 positions in the model compound 52

3.4.1 Reactions at C1-position 52
3.4.2 Reactions at C2 and C3-positions 53
3.4.3 Reactions at C4 and C5-positions 53
3.4.4 Reactions at C6-position 53

3.5 Relative reactivities of carbon atoms at C1-C6 positions in the model compound 54

3.6 Summary 57

Chapter 4 Free-radical Reactions of Methyl 4-*O*-Ethyl-β-D-glucopyranoside with Fenton's Reagent ····· 58

4.1 Introduction 58

4.2 Oxidation of the model compound with Fenton's reagent 59

4.3 Effect of pH on the oxidation of the model compound with Fenton's reagent 60

4.4 Effect of oxygen on the oxidation of the model compound with Fenton's reagent 64

4.5 Summary 69

Chapter 5 Participation of Radical Species in Ozonation of Methyl 4-*O*-Ethyl-β-D-glucopyranoside ····· 71

5.1 Introduction 71

5.2 Reactions of the model compound with ozone itself (Figure 5.1 A) or radical species (Figure 5.1 E) 72

5.3 Effect of initial pH on the ozonation of the model compound (Figure 5.1 B) 73

5.4 Effect of Fe^{2+} ions on the ozonation of the model compound (Figure 5.1 C) 77

5.5 Effect of lignins on the ozonation of the model compound (Figure 5.1 D) 80

5.6 Summary 84

Conclusions **86**

Experimental **89**

References **103**

Publications **109**

Acknowledgment **110**

Introduction

There has been a growing concern about the chlorinated organic compounds present in pulp mill effluents from an environmental point of view. To replace chlorine-based bleaching with ECF (elementary chlorine free) or TCF (totally chlorine free) bleaching, oxygen-containing chemicals such as oxygen, ozone, hydrogen peroxide and peroxy acids have attracted increasing attention as non-chlorine bleaching agents. Of these reagents ozone is the most promising one.

Ozone is one of the most powerful oxidizing agent, which has the electrophilic site being more reactive than the nucleophilic sites (Eckert and Singh 1980) as shown in **Figure 1**. Thus, ozone attacks readily lignin-related aromatic and olefinic structures, and breaks down residual lignin in pulp. The electrophilic attack of ozone leads to the oxidative hydroxylation and demethoxylation and 1,3-dipolar cycloaddition as shown in **Figure 2**. The 1,3-dipolar cycloaddition is the main route of degradation of lignins, which gives muconic acids intermediates (Gierer 1982).

Many investigations have been made to use ozone as a pulp bleaching reagent (Hosoya 1985, Lindholm 1987, 1991, Jacobson *et al.* 1991, Liebergott *et al.* 1992a, 1992b, Byrd *et al.* 1992). The various factors affecting the selectivities of ozone bleaching such as dose of ozone, pulp consistency, pH, temperature and contents of heavy metal ions and residual lignins have been discussed for finding the optimum conditions on the ozone stage.

A commercial ozone bleaching plant was started up by Union Camp in late 1992 in USA. Ozone has started to be used as a bleaching agent in several kraft mills. However, there still remains a problem that ozone reacts not only

with lignin but also with polysaccharides, lowering pulp viscosity and fiber strength.

The reactions of ozone with carbohydrates have been investigated with several model compounds (Katai and Schuerch 1966, Pan *et al.* 1981, Angibeaud *et al.* 1985). Several undesirable reactions of polysaccharides reducing pulp viscosity have been suggested to occur in ozone bleaching. Hydroxyl radicals derived from the decomposition of ozone are also assumed to play an important role in the degradation of polysaccharides in ozone bleaching (Gierer and Zhang 1993).

However, the degradation of carbohydrate during ozone bleaching is not elucidated sufficiently. It is not known what reactions occur and which reactions are the most harmful to polysaccharides and to what degrees these reactions are affected by the reaction conditions. The discussions based on the reaction products from ozonation of carbohydrate under various conditions have not been conducted sufficiently.

In the present investigations, an oxygen-bleached kraft pulp and a cellulose model compound were treated with ozone to elucidate the degradation of polysaccharide during ozone bleaching.

In Chapter 1, an appropriate method to evaluate the extent of the reactions of polysaccharide during ozone bleaching of pulp is described.

In Chapter 2, preparation of a cellulose model compound and analyses of carbonyl sugars from the model compound are described.

In Chapter 3, quantitative analyses of reaction products from ozonation of the model compound are described.

In Chapter 4, free-radical reactions of the model compound with Fenton's reagent are discussed.

In Chapter 5, participation of radical species in the ozonation of the model compound and some future prospects on ozone bleaching of kraft pulp are discussed.

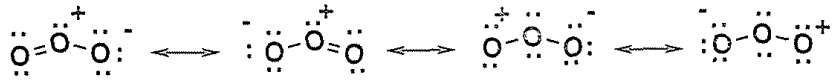


Figure 1 Resonance hybrids of ozone

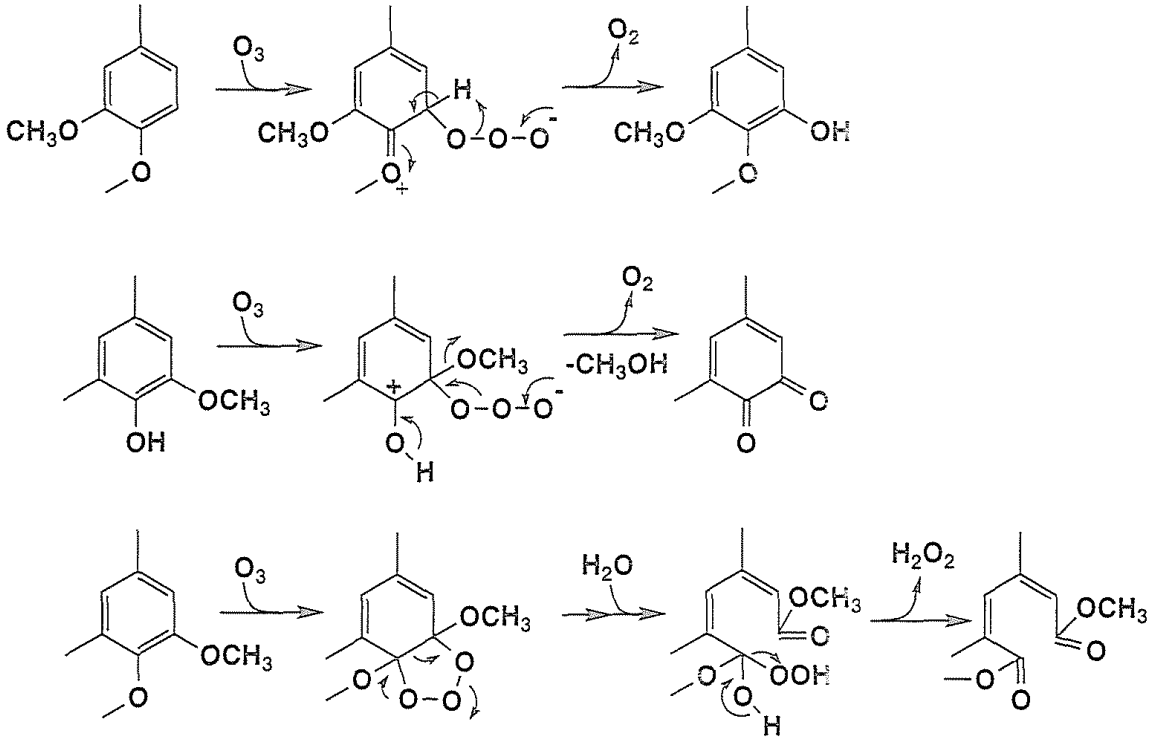


Figure 2 Reactions of ozone with lignins

Chapter 1

Evaluation of Polysaccharide Reactions in Ozone Bleaching of Kraft Pulp

1.1 Introduction

A great number of studies have been made to find the process conditions with high selectivity in ozone bleaching (Byrd *et al.* 1992), and to find the suitable additives to prevent viscosity drops of pulp (Liebergott *et al.* 1992a, 1992b, Kamishima *et al.* 1977, 1982, 1983).

However, many papers so far have reported only their experimental results and detailed discussions based on the reaction mechanism have not been conducted sufficiently.

The objective of the present investigation is to propose an appropriate method to evaluate the extent of the reactions of polysaccharides which induce the reduction of pulp viscosity in ozone bleaching. On the basis of this evaluation method, the mechanisms of several additives to protect carbohydrate degradation will be discussed.

1.2 Basic idea for evaluating the reactions of polysaccharides in ozone bleaching

In this chapter the reactions of polysaccharides with ozone reported hitherto are divided into two groups. One is a direct glycosidic bond cleavage reaction by the insertion of ozone into the anomeric C-H bond (Pathway 1) or

the hydrolysis type reaction (Pathway 2) as shown in **Figure 1.1** (Pan *et al.* 1981) The other is oxidation of hydroxyl groups at C2, C3, or C6 positions in polysaccharides to produce carbonyl groups by ozone itself (Katai and Schuerch 1966) or alternative active-oxygen species such as hydroxyl radicals. Not only the direct glycosidic bond cleavage reaction but also the oxidation of hydroxyl groups is responsible for the viscosity drop of ozone-bleached pulp because carbonyl groups activate glycosidic bond cleavage according to a β-elimination mechanism under alkaline conditions such as the alkaline extraction stage and viscosity measurement as shown in **Figure 1.2**.

In this chapter, the former glycosidic bond cleavage reactions are defined as GC reaction and the latter oxidation reactions are defined as OX reaction. It is important to know to what degrees these two reactions affect the viscosity drop during ozone bleaching and to what degrees these reactions are affected by the reaction conditions, but there are few papers in which the viscosity drop is discussed in detail from this point of view.

As the viscosity drop induced by the carbonyl groups can be hindered by the treatment with sodium borohydride as reported earlier (Gupta and Eckert 1984, Hartler *et al.* 1987), a comparison of the viscosities of pulp measured before and after borohydride treatment is thought to enable us to evaluate the extent of these reactions during ozone bleaching.

This conception is shown schematically in **Figure 1.3**. The values of K and η indicate kappa number and viscosity, respectively. A pulp with K_1 and η_1 is treated with ozone to afford a bleached pulp with K_2 and η_2, and the bleached pulp is treated further with sodium borohydride to give a reduced pulp with η_3. The value of $\Delta\eta_{GC}$ ($= \eta_1 - \eta_3$) corresponds to the viscosity drop caused by the glycosidic bond cleavage during ozone bleaching, and the value of $\Delta\eta_{OX}$ ($= \eta_3 - \eta_2$) corresponds to the secondary viscosity drop by β-elimination during the standard viscosity measurement, caused by the carbonyl groups generated at C2, C3, or C6 positions of polysaccharides during ozone

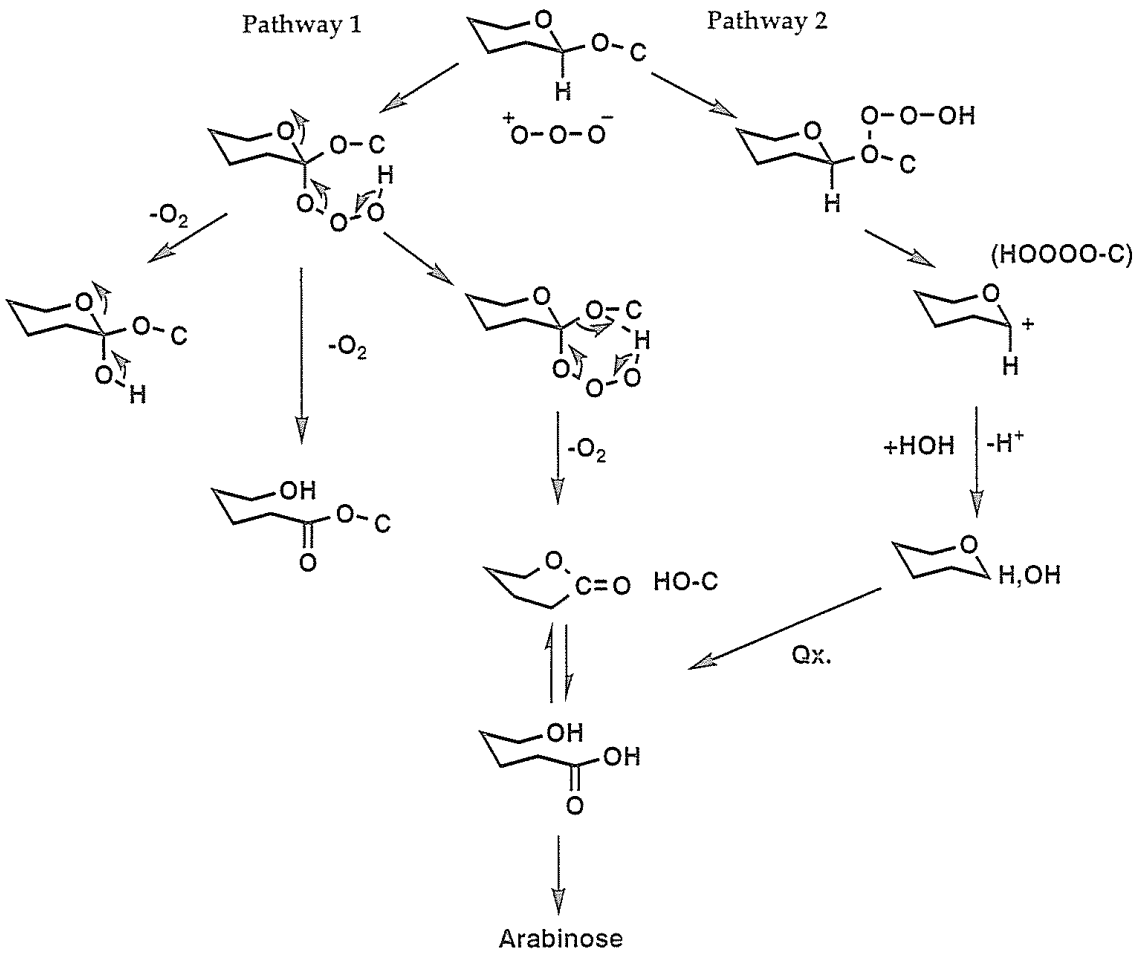


Figure 1.1 Proposed ionic mechanisms in the initial phase of ozonation of β-glycosides (Pan *et al*. 1981)

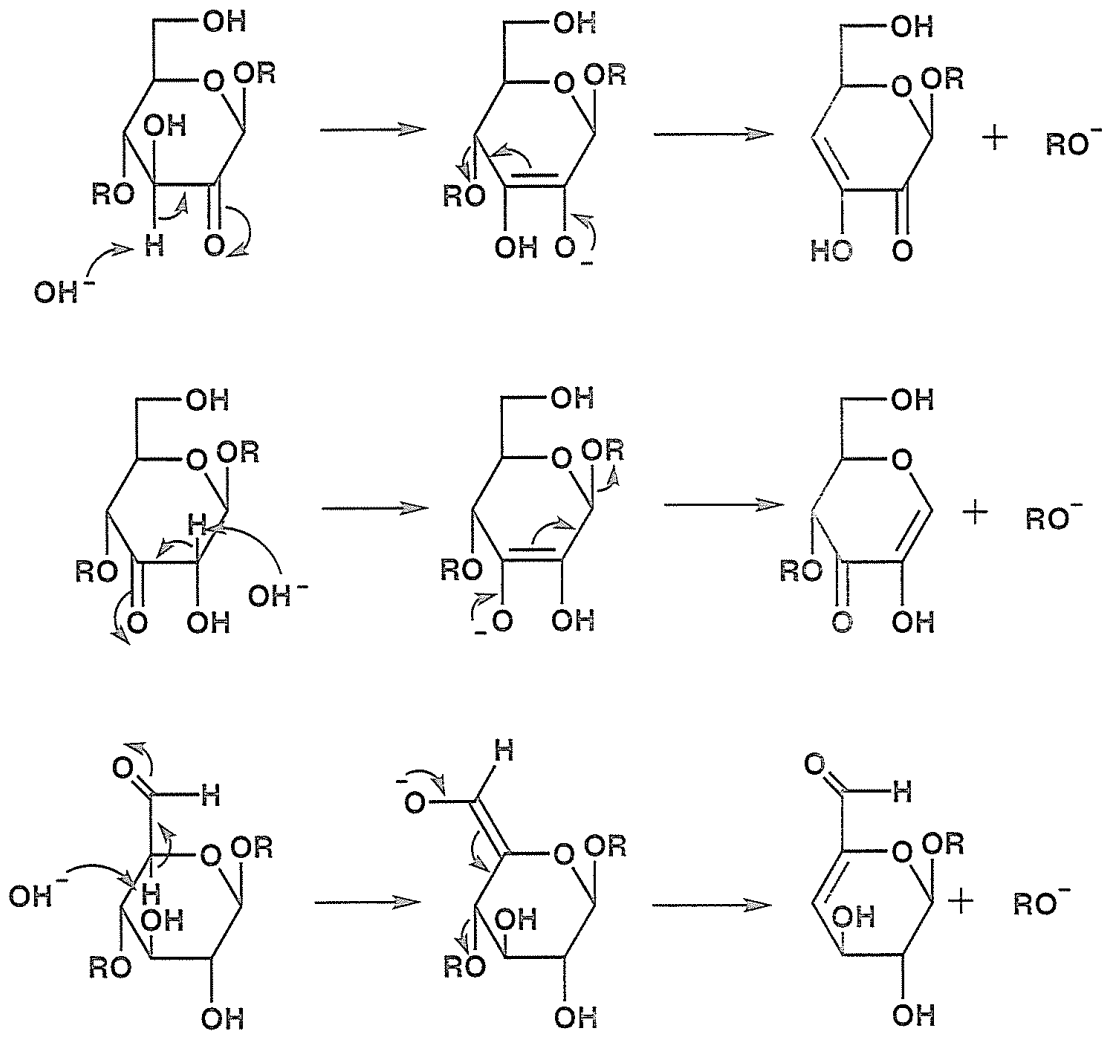


Figure 1.2 Glycosidic bond cleavage caused by carbonyl groups at C2, C3, C6-positions according to a β-elimination mechanism under alkaline conditions

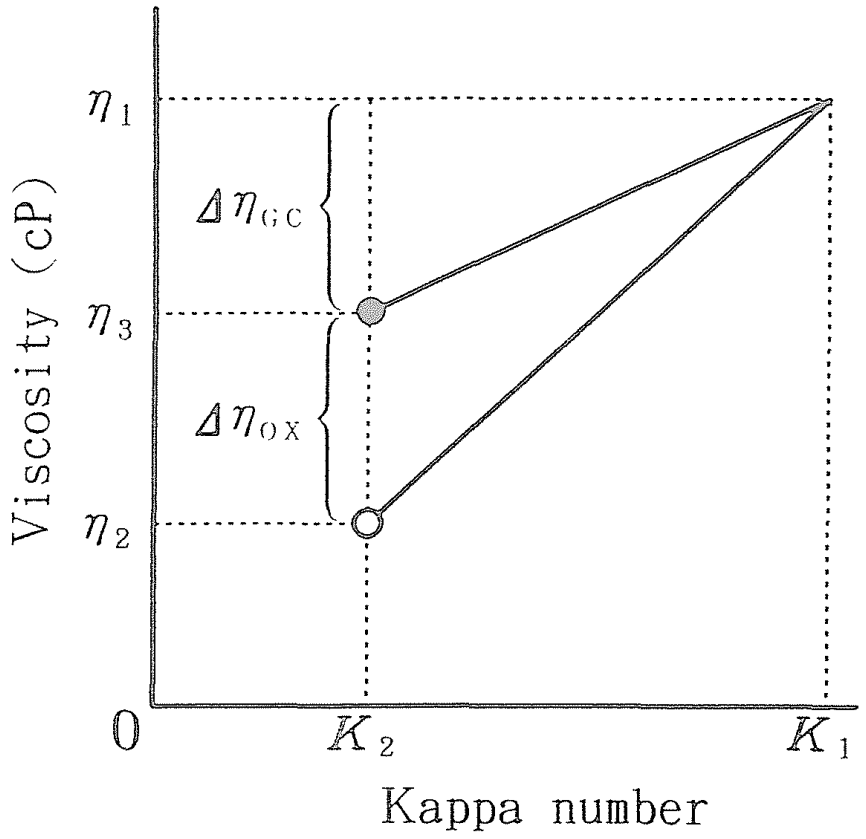
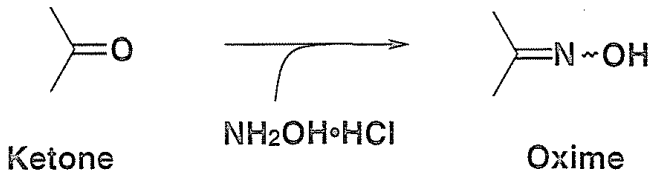


Figure 1.3 Schematic interpretation of viscosity drops in ozone bleaching
Legend: ○: before borohydride treatment, ◉: after borohydride treatment

bleaching. That is, these $\Delta\eta_{GC}$ and $\Delta\eta_{OX}$ values correspond to GC and OX reactions, respectively. Therefore, we may learn the extent of these two reactions in ozone bleaching by evaluating these $\Delta\eta_{GC}$ and $\Delta\eta_{OX}$ values.

Before applying the present conception to the evaluation of the actual experimental results, it is necessary to make sure whether or not the $\Delta\eta_{OX}$ is caused only by carbonyl groups, because carbonyl groups in ozonated cellulose have not been characterized by NMR spectroscopy (Angibeaud 1985). To clarify this point, the ozone-bleached pulp was treated with hydroxylamine hydrochloride instead of borohydride. This reagent readily reacts with carbonyl groups to produce Schiff's base (oxime), which does not induce the β-elimination even under alkaline conditions, as following:



An oxygen-bleached kraft pulp was treated with ozone in methanol for 30 min. The viscosity dropped from 37.6 cP to 27.8 cP. However, the viscosity of the pulp after treatment with borohydride was 34.0 cP. On the other hand, the treatment with hydroxylamine hydrochloride instead of borohydride led to 34.2 cP. Both treatments with these carbonyl reagents led to almost the same viscosities. These results support that the $\Delta\eta_{OX}$ is attributed only to carbonyl groups.

Pan and others (1981) proposed that ozone attacks the glycoside at the anomeric C-H bond to form a hydrotrioxide hemiorthoester by an insertion mechanism and that the fragmentation of the hydrotrioxide yields aldonic acid esters or aldonic acid-δ-lactones as shown in **Figure 1.1**. The conversion to the lactone leads to a shortening of the chain length during ozone treatment, but the conversion to the ester does not. However, it must be noted that, according

to the present definition, the conversion to the ester intermediate is regarded as a GC reaction, not as an OX reaction. This is because the ester intermediate is hydrolyzed under alkaline conditions to induce a viscosity drop, but does not react with sodium borohydride to contribute to the viscosity development of the ozonated pulp; the OX reaction is evaluated only by the viscosity recovery by the treatment with borohydride.

Thus, the author concluded that the extent of the GC and OX reactions to affect the viscosity drop of the pulp in ozone bleaching can be evaluated by $\Delta\eta_{GC}$ and $\Delta\eta_{OX}$ values according to the above strategy.

1.3 Evaluation of GC and OX reactions during ozone bleaching in acidified water and in methanol

Extensive research has been undertaken to find suitable carbohydrate protectors inhibiting the viscosity drop of pulp in ozone bleaching (Liebergott *et al.* 1992a 1992b, Kamishima *et al.* 1977 1982 1983). Various additives such as organic and inorganic acids, neutral organic compounds, and chelating agents were found to inhibit pulp viscosity drops. The protection effects on polysaccharides tentatively have been discussed with various explanations; (1) the removal ability of heavy metal ions catalyzing the formation of hydroxyl radicals: acids and chelating agents; (2) ozone-stabilization: acids; (3) anti-swelling of the pulp: acids (Mbachu and Manlay 1981); (4) radical scavenging ability: several organic materials (Jacobson *et al.* 1991); (5) the different solubilities of polysaccharides and lignin: organic solvents (Kamishima *et al.* 1984), and so forth. The protection mechanism, however, has not been discussed in detail.

The author intended to reinvestigate three ozone-bleaching conditions reported as inhibiting viscosity drops (Jacobson *et al.* 1991, Kamishima *et al.* 1984, Lindholm 1989b), and to evaluate the extent of the GC and OX reactions

under the bleaching conditions by the GC/OX-evaluation method described above for discussing the protection mechanism.

An oxygen-bleached hardwood kraft pulp was treated with ozone at room temperature at low consistency (1%) in four different media, distilled water as a control, water acidified to pH 2.0 with sulfuric or oxalic acid, and methanol. These additives have been reported by Kamishima and others (1984) and by Lindholm (1989b) to protect polysaccharides.

The results obtained in these reaction media are shown in **Figures 1.4-1.6** (viscosity drops vs. kappa numbers). The data obtained in distilled water as a control are shown in each figure by dotted lines for comparisons. The open and solid symbols represent the data for the pulps before and after sodium borohydride reduction, respectively.

All viscosity values are recovered after the reductions with sodium borohydride as reported earlier. The viscosity in distilled water linearly decreases with the decrease of the kappa number, whereas in other cases shown in **Figures 1.4** and **1.5**, the decrease describes a parabola. Lindholm (1989b) reported that the addition of oxalic acid clearly improved the selectivity both at low and high consistencies, and that acidification to low enough pH with sulfuric acid gave almost as good selectivity as with oxalic acid, but nobody referred in more detail to viscosity drops at low kappa number levels. The present data shows that the viscosity drops in aqueous sulfuric and oxalic acid solutions are inhibited only above kappa numbers of about 4.0 and 5.5, respectively, but rather accelerated below these kappa numbers. These results suggest that the inhibition effects of the viscosity drops in these acidic media depend on the amount of residual lignin, probably also on its properties.

As shown in **Figure 1.6**, the viscosity drop in methanol was extremely smaller than those in aqueous media as expected from the data reported to date, but strangely, the feature below the kappa number of about 5.5 was

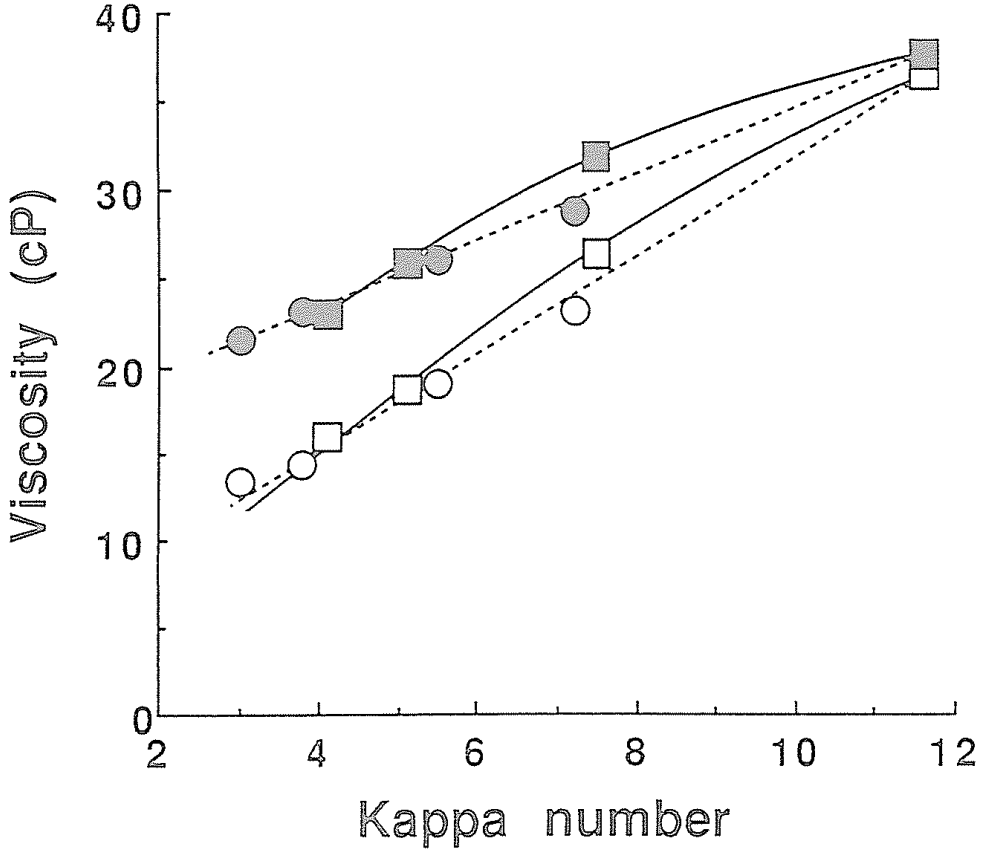


Figure 1.4 Effects of sulfuric acid on selectivity in ozone bleaching
Legend: □,■: Sulfuric acid (pH2), ○,●: Control (distilled water)
Note: Open and solid symbols show the data for the pulps before and after borohydride treatment, respectively.

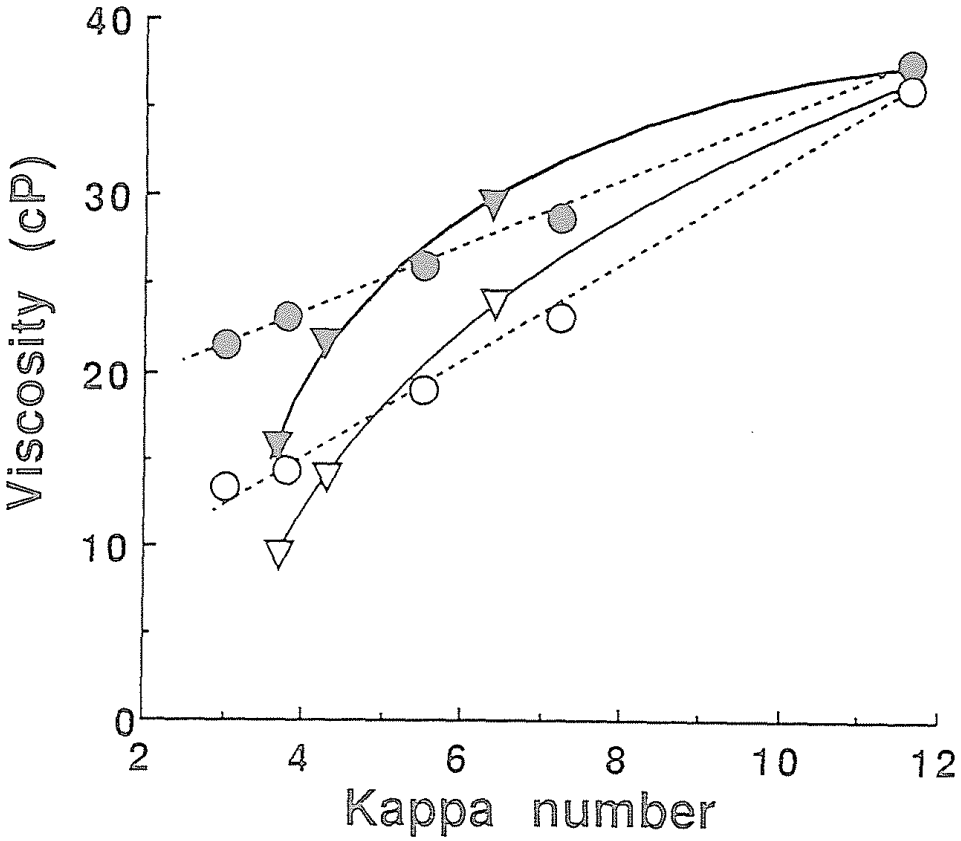


Figure 1.5 Effects of oxalic acid on selectivity in ozone bleaching
Legend: ▽,▼: Oxalic acid (pH2), ○,●: Control (distilled water)
Note: Same as in Figure 1.4.

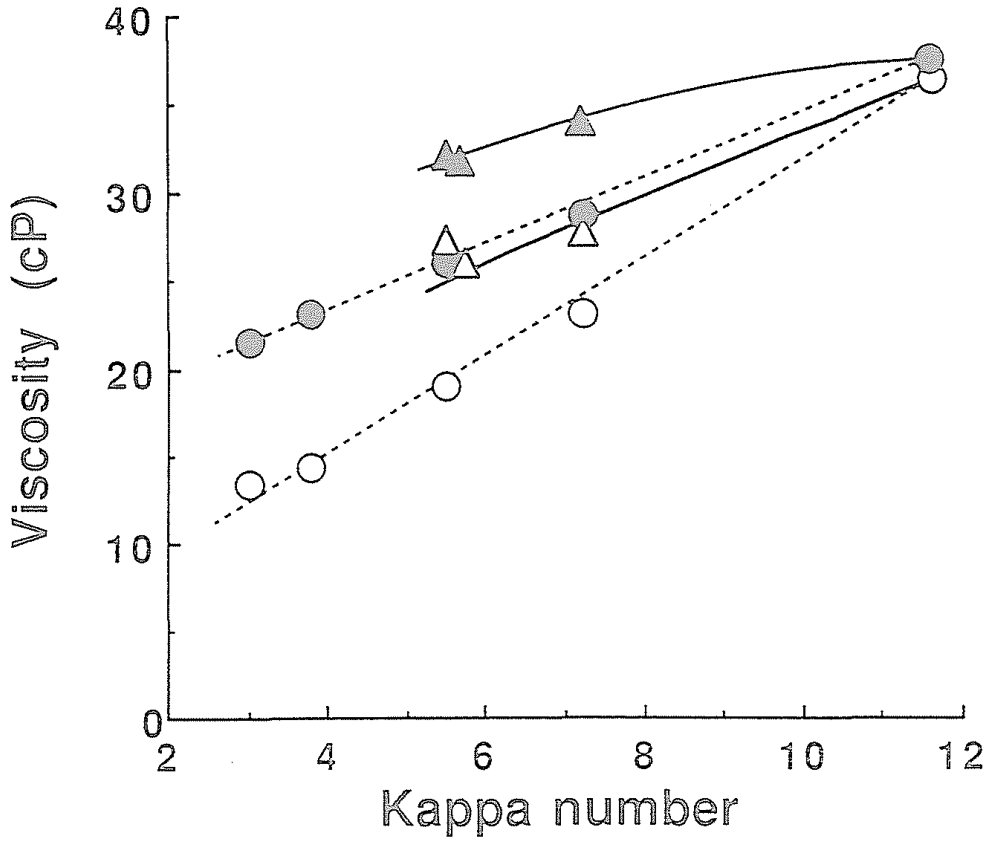


Figure 1.6 Effects of methanol on selectivity in ozone bleaching
Legend: △,▲: Methanol, ○,●: Control (distilled water)
Note: Same as in Figure 1.4.

quite different from those in the aqueous media. That is, neither viscosity nor kappa number decrease so much even when the ozonation was done again after replacement with fresh methanol.

However, the residual lignin below kappa number 5.5 was substantially removed in the following sequence: the pulp ozonated in methanol for 30 min (viscosity: 27.6 cP, kappa number: about 7) was reduced with 5% sodium borohydride for 30 min at room temperature. The viscosity value of the pulp increased to 34.7 cP, and the kappa number decreased to 3.8. The pulp was treated again with ozone for 30 min in methanol, and the viscosity decreased to 30.8 cP, and the kappa number decreased to 2.9.

The removal of the residual lignin by the above sequence is thought to proceed by the alkaline-extraction of the residual lignin, and/or by conversion of the residual lignin into an ozone-sensitive lignin by the borohydride reduction, or by the improvement of the accessibility of ozone for the effective delignification by an alkaline-swelling of the pulp fiber.

Table 1.1 Effects of various additives on the GC and OX reactions in ozone bleaching of kraft pulp

Additives	30 min[a)]		60 min[a)]		90 min[a)]	
	$\Delta\eta_{GC}$	$\Delta\eta_{OX}$	$\Delta\eta_{GC}$	$\Delta\eta_{OX}$	$\Delta\eta_{GC}$	$\Delta\eta_{OX}$
Control (water)	8.8	4.4	14.5	7.6	16.0	7.1
Sulfuric acid	5.8	4.2	11.7	6.1	14.7	5.8
Oxalic acid	8.0	4.5	15.8	6.5	21.9	4.8
Methanol	2.9	5.9	4.7	4.5	5.2	5.1

a) Reaction times.

Legend: $\Delta\eta_{GC}$: Viscosity drop caused by GC reaction during ozone bleaching (cP), $\Delta\eta_{OX}$: Viscosity drop caused by OX reaction during standard viscosity measurements (cP)

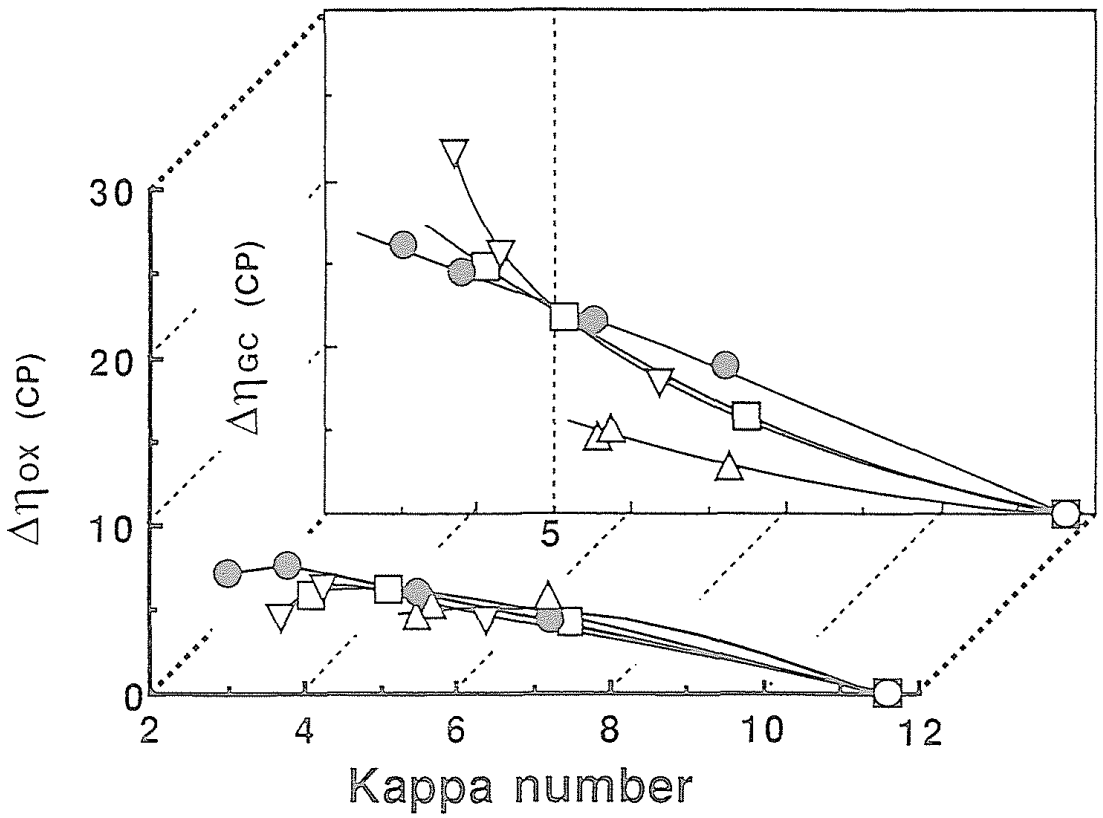


Figure 1.7 Effects of various additives on the GC and OX reactions in ozone bleaching

Legend: □: Sulfuric acid (pH2), ▽: Oxalic acid (pH2), △: Methanol, ●: Control (distilled water); $\Delta\eta_{GC}$, $\Delta\eta_{OX}$: see Table 1.1.

Table 1.1 summarizes $\Delta\eta_{GC}$ and $\Delta\eta_{OX}$ values calculated from the data shown in **Figures 1.4-1.6**. These $\Delta\eta_{GC}$ and $\Delta\eta_{OX}$ values were plotted against kappa numbers as shown in **Figure 1.7**.

In **Figure 1.7**, solid circles show the control experiment conducted in distilled water. The $\Delta\eta_{OX}$ value does not increase much with the decrease of the kappa number: the value tends to level off below kappa number 5. The value is not so affected by the reaction media, which is different from the $\Delta\eta_{GC}$ value.

Thus, neither acidified water nor methanol is considered to inhibit the OX reaction, i. e. the oxidation of the hydroxyl groups to form the carbonyl groups under our experimental conditions, where oxygen gas containing ozone was bubbled continuously through the pulp suspension at low consistency.

There is no possibility of the viscosity drop caused by the acid-catalyzed β-elimination induced by carbonyl groups during the ozone treatment in acidic media. The ozone-bleached pulp (24.8 cP, 28.7 cP, before and after borohydride treatment, respectively) was treated with water acidified to pH 2.0 with sulfuric acid for 90 min without ozonation, and the acid-treated pulp obtained gave 23.8 cP and 28.0 cP before and after the borohydride treatment, respectively, which are almost the same values as those before the acid treatment.

On the other hand, the value of $\Delta\eta_{GC}$ increased clearly with the decrease of the kappa number. The value is much greater than that of $\Delta\eta_{OX}$ at the same kappa number. It is noteworthy that the values of $\Delta\eta_{GC}$ in methanol and acidified water are lower than those in distilled water above kappa number 5, and that especially in methanol the value is much lower. These data clearly indicate that the inhibition effects of methanol and acidified water on the viscosity drop are attributable to the inhibition of the GC reaction, not to that of the OX reaction. Both oxalic and sulfuric acids accelerate viscosity drop below kappa number 5 under the present bleaching conditions. The large

increase of $\Delta\eta_{GC}$ in aqueous oxalic acid medium is not explained by a simple hydrolysis catalyzed by oxalic acid because a viscosity drop of an oxygen-bleached pulp was not observed in oxalic acid medium at pH 2 without ozone bubbling. These results suggest that ozone bleaching in these media is not favorable for pulps with kappa number below 5.

Lindholm (1989b) investigated the effects of various additives and pretreatments on the effectivenesses and selectivities in high (35%) and low (1%) consistency ozone bleaching of pine (*Pinus* sp.) kraft pulp. The author attempted to evaluate the results of Lindholm by the present GC/OX-evaluation method. The results are shown in **Figure 1.8** (high consistency) and **Figure 1.9** (low consistency). It is interesting that the $\Delta\eta_{OX}$ value is not so affected by the additives and pretreatments, and scarcely is affected by the pulp consistency. The value of $\Delta\eta_{OX}$ remains at about 130 below kappa number 10. On the other hand, the value of $\Delta\eta_{GC}$ is very much affected by the additives and pretreatment, and the value is much greater than that of $\Delta\eta_{OX}$. These tendencies at low consistency agree with our results shown in **Figure 1.7**.

The effects of the additives and pretreatments at low consistency on the GC reaction are much larger than that at high consistency. The low consistency bleaching seems to be better when we expect the effective functions of the additives and pretreatments. **Figures 1.8** and **1.9** clearly show that the improved selectivity at low consistency is attributable to the inhibition of the GC reaction and not to the OX reaction, as is implied by Lindholm (1989a).

If other new reactions during ozone bleaching, for example, the fate of the carbonyl groups formed by the OX reaction, are found by further investigation, it will be necessary to modify the present evaluation method, because the method is based only on the reactions of ozone with

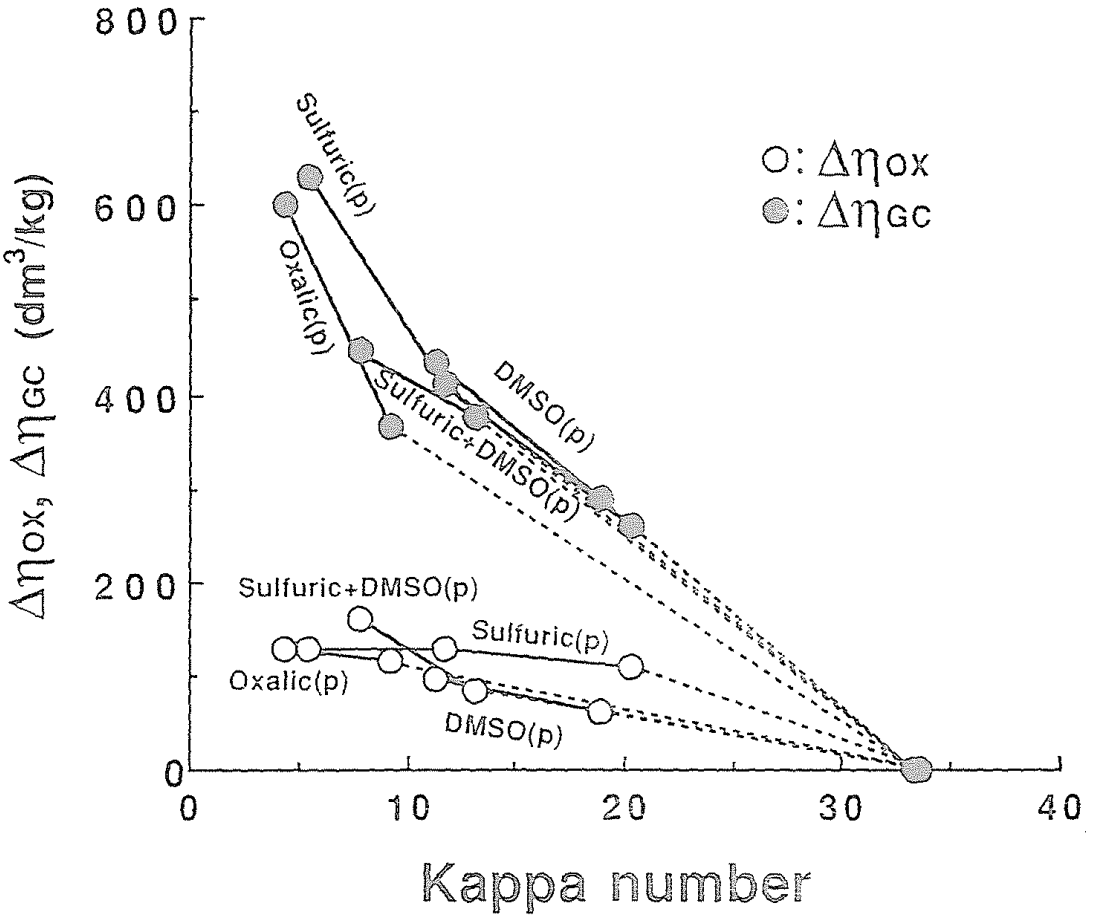


Figure 1.8 Reinvestigation of the experimental results of Lindholm (1989a) in high consistency ozone bleaching

Notes: Legend is same as in Table 1.1. (p) shows pre-washing.

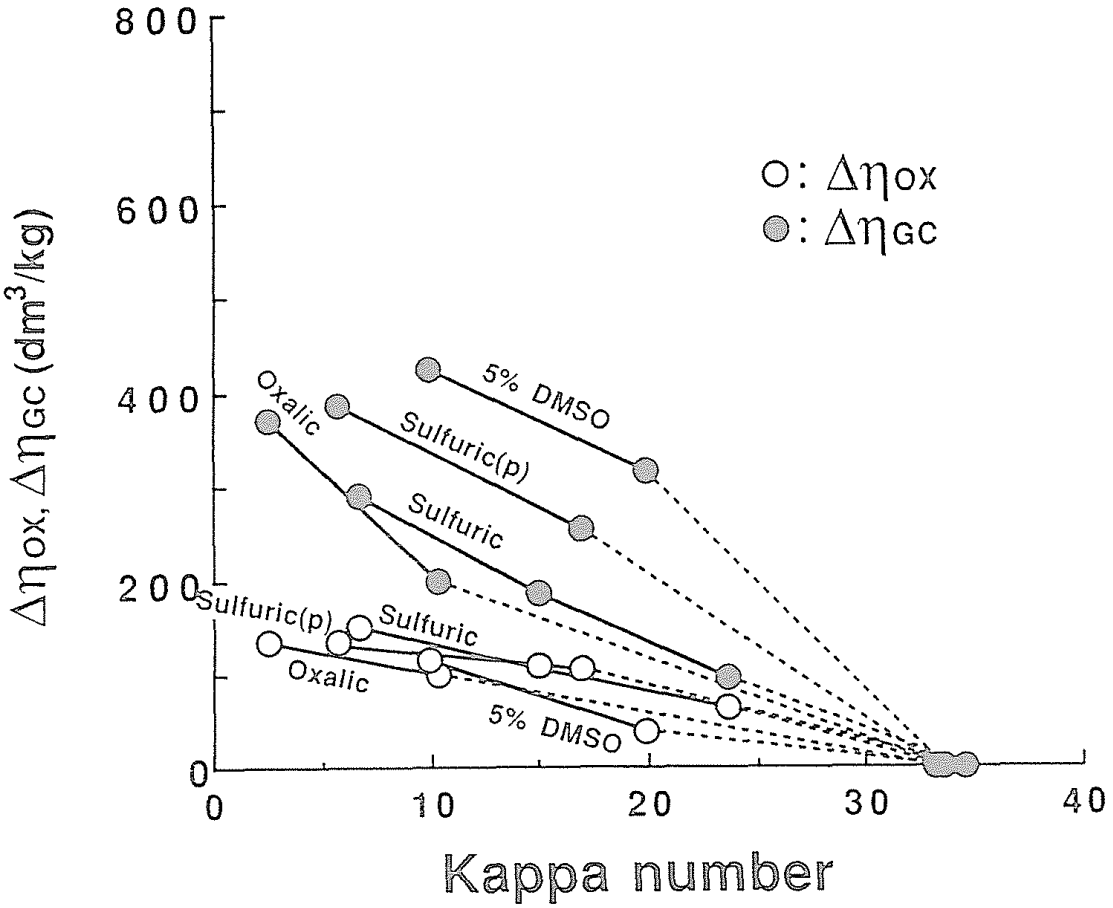


Figure 1.9 Reinvestigation of the experimental results of Lindholm (1989a) in low consistency ozone bleaching

Notes: Legend is same as in Table 1.1. (p) shows pre-washing.

carbohydrates reported to date. It is thought that this evaluation method makes it possible for us to discuss viscosity drops in more detail.

1.4 Summary

Several undesirable reactions of ozone with polysaccharide inducing viscosity drop of pulp have been reported to date. In this chapter, the author divided these reactions into two categories: glycosidic bond cleavage (GC reaction) and oxidation (OX reaction) of hydroxyl groups to carbonyl groups. The author proposed an appropriated method (GC/OX evaluation method) to evaluate the extent of these GC and OX reactions under conditions reported to inhibit viscosity drops in ozone bleaching.

As a result, the following conclusions were reached: 1) the viscosity drop caused by GC reaction was greater than that by OX reaction; 2) OX reaction is not affected by the reaction media; 3) GC reaction is affected by the reaction media; 4) the inhibition effect of the viscosity drop by methanol and acidified water is attributed to the inhibition of GC reaction, not to that of OX reaction. The present evaluation method was applied to the experimental results of Lindholm, and conclusions similar to those in 1)- 3) were obtained.

Chapter 2

Preparation and Analyses of Methyl 4-*O*-Ethyl-β-D-glucopyranoside and Its Acetylated Carbonyl Sugars

2.1 Introduction

To elucidate the degradation mechanism of cellulose during ozone bleaching, low molecular weight model compounds, such as glucose (Angibeaud *et al.* 1985), methyl α-D-glucopyranoside (Katai and Schuerch 1966), methyl β-D-glucopyranoside (Pan *et al.* 1981), and cellobiose (Angibeaud *et al.* 1985) have been used, because they are commercially available. A 1,4-substituted glucose derivative is more suitable for this purpose, because it has a protective group at 4-*O*-position. The reactivity of the inner glucose repeating unit in cellulose molecule can be elucidated by use of this derivative.

Oxidation of polysaccharide is one of the predominant reactions which cause degradation of polysaccharide and viscosity drop during bleaching. In most case hydroxyl groups are oxidized to produce carbonyl groups in the molecule. Oxidation of hydroxyl groups of cellulose during ozone bleaching have also been reported (Katai and Schuerch 1966).

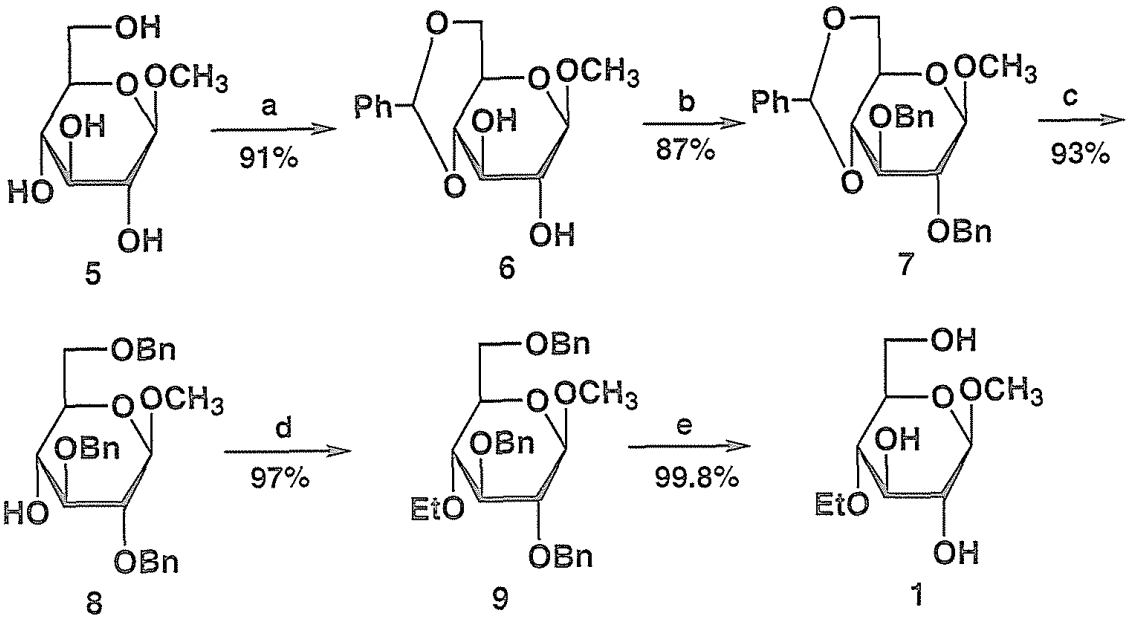
In Chapter 2, the author describes the synthesis and analysis of methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) selected as a model compound for cellulose, and acetylated carbonyl sugars (**2**-**4**) expected to be derived from the model compound. The carbonyl sugars were converted into *O*-methyloximes and analyzed by gas chromatography and mass spectrometry.

2.2 Synthesis of methyl 4-*O*-ethyl-β-D-glucopyranoside

A model compound, methyl 4-*O*-methyl-β-D-glucopyranoside has been synthesized by the methylation of the 2,3,4-tri-*O*-acetylated derivative *via* acyl migration (Bouveng *et al.* 1957) or the methylation of the 2,3-di-*O*-benzylated 6-*O*-tritylated derivative (McGilvray 1952). These synthetic routes give low yields or are rather tedious, because the yield of the direct methylation of the acetylated derivative in alkaline conditions is 45 %, and many reaction steps are needed for the tritylated and benzylated derivative. The author developed a synthetic route for the 1,4-substituted model compound, methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) as shown in **Figure 2.1**. This route is simple and gives high yields. Here, ethyl group was chosen for the substituent at 4-*O*-position, because the extents of elimination of protecting group at 1-*O*- or 4-*O*-position by ozonation can be elucidated separately by gas chromatography and mass spectrometry.

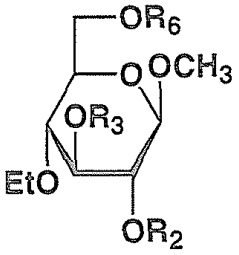
Commercially available methyl β-D-glucopyranoside (**5**) was converted into methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**6**) in a 91 % yield, which was then benzylated to afford methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**7**) in a 87 % yield. Reductive cleavage of the 4,6-*O*-benzylidene acetal derivative (**7**) with sodium cyanoborohydride and trimethylsilyl chloride in acetonitrile afforded methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**) in a 93 % yield (Kamitakahara *et al.* 1994).

Ethylation of compound **8** with ethyl iodide and sodium hydride afforded methyl 2,3,6-tri-*O*-benzyl-4-*O*-ethyl-β-D-glucopyranoside (**9**) in a 97 % yield. The benzyl groups were removed with palladium carbon under H_2 to afford methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) in an almost quantitative yield. The overall yield of compound **1** from compound **5** was 71 %.



a) $C_6H_5CH(OMe)_2$ / p-TsOH / DMF / 50°C / 50min,
b) $C_6H_5CH_2Br$ / NaH / Bu_4NI / DMF / r.t. / 1.5h,
c) $NaCNBH_3$ / TMSCl / CH_3CN / r.t. / 2h,
d) EtI / NaH / DMF / r.t. / 1h,
e) 10%Pd-C / H_2 / EtOH / AcOH / 50°C / 4h.

Figure 2.1 Synthetic route for methyl 4-*O*-ethyl β-D-glucoside



10: R_2= R_3=R_6=Ac
11: R_2=H, R_3=R_6=Ac
12: R_2=Ac, R_3=H, R_6=Ac
13: R_2=R_3=Ac, R_6=H
14: R_2=R_3=Ac, R_6=Tr

Tr = $C(C_6H_5)_3$

Figure 2.2 Regiospecifically acetylated model compounds

2.3 Preparation of carbonyl sugars by oxidation of regio-specifically acetylated model compounds

2.3.1 Regioselective acetylation of the model compound

It is difficult to oxidize 1,2-diols such as compound **1** to α-hydroxy ketones, because both hydroxyl groups are oxidized to α-diketones, or carbon-carbon cleavage takes place. In fact, methyl 4-*O*-methyl-β-D-glucopyranoside has been oxidized directly with chromic acid to afford 2-keto, 3-keto, and 6-aldehyde derivatives (Assarsson and Theander 1964) only in low yields: 0.2, 2.6, and 3.1 %, respectively. Therefor, the author tried the oxidation of partially acetylated model compounds as shown in **Figure 2.2**.

Regioselective acetylation of compound **1** was performed with acetyl chloride (8.5eq) and 2,6-lutidine (8.5eq) in ethyl acetate under reflux for 12h (Nishimura *et al.* 1993), to afford methyl 2,3,6-tri-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**10**), methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**11**), and methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**12**) in 30, 20 and 50 % yields, respectively. These products were separated on a silica gel column. The difference in their yields between compounds **11** and **12** may be explained as follows: 1) Generally, 2-OH is more reactive than 3-OH for a nucleophilic substitution, because the former is more easily ionized under basic conditions. 2) The bulky salt of acetyl chloride and 2,6-lutidine is more accessible to 2-*O*-position than 3-*O*-position, because 4-*O*-position is substituted for more bulky ethyl group.

Methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (**13**) was prepared from methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-6-*O*-trityl-β-D-glucopyranoside (**14**) by the detritylation with *p*-toluenesulfonic acid.

2.3.2 Preparation of carbonyl sugars

Methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*arabino*-hexopyranosidulose (**2**), methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*ribo*-hexopyranoside-3-ulose (**3**), and methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-*gluco*-hexodialdo-1,5-pyranoside (**4**) were prepared from the corresponding partially acetylated derivatives (**11-13**) by oxidation with pyridinium chlorochromate (PCC). These carbonyl sugars are shown in **Figure 2.3**. The ^{1}H-and ^{13}C-NMR spectral data of acetylated carbonyl sugars are shown in **Table 2.1**.

The 2-hydroxyl derivative (**11**) was treated with PCC at room temperature for 31 h. After removing the unreacted starting material **11** (Rf: 0.24) by preparative thin layer chromatography (PTLC) (1/1 ethyl acetate/*n*-hexane), the expected compound **2** was obtained as a syrup in a 64 % yield. The tailing of chromatographic spot of the product with Rf value 0.15 suggested that the product was a hydrate form as reported by Baker *et al.* (1972), because carbonyl compounds usually have larger Rf value than those of the corresponding alcohols on silica-gel TLC plate developed with normal phase. The ^{13}C-NMR signal assigned to ketone carbon (C2) was observed at 192.2 ppm. The C1 signal was observed at 100.2 ppm. The ^{1}H-NMR signals for C1-, C3-, and C4-protons were observed at 4.82 ppm (singlet), 5.38 ppm (doublet, $J_{3,4}$=9.5), 3.74 ppm (triplet, $J_{4,5}$=9.5), respectively. The ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the 2-keto derivative (**2**).

The 3-hydroxyl derivative (**12**) was treated with PCC at room temperature for 35 h to afford 3-keto derivative (**3**) in 79 % yield. The ^{13}C-NMR signal attributed to ketone carbon (C3) was observed at 198.3 ppm, and the C1 signal was observed at 102.4 ppm. The ^{1}H-NMR signals for C1-, C2- and C4-protons were observed at 4.52 ppm (doublet, $J_{1,2}$=8.0), 5.12 ppm (double doublet, $J_{2,4}$=1.5), and 4.00 ppm (double doublet, $J_{4,5}$=10), respectively. The ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the 3-keto derivative (**3**).

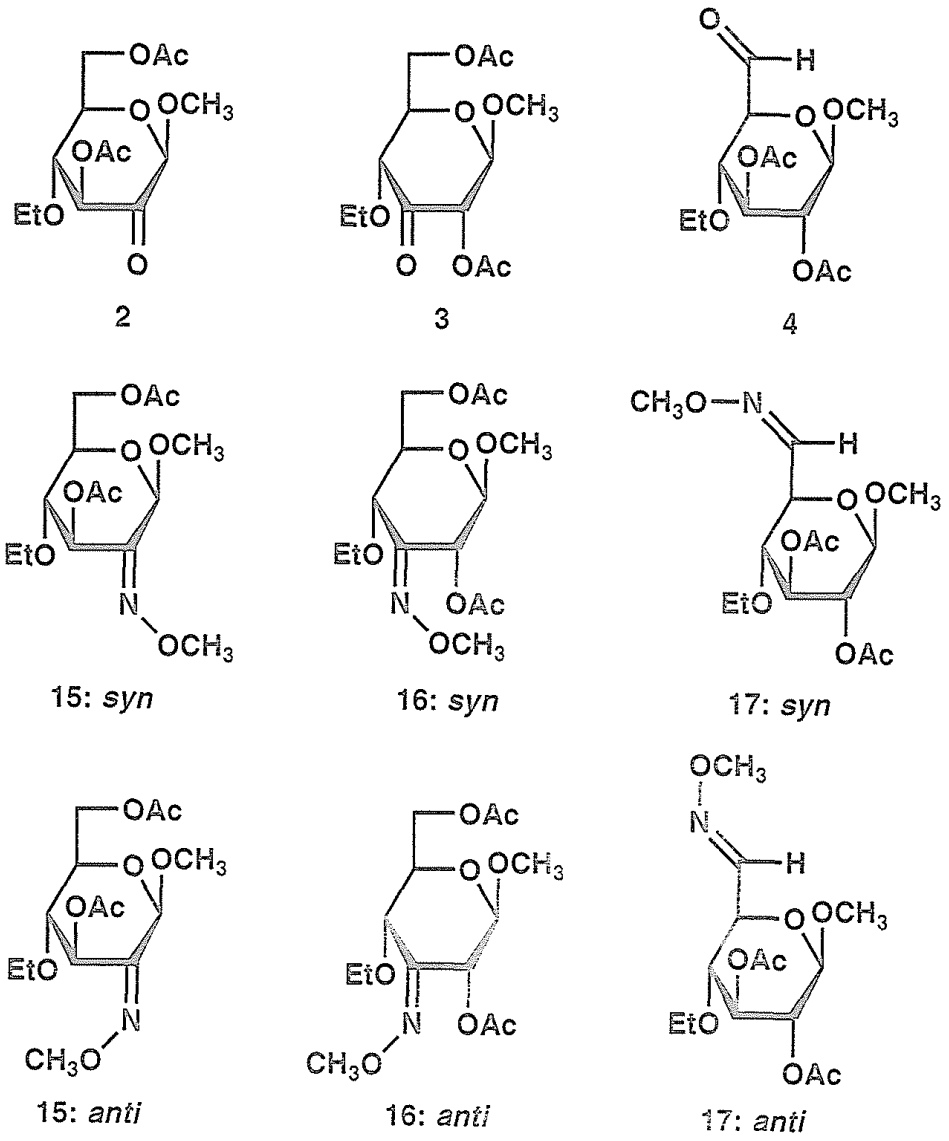


Figure 2.3 Synthesized acetylated carbonyl sugars and their *O*-methyloximes

Table 2.1 ^{1}H and ^{13}C-NMR spectral data of acetylated carbonyl sugars.

Compound	2	3	4
	^{1}H-NMR		
	Chemical Shift (ppm)		
1-H	4.82s	4.52d	4.54d
2-H	-	5.12dd	4.90t
3-H	5.38d	-	5.22t
4-H	3.74t	4.00dd	
5-H	3.93m	3.63m	3.96d
6-H_a	4.31dd	4.35dd	9.74s
6-H_b	4.48dd	4.46dd	-
C-OCH_3	3.58s	3.61s	3.56s
-OCH_2CH_3	1.16t	1.25t	1.16t
-OCH_2CH_3		3.46m	
		3.89m	
-$OCOCH_3$	2.10s	2.16s	2.08s
	2.22s	2.24s	
	Coupling Constants (Hz)		
$J_{1,2}$	-	8.0	8.0
$J_{2,3}$	-	-	8.0
$J_{3,4}$	9.5	-	8.0
$J_{4,5}$	9.5	10.0	9.0
$J_{5,6a}$	5.0	4.5	-
$J_{5,6b}$	2.0	2.5	-
$J_{6a,6b}$	12.0	12.0	-
$J_{2,4}$	-	1.5	-
	^{13}C-NMR		
	Chemical Shift (ppm)		
C-1	100.2	102.4	101.6
C-2	192.2		
C-3		198.3	
C-6			196.3

The 6-hydroxyl derivative (**13**) was easily oxidized by PCC at room temperature within 2.5 h to afford 6-aldehyde derivative (4) in a 62 % yield under the optimum reaction conditions. When the treatment was prolonged, side reactions occurred and the expected product was not obtained. The ^{13}C-NMR signal attributed to aldehyde carbon (C6) was observed at 196.3 ppm, and the C1 signal was observed at 101.6 ppm. The ^{1}H-NMR signal for aldehyde proton (C6-proton) was observed at 9.74 ppm, and the signal for C5-proton was observed at 3.96 ppm (doublet, $J_{4,5}$=9.0). The ^{1}H-NMR and ^{13}C-NMR spectra were consistent with the 6-aldehyde derivative (4).

2.4 Preparation of *O*-methyloximes (15-17) of carbonyl sugars (2-4) and analyses by gas chromatography and mass spectrometry

It is difficult to analyze directly the mixtures of carbonyl sugars obtained by oxidation of glycoside, because they are extremely sensitive under alkaline conditions at room temperature or under neutral and slightly acidic conditions at elevated temperature (Theander 1965). Larm (1976) investigated the stabilization method for the carbonyl sugars obtained by bromine oxidation of methyl β-D-glucoside, and established to analyze quantitatively as trimethylsilylated *O*-methyloxime derivatives. The *O*-methyloxime derivatives of steroid ketones also have been used for analytical and structural studies by gas chromatographic and mass spectrometric techniques (Horning *et al.* 1968). Oxime or *O*-methyloxime derivatives of aldoses (Sweely *et al.* 1963) and ketoses (Petersson 1974) have been used for quantitative analysis by gas chromatography. The oxime or *O*-methyloxime derivatives of carbonyl sugars are stable to alkali, and their enolization does not occur in contrast to the behavior often observed for ketones.

The acetylated carbonyl sugars (**2-4**) were treated with methoxylamine hydrochloride and pyridine to afford *O*-methyloximes (**15-17**), respectively. The geometric isomers of the *syn* and *anti* types were formed during the reaction of carbonyl sugars (**2-4**) with methoxylamine, as shown in **Figure 2.3**. The ^{1}H-NMR spectral data of *O*-methyloximes **15-17** are shown in **Table 2.2**. The configurational assignment of *syn* and *anti* forms of oximes based on ^{1}H-NMR spectral data has been reported (Lemieux *et al.* 1973). The deshielding of a vicinal hydrogen by the oxime hydroxyl allows an assignment of configuration. For example, the C1-proton of the *syn*-*O*-methyloxime **15** appears in 0.51 ppm lower magnetic field than that of the *anti*-*O*-methyloxime **15**. On the other hand, the C3-proton of the *syn*-*O*-methyloxime **15** appears in 0.51 ppm higher magnetic field than that of *anti*-*O*-methyloxime **15**. The configuration of *syn* and *anti* forms of *O*-methyloximes **16** and **17** also can be assigned by ^{1}H-NMR spectral data.

The approximate *syn/anti* ratios of *O*-methyloximes **15-17** also can be determined by ^{1}H-NMR analyses of mixtures of the isomers; by the ratios of the peak areas of $NOCH_3$ protons for *O*-methyloxime **15**, C2-protons for *O*-methyloxime **16**, and C6-protons for *O*-methyloxime **17**. The *syn/anti* ratios of the *O*-methyloximes **15**, **16** and **17** were 1.3, 2.0 and 0.1, respectively.

The gas chromatograms of *O*-methyloximes **15-17** are shown in **Figure 2.4**. The *syn* and *anti* forms of *O*-methyloxime **15** were inseparable by gas chromatography. However, the *syn* and *anti* forms of *O*-methyloximes **16** and **17** were separable. The *syn/anti* ratios of *O*-methyloximes **16** and **17** measured by gas chromatography are in good agreement with the results by ^{1}H-NMR analysis. The peak of *O*-methyloxime **15** overlapped with *anti*-*O*-methyloxime **16** and *syn*-*O*-methyloxime **17** under our analytical conditions. The *syn/anti* ratios were variable under slightly acidic conditions at room temperature.

Mass spectra (CI-MS) of the *O*-methyloxime **15-17** are shown in **Figures 2.5-2.7**, respectively. The mass spectra of *syn* and *anti* forms of *O*-

Table 2.2 ^{1}H-NMR spectral data of *O*-methyloximes **15-17**

Compound	**15**		**16**		**17***	
	syn	*anti*	*syn*	*anti*	*syn*	*anti*
			Chemical Shift (ppm)			
1-H	5.51s	5.00s	4.84d	4.84d		4.43d
2-H	-	-	5.87dd	5.24dd		4.88dd
3-H	5.49d	6.00d	-	-	5.17t	5.16t
4-H	3.67dd	3.98dd	3.94d	4.70d	3.38t	3.45t
5-H	3.74-3.84m	3.82m	4.22m	(4.11-4.24m)	4.72dd	3.96dd
6-H_a	4.33dd	4.28dd	4.12dd		6.72d	7.35d
6-H_b	4.38dd	4.40dd	4.27dd		-	-
C-OCH_3	3.53s	3.50s	3.47s	3.46s		3.49s
N-OCH_3	3.94s	3.91s	3.93s	3.94s	3.92s	3.90s
-$OCH_2C\underline{H}_3$	1.19t	1.15t	1.22t	1.21t		1.10t
-$OC\underline{H}_2CH_3$	3.57m 3.79m	3.52-3.69m	3.42m 3.62m	3.43-3.53m		3.51-3.66m
-$OCOCH_3$	2.10s 2.12s	2.10s 2.12s	2.08s 2.09s	2.08s 2.12s		2.04s 2.06s
			Coupling Constants (Hz)			
$J_{1,2}$	-	-	4.4	2.2		7.8
$J_{2,3}$	-	-	-	-		9.7
$J_{3,4}$	4.5	7.0	-	-	9.1	9.4
$J_{4,5}$	6.5	8.4	2.3	1.6	9.4	9.5
$J_{5,6a}$	6.4	5.1	5.2		6.7	6.9
$J_{5,6b}$	4.9	3.6	4.7		-	-
$J_{6a,6b}$	11.6	11.8	10.0		-	-
$J_{2,4}$	-	-	0.6	0.9	-	-

*The chemicl shifts and the coupling constants were read from the *syn* and *anti* mixture.

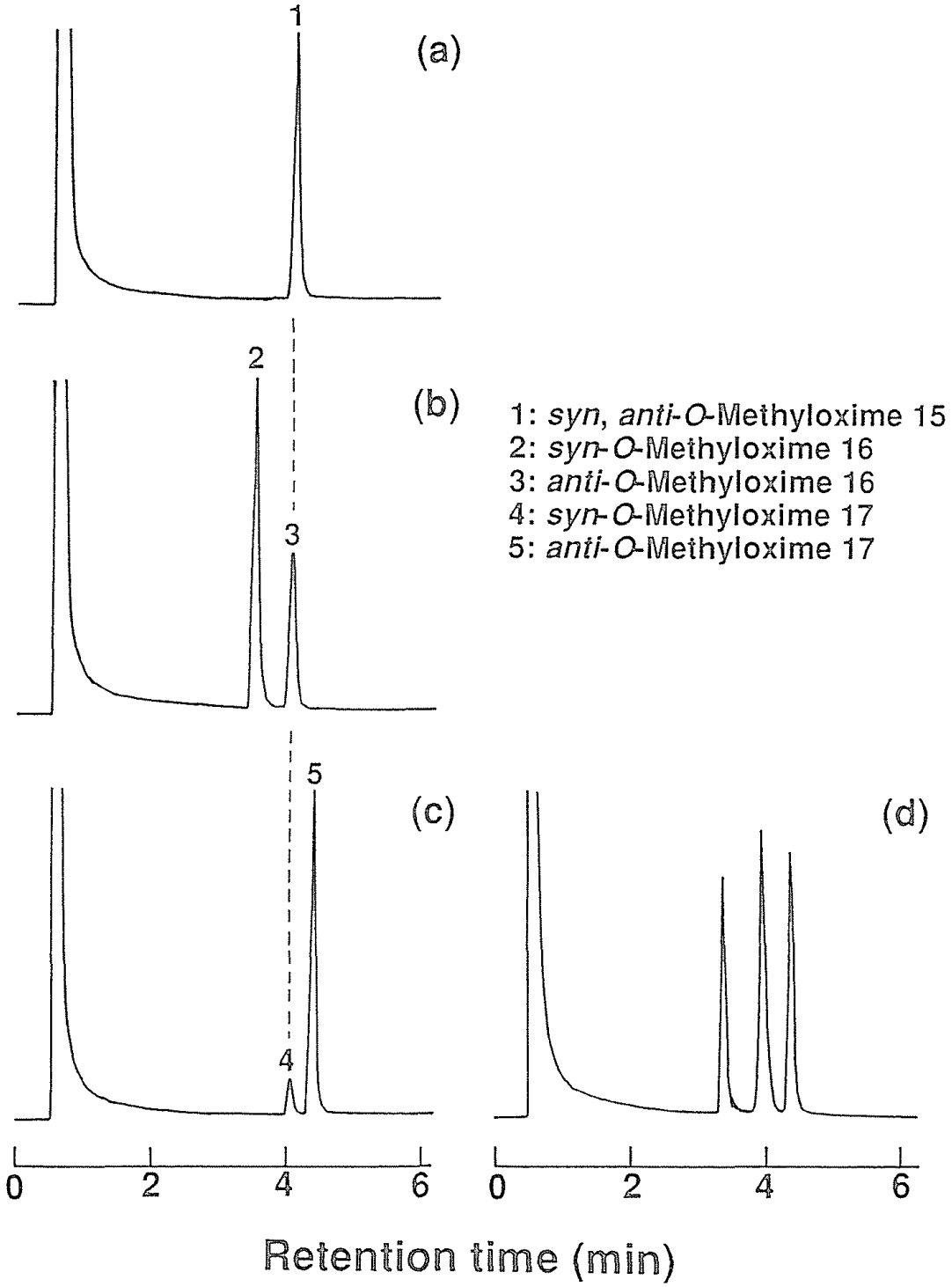


Figure 2.4 Gas chromatograms of *O*-methyloximes, (a): *O*-methyloxime **15**, (b): *O*-methyloxime **16**, (c): *O*-methyloxime **17**, and (d): mixture of *O*-methyloximes **15**-**17**

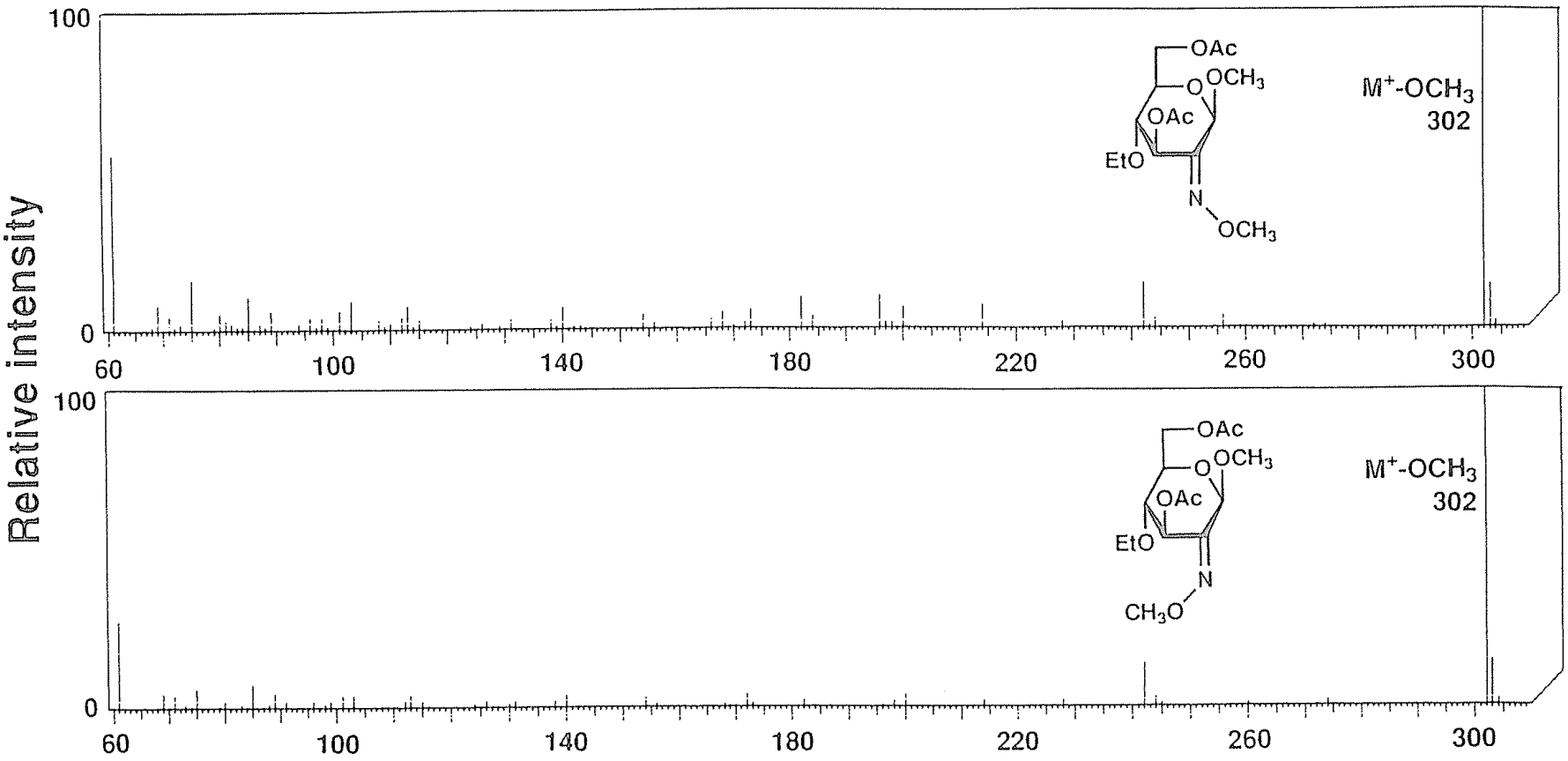


Figure 2.5 Mass spectra of *O*-methyloxime **15**

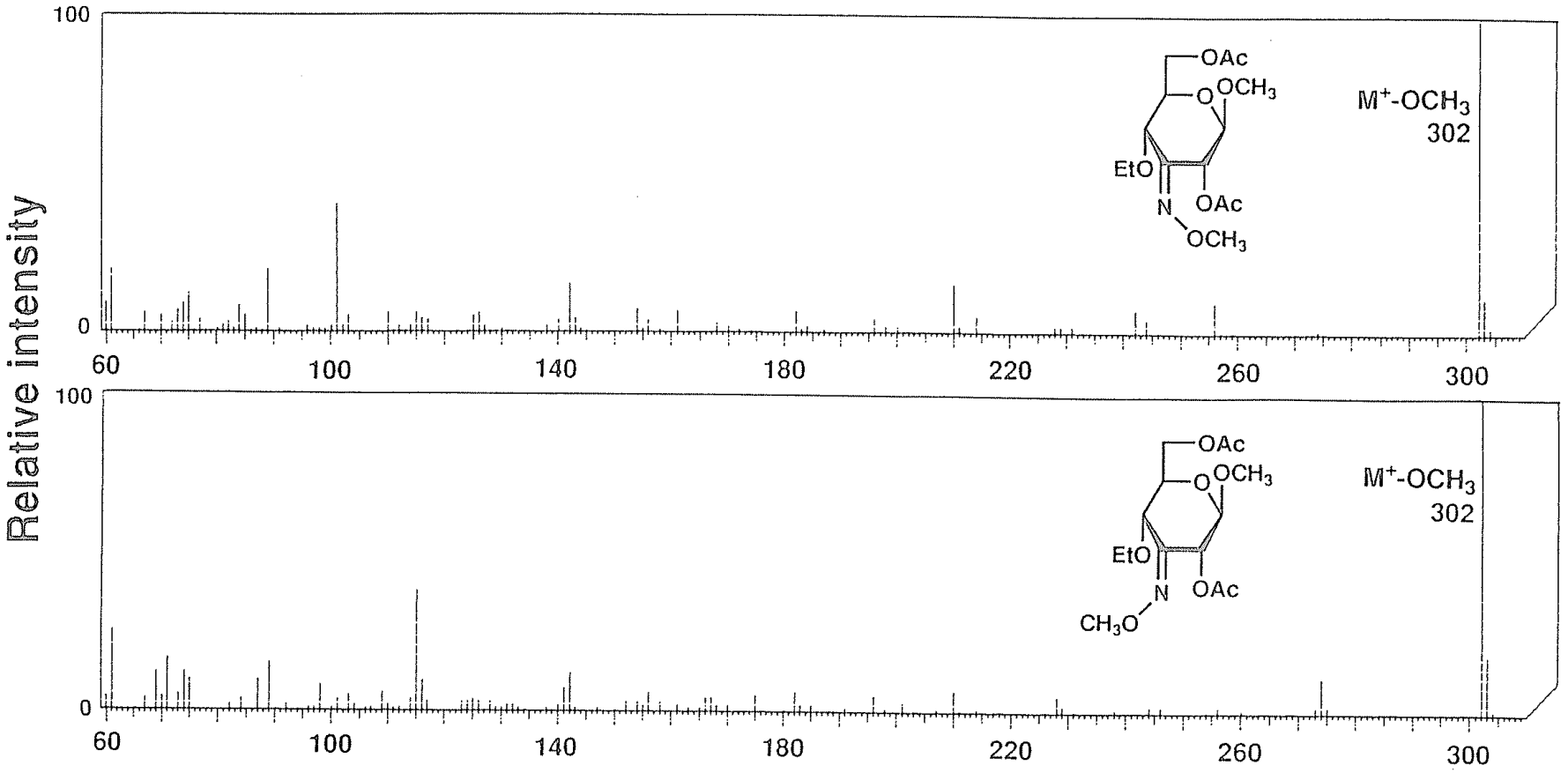


Figure 2.6 Mass spectra of *O*-methyloxime **16**

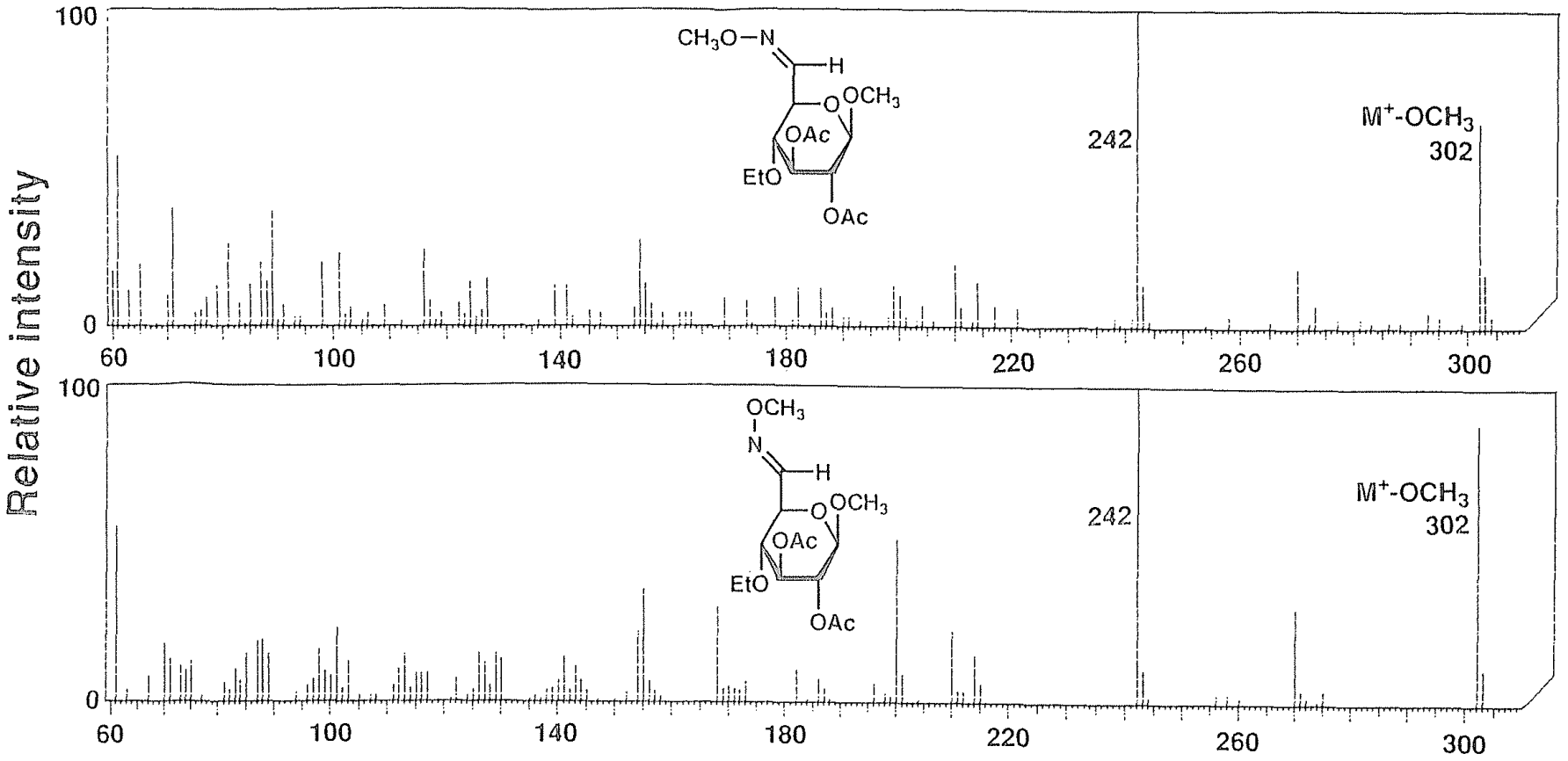


Figure 2.7 Mass spectra of *O*-methyloxime **17**

methyloximes **15-17** were similar to each other. The mass spectra of *O*-methyloximes **15-17** did not show molecular ion peak at m/e 333, but showed distinct peak at m/e 302 attributed to fragment (M^+-OCH_3). The mass spectra of *O*-methyloxime **17** showed a characteristic ion peak at m/e 242 (M^+-OCH_3-HOAC).

Thus, it was found that the ulose derivatives can be analyzed by GC-MS after *O*-methyloximation and subsequent acetylation. In Chapter 1, the author described that the viscosity drop caused by GC (glycosidic bond cleavage) reaction was greater than that by OX (oxidation) reaction during ozone bleaching of kraft pulp (Kishimoto *et al.* 1993). These reactions of polysaccharide with ozone can be elucidated in more detail by analyzing the ulose derivatives from ozonation of methyl 4-*O*-ethyl-β-D-glucopyranoside. The present method may be also applied to the investigations of the oxidation mechanisms with various reagents such as chlorine, oxygen and Fenton's reagent.

2.5 Summary

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was prepared as a model compound for cellulose to investigate the reactions of polysaccharides during ozone bleaching. The model compound was converted into acetylated carbonyl sugars: methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*arabino*-hexopyranosidulose (**2**), methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*ribo*-hexopyranoside-3-ulose (**3**), methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-*gluco*-hexodialdo-1,5-pyranoside (**4**). These carbonyl sugars were converted into *O*-methyloximes and analyzed by gas chromatography and mass spectrometry. Thus, it was found that the ulose derivatives which would be formed in ozonation of model compound **1** can be analyzed by GC-MS after *O*-methyloximation and subsequent acetylation.

Chapter 3

Quantitative Analyses of Reaction Products from Ozonation of Methyl 4-*O*-Ethyl-β-D-glucopyranoside

3.1 Introduction

Ozone inserts into glycosidic carbon-hydrogen bonds (Deslongchamps *et al.* 1974). Ozone-insertion into carbon-hydrogen bonds also occurs with alcohols, aldehydes, ethers, acetals, and even secondary and tertiary carbon-hydrogen bonds in hydrocarbons (Eckert and Singh 1980, Gierer 1982) as shown in **Figure 3.1**. The reactive hydroxyl radicals are also assumed to play an important role in the degradation of polysaccharides in ozone bleaching (Gierer and Zhang 1993).

In Chapter 1 the author divided the reactions of polysaccharides inducing viscosity drop during ozone bleaching of kraft pulp into two groups: GC (glycosidic bond cleavage) reaction and OX (oxidation) reaction, and proposed the method to evaluate the extent of these reactions. It is necessary to confirm what kinds of reactions are included with GC and OX reactions. These undesirable reactions can be elucidated in more detail by use of the low molecular weight model compound.

In Chapter 2 the author described the preparation and analytical method of cellulose model compound, methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) and the acetylated carbonyl sugars which would be formed by ozonation of model compound **1** (Kishimoto *et al.* 1995). In the present study, model compound **1** was treated with ozone in distilled water at room temperature.

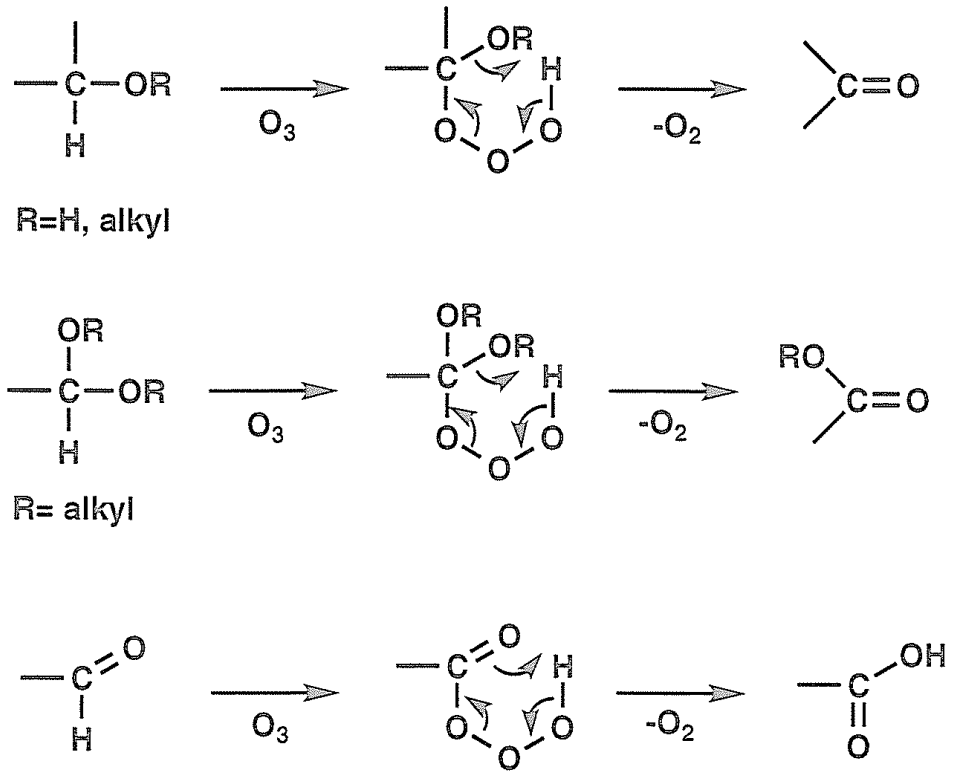


Figure 3.1 Ozone insertion reactions with alcohol, ether, acetal and aldehyde

The reaction products were analyzed by ^{1}H and ^{13}C-NMR spectroscopy and gas chromatography. The relative reactivities of carbon atoms at C1-C6 positions in model compound **1** during ozonation in distilled water were evaluated by the quantitative analysis of the reaction products.

3.2 Analysis of reaction products by NMR spectroscopy

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was used as a model for cellulose, and treated with ozone in distilled water (initial pH 5.7) at room temperature for 2h. The recovery of the model compound was about 50%. The reaction products were freeze-dried and analyzed by NMR spectroscopy. The ^{1}H and ^{13}C-NMR spectra of both ozonated and unozonated model compounds are shown in **Figures 3.2-3.5**.

As shown in **Figure 3.3**, the broadening of the ^{1}H-NMR signals assigned to C2-C6 protons at 3.2-4.0 ppm indicates the degradation and modification of model compound **1**. Increase of peak area for free-OH protons appeared at 4.77 ppm after ozonation shows the elimination of ethyl or methyl groups in model compound **1**. The ^{1}H-NMR signals assigned to aldehyde protons were observed at 8.22 and 8.44 ppm, although it was very small. These signals shows the oxidation of primary hydroxyl groups at C6 position. As shown in **Figure 3.5**, the multiplicity of the ^{13}C-NMR signals assigned to C1-C6 also indicate the degradation of model compound **1**, but the distinct signals assignable to carbonyl carbons were not observed. Thus, it was found that analyses of the reaction mixtures by NMR spectroscopy are difficult, even when the low molecular weight model compound **1** was used. It is much more difficult to analyze the ozonated cellulose by NMR spectroscopy.

3.3 Analysis of reaction products by gas chromatography

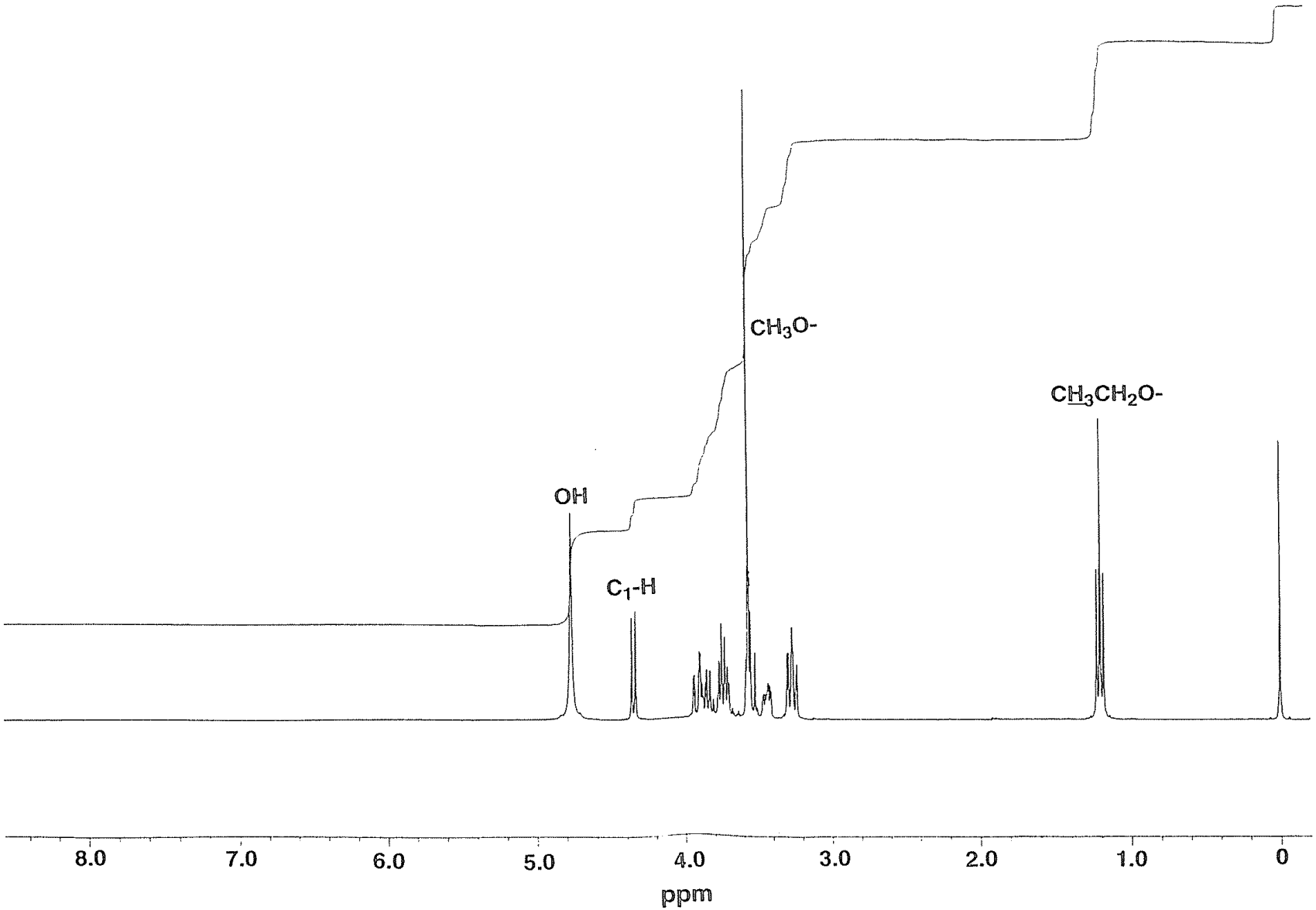


Figure 3.2 ^{1}H-NMR spectrum of methyl 4-*O*-ethyl-β-D-glucoside in D_2O.

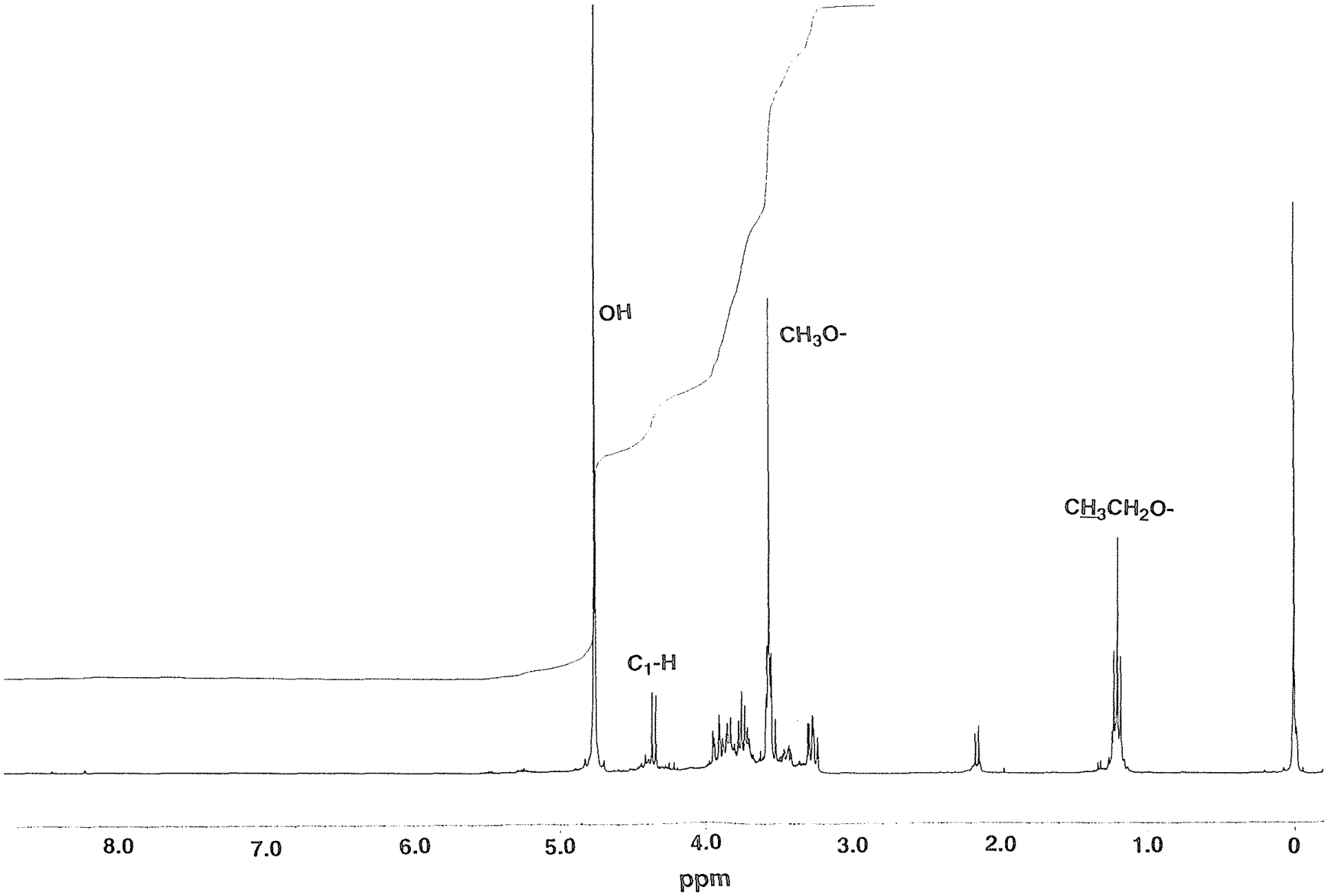


Figure 3.3 ^{1}H-NMR spectrum of the reaction products from ozonation of methyl 4-*O*-ethyl-β-D-glucoside in D_2O.

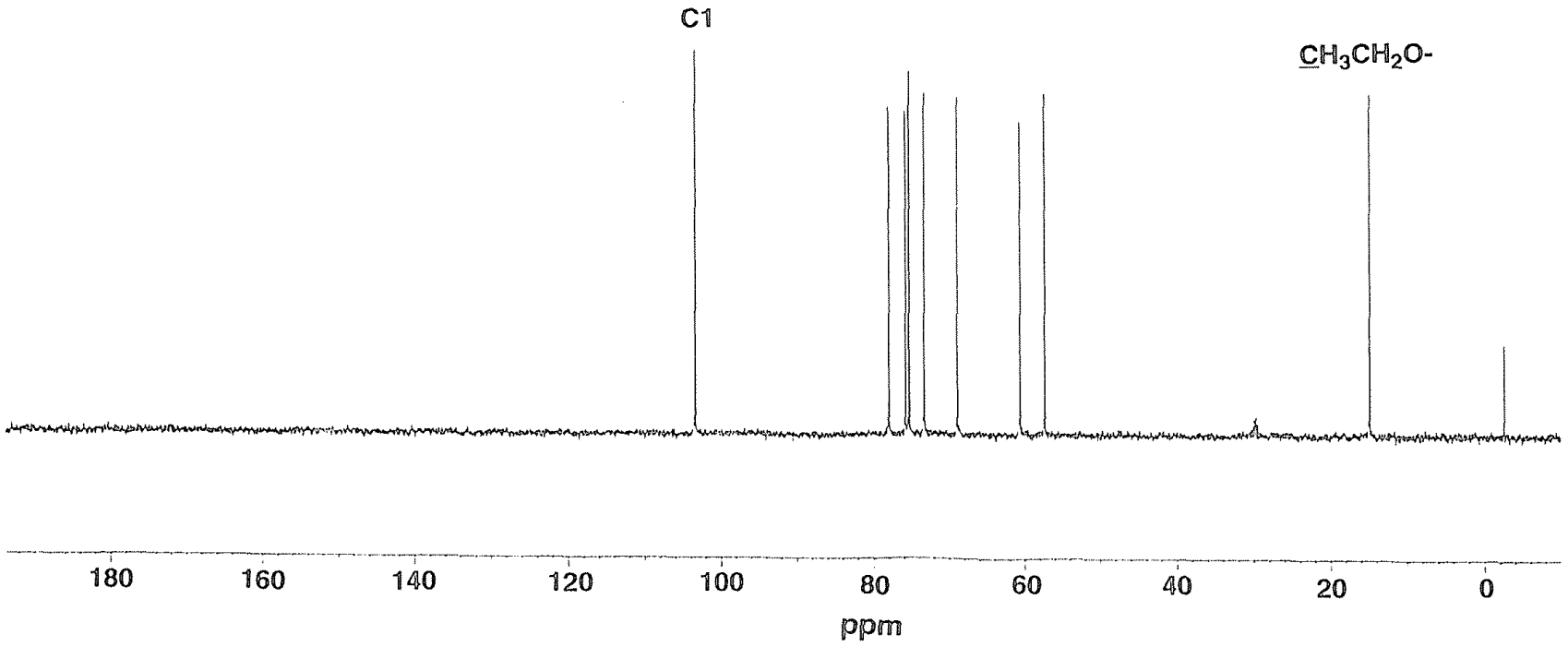


Figure 3.4 ^{13}C-NMR spectrum of methyl 4-*O*-ethyl-β-D-glucoside in D_2O

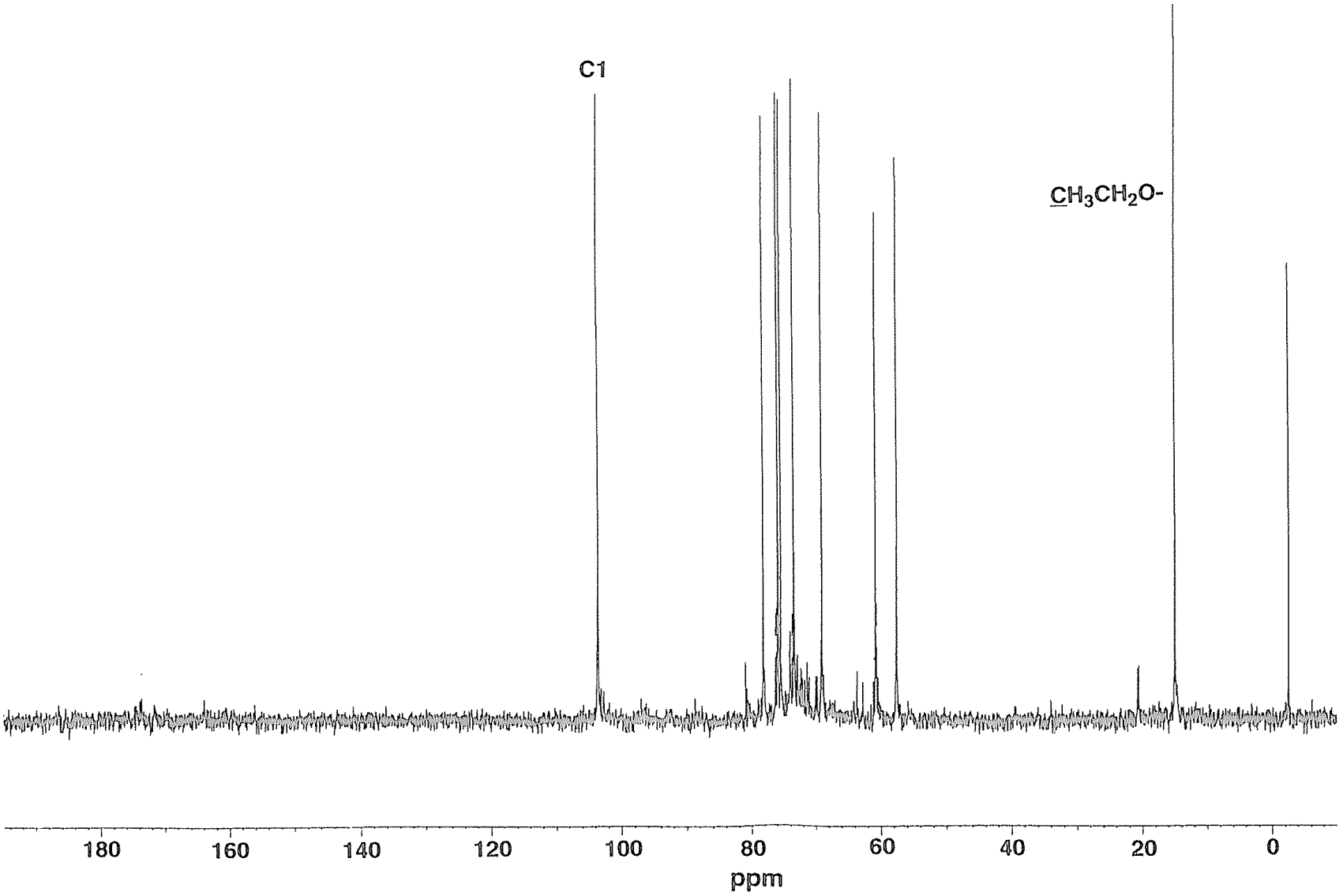


Figure 3.5 ^{13}C-NMR spectrum of the reaction products from ozonation of methyl 4-*O*-ethyl-β-D-glucoside in D_2O.

Model compound **1** was treated with ozone in distilled water. The standard analytical scheme was illustrated in **Figure 3.6**. The ozonated reaction mixture was divided into two fractions for separately analyzing neutral and acidic sugars. In the present study, mono saccharides with five to six carbon atoms were analyzed. The neutral sugars containing carbonyl sugars formed by ozonation of model compound **1** were *O*-methyloximated according to the method of Larm (1976) and acetylated. These acetylated *O*-methyloximes derivatives can be analyzed by gas chromatography (Kishimoto *et al.* 1995). Lactone-forming acidic sugars were treated with 0.1 M sodium hydroxide solution to give sodium salts and then trimethylsilylated (Petersson 1974). The acidic sugars were separated from neutral sugars with a strongly basic anion-exchange resin, because the peak of 4-*O*-ethyl-D-gluconic acid (**18**) overlapped with that of methyl β-D-glucoside (**5**) on gas chromatographic analysis. The neutral sugars were recovered quantitatively after the anion-exchange column separation, but the acidic sugars were not recovered quantitatively. Thus, 4-*O*-ethyl-D-gluconic acid (**18**) was quantitatively analyzed by the reduction of the peak area of methyl β-D-glucoside (**5**) from the overlapped peak area.

The reaction products from ozonation of model compound **1** are shown in **Figure 3.7**. The gas chromatograms of acetylated *O*-methyloximes of neutral sugars and trimethylsilylated acidic sugars are shown in **Figures 3.8** and **3.9**, respectively. These reaction products were determined by comparison with retention times of authentic samples, but 3-*O*-ethyl-D-arabinose (**28**) was assigned only by GC-MS spectrometry, because authentic samples were not obtained. The peaks 1 and 2 in **Figure 3.8** corresponds to *syn* and *anti* isomers of acetylated *O*-methyloximes of 3-*O*-ethyl-D-arabinose (**28**). Mass spectrum (m.s.) of peak 1 is similar to that of peak 2: the ion peaks at *m/e* 334 (10%) and *m/e* 274 (100%) were attributed to (M^++H) and (M^++H-HOAc), respectively.

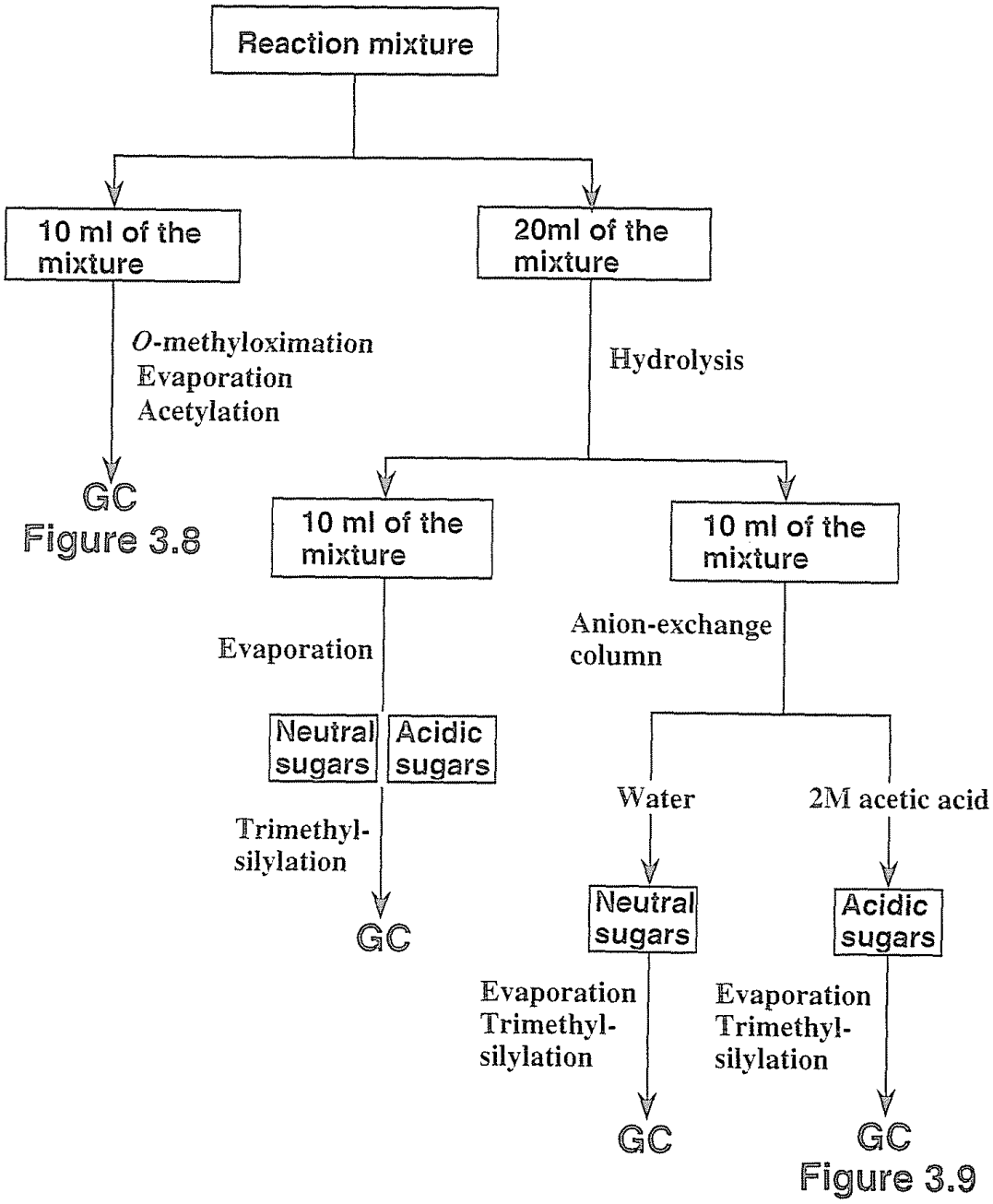


Figure 3.6 Standard analytical method for reaction products from ozonation of methyl 4-*O*-ethyl-β-D-glucopyranoside

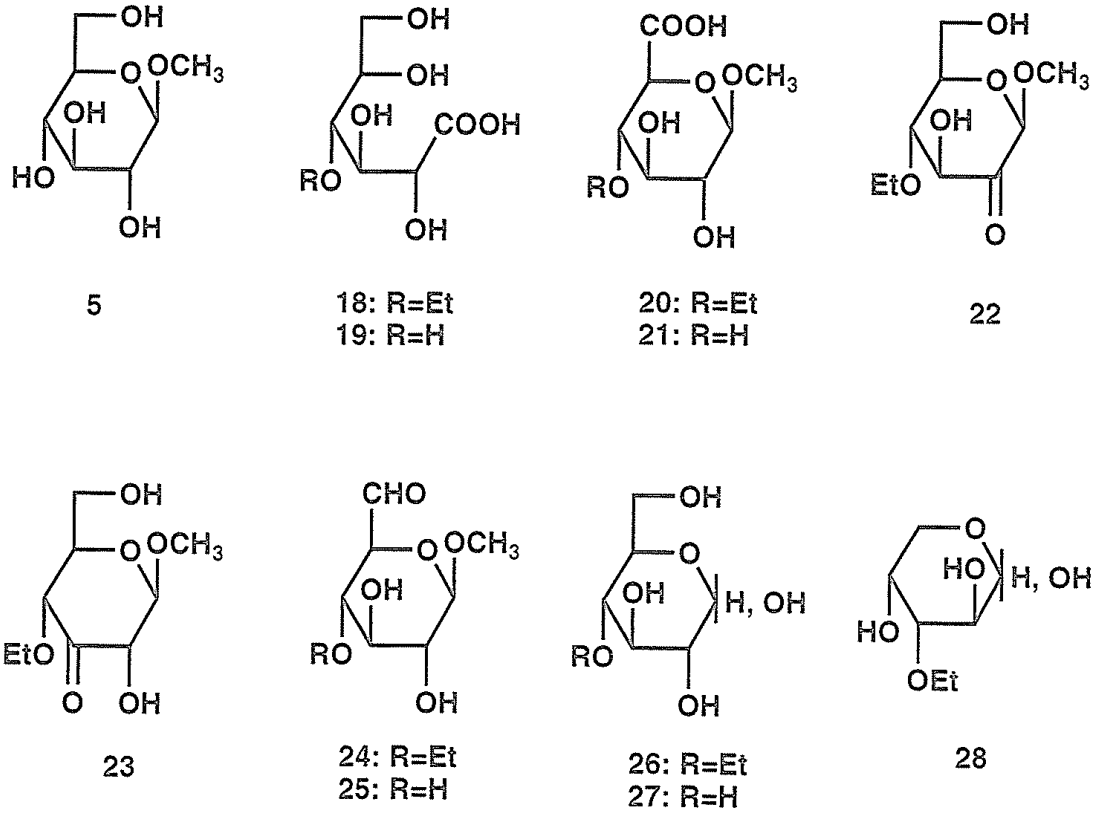


Figure 3.7 Reaction products from ozonation of methyl 4-*O*-ethyl-β-D-glucoside

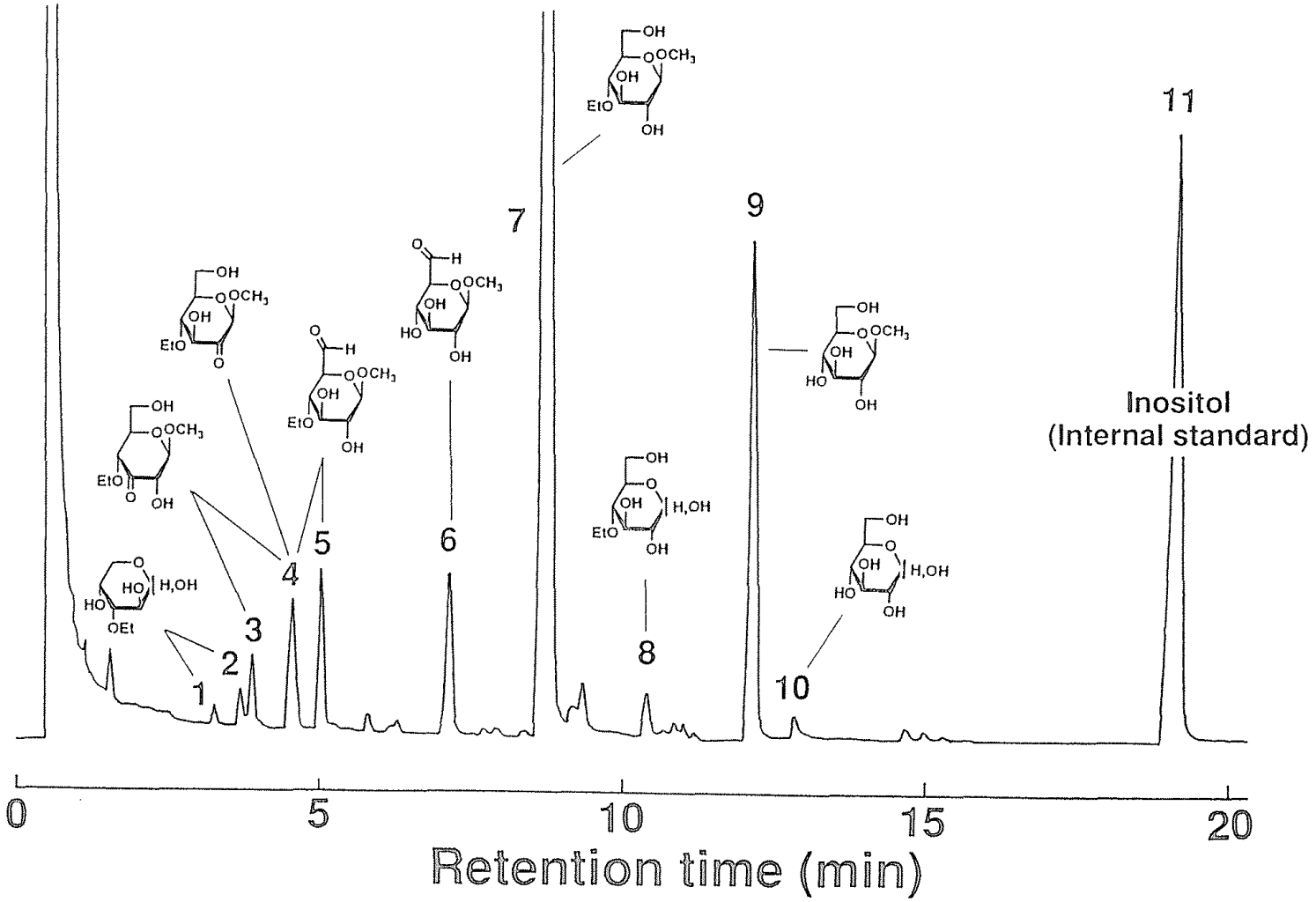


Figure 3.8 Gas chromatogram of the acetylated *O*-methyloximes of neutral sugars from ozonation of methyl 4-*O*-ethyl-β-D-glucoside.

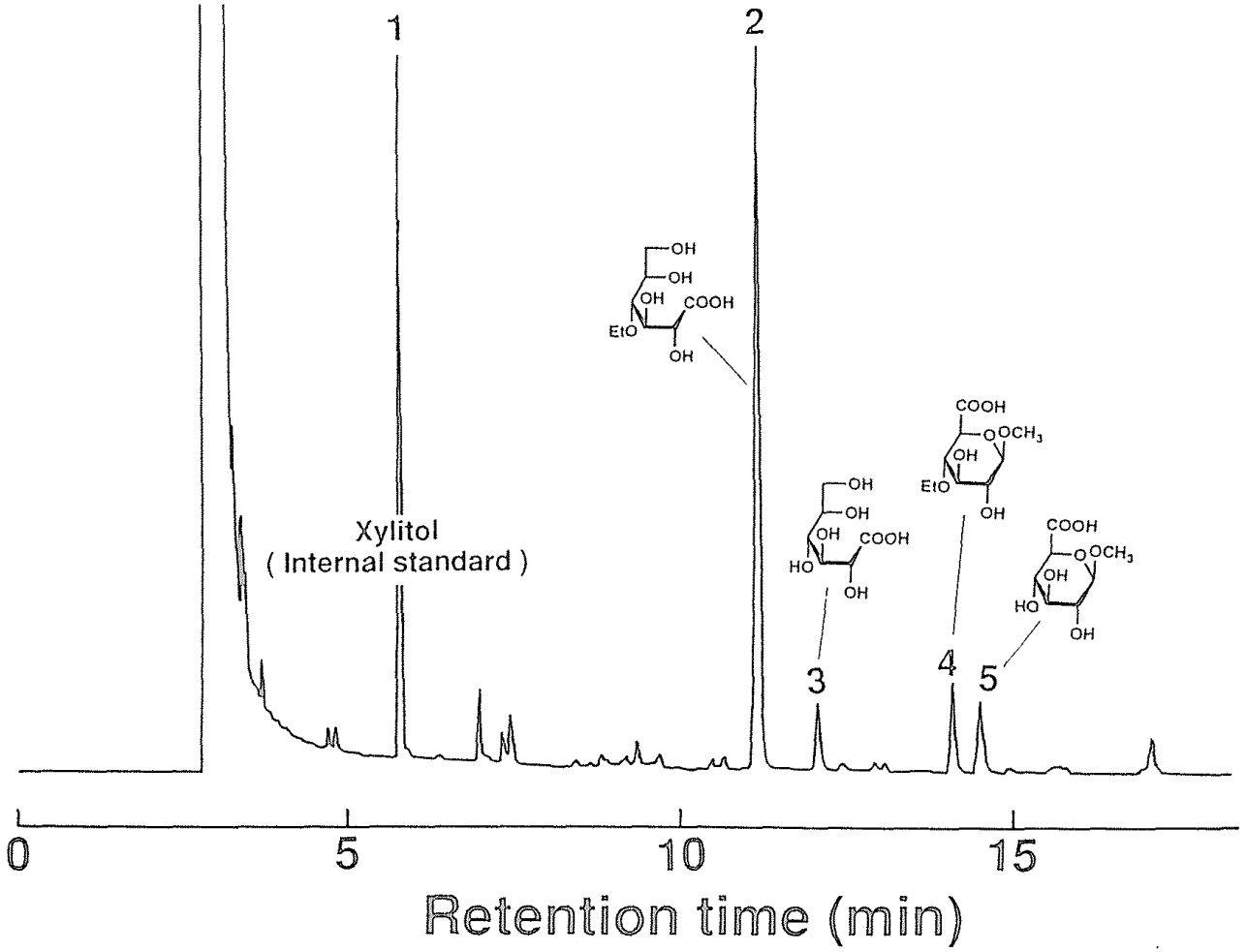


Figure 3.9 Gas chromatogram of the trimethylsilylated acidic sugars from ozonation of methyl 4-*O*-ethyl-β-D-glucoside.

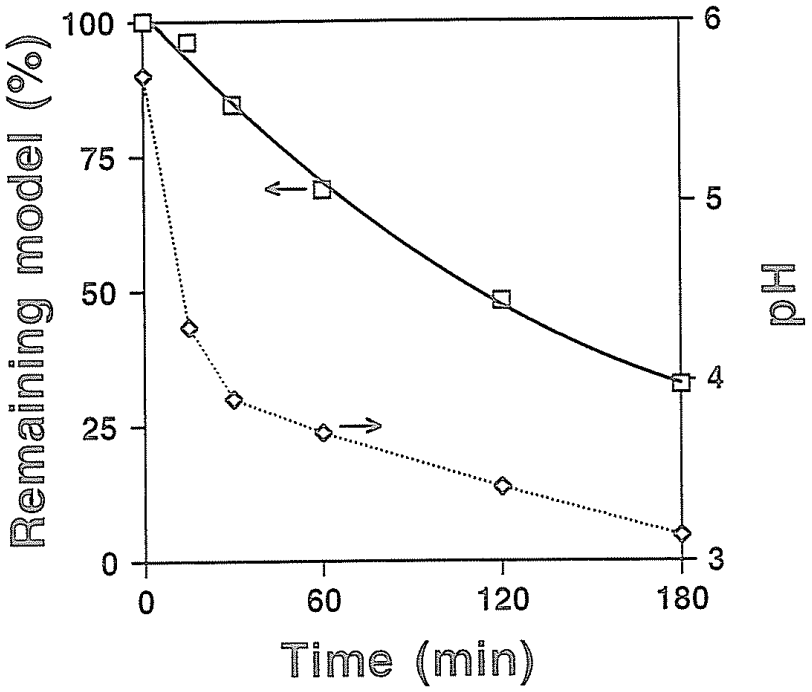


Figure 3.10 Ozonation of methyl 4-*O*-ethyl-β-D-glucoside in distilled water at room temperature

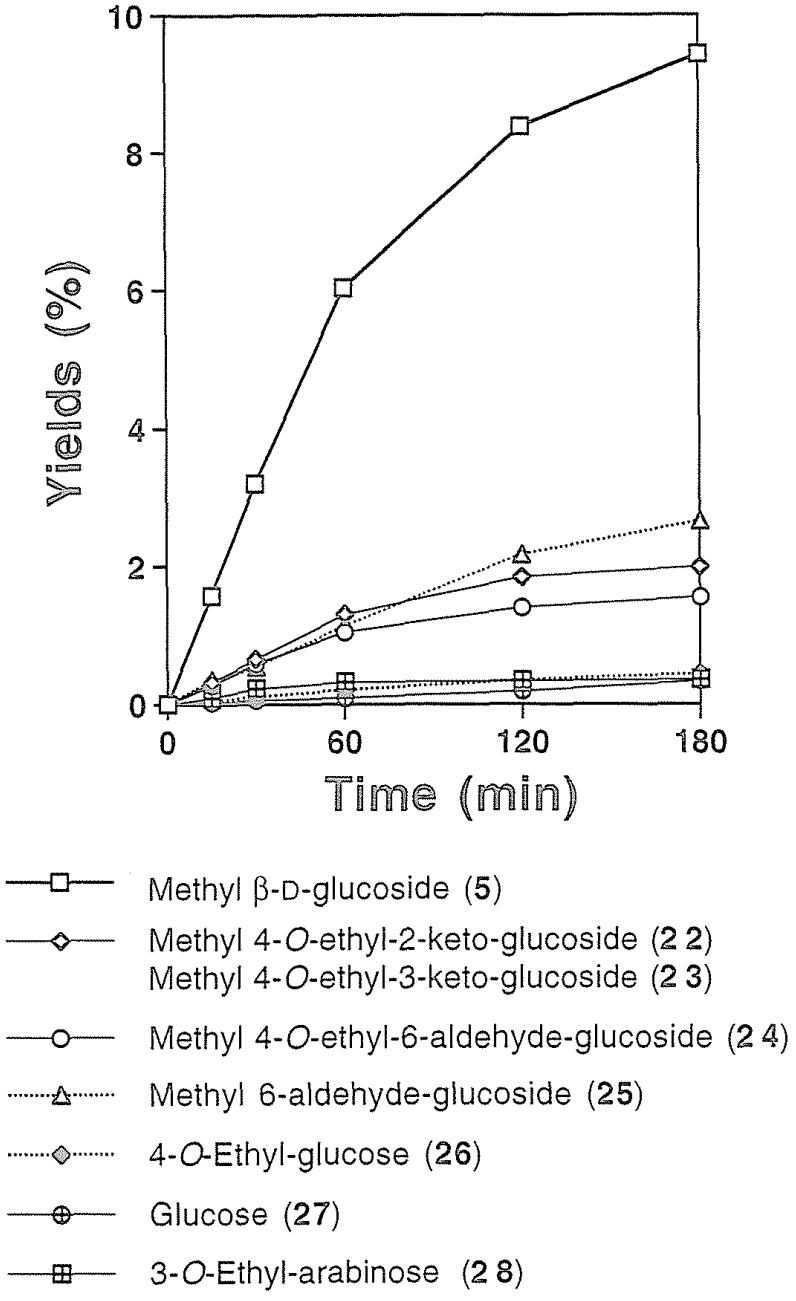


Figure 3.11 Neutral sugars from ozonation of methyl 4-*O*-ethyl-β-D-glucoside

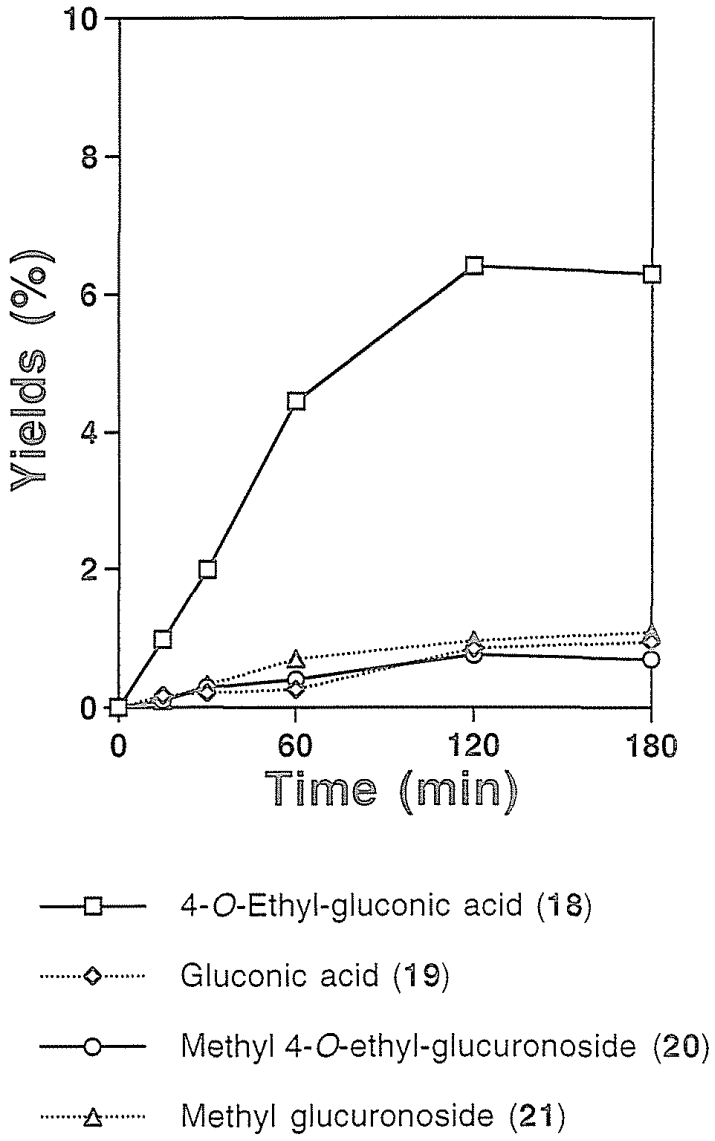


Figure 3.12 Acidic sugars from ozonation of methyl 4-*O*-ethyl-β-D-glucoside

The amount of methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) and the pH change of the reaction mixture are shown in **Figure 3.10**. The pH of the reaction mixture decreased with the progress of degradation of model compound **1**. The reduction of pH value was explained by the formation of acids. The yields of neutral and acidic sugars from ozonation of model compound **1** are shown in **Figures 3.11** and **3.12**, respectively.

3.4 Reactions at C1-C6 positions in the model compound

3.4.1 Reactions at C1-position

The major reaction products was 4-*O*-ethyl-D-gluconic acid (**18**), but only trace amounts of methyl 4-*O*-ethyl-D-gluconate were detected after direct acetylation of the reaction products without *O*-methyloximation. Deslongchamps (1974) suggested that only β-anomer reacts with ozone to produce methyl gluconate and not gluconic acid-δ-lactone in ozonation of per-acetylated methyl D-glucoside in acetic anhydride as shown in **Figure 3.13**. Angibeaud *et al.* (1985) detected only trace of methyl gluconate in ozonation of both methyl α- and β-D-glucoside in both buffered and unbuffered solutions. Thus, the mechanism proposed by Deslongchamps does not fit to the present results in aqueous solutions.

A small amount of 4-*O*-ethyl-β-D-glucose (**26**) was detected, which may be explained by the oxidative elimination of methyl group at 1-*O*-position by ozone or the previously proposed ozone-catalyzed hydrolysis (Katai and Schuerch 1966) besides the acid-catalyzed hydrolysis. A small amount of arabinose derivative (**28**) was also detected, which may be explained by the reaction similar to the Ruff degradation as reported previously (Katai and Schuerch 1966; Pan *et al.* 1981; Angibeaud *et al.* 1985).

3.4.2 Reactions at C2 and C3-positions

Methyl 4-*O*-ethyl-2-keto-glucoside (**22**) and methyl 4-*O*-ethyl-3-keto-glucoside (**23**) were detected in our model experiment. Identification of these carbonyl glucosides is the direct conclusive proof of the carbonyl groups formation at C2 or C3-position by ozonation of glucosides. These carbonyl groups activate glycosidic bond cleavage via β-elimination mechanism under alkaline conditions such as in the alkaline extraction stage. Diketo compounds which are intermediate reaction products in oxygen-alkali oxidation of glycosides (Ericsson 1974) were not detected.

3.4.3 Reactions at C4 and C5-positions

Another major product was methyl β-D-glucoside (**5**). Furthermore, methyl 4-*O*-methyl-β-D-glucopyranoside was also treated with ozone to give methyl β-D-glucoside (**5**), even though in low yield. On the other hand, methyl β-D-glucoside has not been observed by oxidation of methyl 4-*O*-methyl-β-D-glucopyranoside with both oxygen in alkali solution and hydrogen peroxide (Ericsson and Malinen 1974). Therefore, ethyl and methyl groups at 4-*O*-position were eliminated by ozone itself. In fact, ozone generally attacks ether to give carbonyl compounds as reported by Bailey and Lerdal (1978). The similar reactions of ozone at C4- or C5-positions would yield 4-keto or 5-keto compounds. However, only trace amounts of compounds which may be assigned to 4-keto compounds (retention time 7.5 - 8.6 min) were detected. These products may be also formed from the major product, methyl β-D-glucoside (**5**). The 5-keto compound was not detected, too. These results indicate that the oxidation at C4 or C5 has little participation in the degradation of cellulose by ozone.

3.4.4 Reactions at C6-position

Methyl 4-*O*-ethyl-6-aldehyde-glucoside (**24**), methyl 6-aldehyde-glucoside (**25**), methyl 4-*O*-ethyl-β-D-glucuronoside (**20**), and methyl β-D-glucuronoside (**21**) were formed in our model experiment. The yields of aldehyde derivatives (**24**, **25**) were higher than those of carboxyl acids (**20**, **21**). These carbonyl groups also activate glycosidic bond cleavage under alkaline conditions.

3.5 Relative reactivities of carbon atoms at C1-C6 positions in the model compound

The reaction products at C1-C6 positions may be divided into four groups according to the functional groups (acetal, secondary alcohol, primary alcohol and ether) of the reaction sites as shown in **Figure 3.14**. The formation of these reaction products could be explained by the insertion of ozone or the radical attack at the carbon-hydrogen bonds. **Figure 3.15** shows the total yields of reaction products at C1 (acetal), C2 or C3 (secondary alcohols), and C6 (primary alcohol) from ozonation of methyl 4-*O*-ethyl-β-D-glucoside (**1**). These yields are linear with reaction time up to 60 min. It is thought that the initial slopes of these plots correspond to the relative reactivities of carbon atoms at C1-C6 positions. The oxidation at C4 or C5 (ether) has little participation in the degradation of model compound **1** as described above. These results indicate that the order of relative reactivities of carbon atoms at C1-C6 positions during ozonation in distilled water at room temperature is as the following: C1 (acetal) > C6 (primary alcohol) > C2, C3 (secondary alcohols) > C4, C5 (ethers).

The highest reactivity at C1-position indicates that the glycosidic bond cleavage is one of the predominant reactions of polysaccharides during ozone bleaching of kraft pulp. In Chapter 1, the author described that the viscosity drop caused by GC (glycosidic bond cleavage) reaction was greater than that

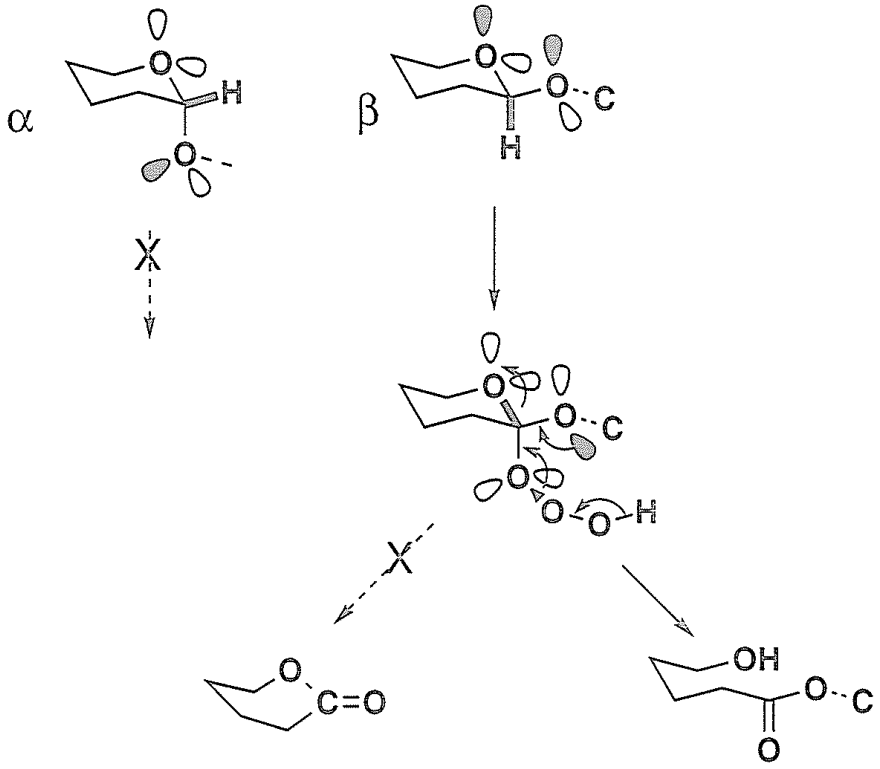


Figure 3.13 Orbital assisted reactions in ozonation of glycopyranosides

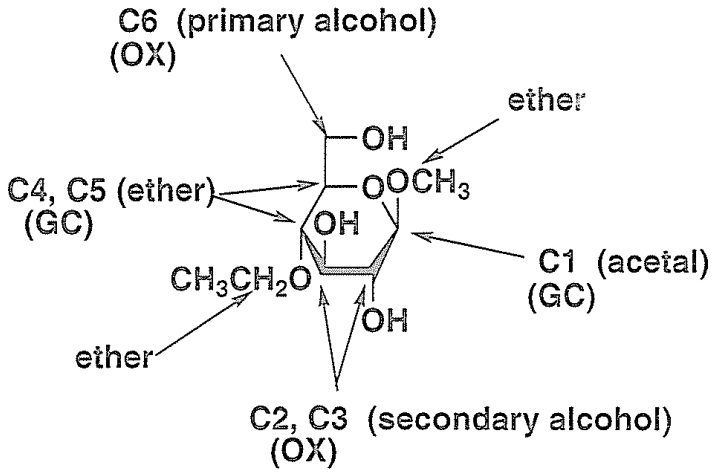


Figure 3.14 Reaction sites of methyl 4-*O*-ethyl β-D-glucopyranoside during ozonation.

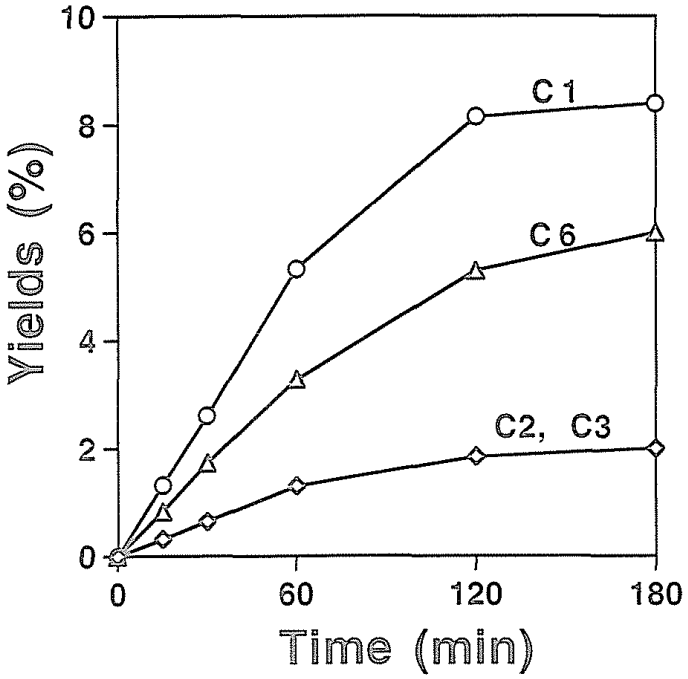


Figure 3.15 Total yields of reaction products at C1, C2, C3 and C6 positions from ozonation of methyl 4-*O*-ethyl-β-D-glucoside

by OX (oxidation) reaction, and that GC reaction was very much affected by additives such as methanol and oxalic acid in ozone bleaching of kraft pulp (Kishimoto *et al.* 1993). The effects of reaction conditions such as pH, temperature and additives on GC or OX reactions during ozone bleaching of kraft pulp could be elucidated in more detail by the model experiment.

3.6 Summary

Methyl 4-*O*-ethyl-β-D-glucopyranoside was used as a model for cellulose and treated with ozone in distilled water (pH 5.7). The reaction products were analyzed by NMR spectroscopy and gas chromatography. The ^{1}H and ^{13}C-NMR spectra of the ozonated model compound suggests its degradation, but the detailed results could not be obtained. Thus, gas chromatography is found to be useful for the quantitative analysis of such reaction products. The following products were determined by gas chromatographic analysis: methyl β-D-glucoside, 4-*O*-ethyl-D-gluconic acid, gluconic acid, methyl 4-*O*-ethyl-glucuronoside, methyl glucuronoside, methyl 4-*O*-ethyl-2-keto-glucoside, methyl 4-*O*-ethyl-3-keto-glucoside, methyl 4-*O*-ethyl-6-aldehyde-glucoside, methyl 6-aldehyde-glucoside, 4-*O*-ethyl-glucose, glucose, and 3-*O*-ethyl-arabinose. The oxidation at C4 or C5 positions has little participation in the degradation of the model compound. The formation of these products could be explained by the insertion of ozone or the radical attack at the carbon-hydrogen bonds in the model compound. The order of relative reactivities of carbon atoms at C1-C6 positions in the model compound during ozonation in distilled water was found from quantitative analysis of the reaction products: C1 (acetal) > C6 (primary alcohol) > C2, C3 (secondary alcohols) > C4, C5 (ethers). Inhibition of these undesirable reactions is one of the key to the prevention of the viscosity drop in ozone bleaching of kraft pulp.

Chapter 4

Free-radical Reactions of Methyl 4-*O*-Ethyl-β-D-glucopyranoside with Fenton's Reagent

4.1 Introduction

It has been assumed that the low selectivity in ozone bleaching is, at least partly, due to the formation of reactive radical species by the decomposition of ozone and organic peroxide intermediates, especially in the presence of heavy metal ions (Gierer and Zhang 1993). The formed active oxygen species such as hydroxyl radicals (HO∘), alkoxyl radicals (RO∘), hydroperoxyl radicals (HOO∘), and peroxyl radicals (ROO∘), abstract hydrogen atoms from carbon-hydrogen bonds activated by α-oxygen atoms in polysaccharide, introducing carbonyl groups in it. Hydroxyl radicals and alkoxyl radicals are the most reactive species.

Therefore, it is worthwhile to study in more detail the degradation of polysaccharide by radical species. The γ-irradiation techniques have been widely used for the study of free-radical reactions. Many meritorious investigations have been conducted by use of γ-radiolysis of aqueous solution of carbohydrate (von Sonntag 1980). The techniques have been also used in the field of non-chlorine bleaching chemistry in recent years (Reitberger *et al.* 1988, Ek *et al.* 1989, Gierer *et al.* 1992, 1994).

The combination of hydrogen peroxide and a ferrous sulfate, "Fenton's reagent" is also accepted as a convenient source for hydroxyl radicals ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\circ}OH$), (Snook and Hamilton 1974, Walling 1975). The

reaction of carbohydrate with Fenton's reagent has been investigated (Moody 1964). However, the degradation of glycosides have received relatively little attention and primary reaction products have not been determined sufficiently. The author reinvestigated in more detail the degradation of glucosides by Fenton's reagent: the combination of hydrogen peroxide and heavy metal ion is a good model system for the source of radicals in non-chlorine bleaching processes.

In the present investigation, the cellulose model compound, methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was treated with Fenton's reagent at room temperature. The amount of residual model compound and the relative reactivities of carbon atoms at C1-C6 positions in model compound **1** were determined by gas chromatographic analyses as described in Chapter 3. The effects of pH and oxygen on the degradation of model compound **1** were investigated.

4.2 Oxidation of the model compound with Fenton's reagent

Methyl 4-*O*-ethyl-β-D-glucopyranoside was treated with hydrogen peroxide in the presence of Fe^{2+} ions at room temperature under air at initial pH 3.5 for 2 h. The pH of the reaction mixture fell and reached to final pH 2.8.

The reaction products were analyzed by gas chromatography. The following oxidation products were identified: methyl β-D-glucoside (**5**) (9.3%), 4-*O*-ethyl-D-gluconic acid (**18**) (2.6%), gluconic acid (**19**) (0.7%), methyl 4-*O*-ethyl-glucuronoside (**20**) (0.1%), methyl glucuronoside (**21**) (0.6%), methyl 4-*O*-ethyl-2-keto-glucoside (**22**), methyl 4-*O*-ethyl-3-keto-glucoside (**23**) (2.8%; total yields of **22** and **23**), methyl 4-*O*-ethyl-6-aldehyde-glucoside (**24**) (2.0%), methyl 6-aldehyde-glucoside (**25**) (2.6%), 4-*O*-ethyl-glucose (**26**) (0.8%), and glucose (**27**) (0.7%). These reaction products were almost the same as those from

ozonation of model compound **1** as shown in **Figure 3.7**. Reaction products at C4 or C5 were not detected. Arabinose derivatives formed by ozonation were not detected either.

The treatment of model compound **1** only with hydrogen peroxide or only with iron (II) sulfate for 2 h as control experiments led to the recovery of the model compound unchanged. These results indicate that both hydrogen peroxide and Fe^{2+} ions are required for the degradation of carbohydrates.

The reaction products were formed by the abstraction of hydrogen atoms from carbon-hydrogen bonds in the model compound by radical species. The main product was methyl β-D-glucoside (**5**), which was formed by the oxidative elimination of ethyl group. The 2-keto, 3-keto and 6-aldehyde derivatives, and glucose have been already determined from oxidation of methyl β-D-glucopyranoside with Fenton's reagent (de Belder *et al.* 1963). However, the acid fraction has not been analyzed and further detailed investigations have not been conducted.

Instead of iron (II) sulfate, manganese (II) sulfate or copper (II) sulfate was used for the treatment of model compound **1** with hydrogen peroxide at room temperature at pH 3.5 for 2 h, but compound **1** was recovered almost unchanged. The Fe^{2+} ions may be the most harmful to polysaccharide in the presence of peroxides during non-chlorine bleaching: iron, manganese and copper are present abundantly in unbleached pine kraft pulp (Sjöström 1980).

4.3 Effect of pH on the oxidation of the model compound with Fenton's reagent

Fenton reaction is generally performed under acidic or neutral conditions. The degradation of carbohydrate with hydrogen peroxide in the presence of heavy metal ions under alkaline conditions have been investigated

(Isbell *et al.* 1976, Isbell and Czubarow 1990). However, the effect of pH on the degradation of glycosides with Fenton's reagent is not investigated sufficiently.

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was treated with Fenton's reagent at initial pH 2.1-10.9 under air. The pH of the reaction mixture was adjusted to 2.1 and 3.5 with H_2SO_4, and to 6.3 and 10.9 with NaOH, after the $FeSO_4/H_2SO_4$ solution was added as described in the experimental section. The addition of solid $FeSO_4$ instead of the $FeSO_4/H_2SO_4$ solution resulted in the initial pH 4.7.

The amount of recovered model compound **1** increased slightly with an increase of initial pH up to 4.7 as shown in **Figure 4.1**. The degradation of model compound **1** at pH 6.3 and 10.9 was drastically inhibited. The model compound was recovered almost unchanged at pH 10.9, and the degradation products (**5**, **18-27**) were not detected. This is probably because the solubility of Fe^{2+} and Fe^{3+} ions decreases with an increase of initial pH of the reaction mixture: in fact, a brown precipitate appeared at pH 6.3 and 10.9. The pH of the reaction mixture fell at initial pH 3.5, 4.7, 6.3 and 10.9 and reached to final pH 2.8, 2.7, 3.9 and 10.0, respectively, but did not change at initial pH 2.1.

The relative reactivities of carbon atoms at C1-C6 were evaluated by the distribution ratios of the oxidation products at C1, C2, C3 and C6 positions. As shown in **Figure 4.2**, the relative reactivities were C1 (39%) > C6 (38%) > C2, C3 (23%) > C4, C5 at initial pH 2.1, C6 (41%) > C1 (37%) > C2, C3 (22%) > C4, C5 at initial pH 3.5, C6 (52%) > C2, C3 (29%) > C1 (19%) > C4, C5 at initial pH 4.7, and C6 (57%) > C2, C3 (38%) > C1 (5%) > C4, C5 at initial pH 6.3. The relative reactivity at C1, that is, glycosidic bond cleavage decreased as the initial pH increased, whereas those at C2, C3 and C6 increased.

Although pH-dependence of the products ratio in oxidation with Fenton's reagent has been reported by Snook and Hamilton (1974), the inhibition of glycosidic bond cleavage at higher pH has not been reported and cannot be

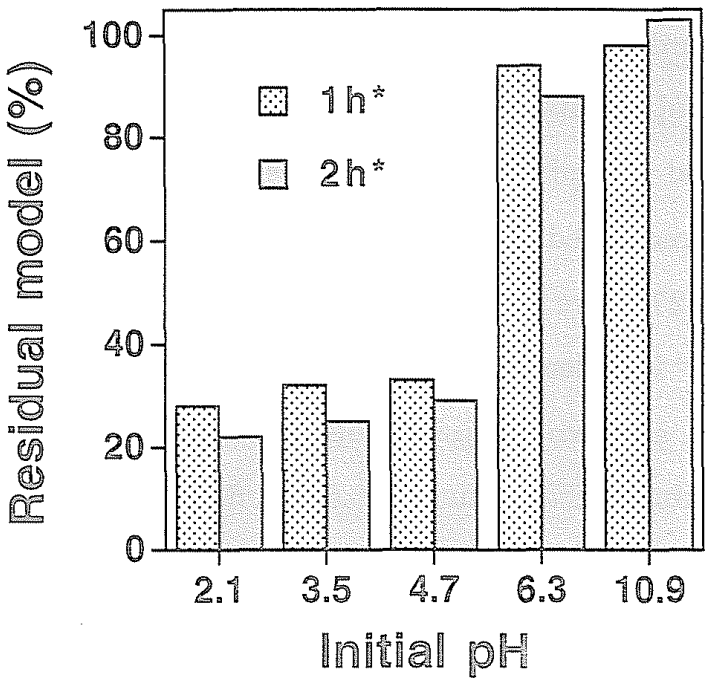


Figure 4.1 Effect of initial pH on the oxidation of methyl 4-*O*-ethyl-β-D-glucopyranoside with Fenton's reagent under air. (Conditions: model: 4.5 mmol/l; H_2O_2: 42 mmol/l; $FeSO_4$: 1.7 mmol/l; 25°C; pH 2.1, 3.5: H_2SO_4; pH6.3, 10.9: NaOH). *Reaction time.

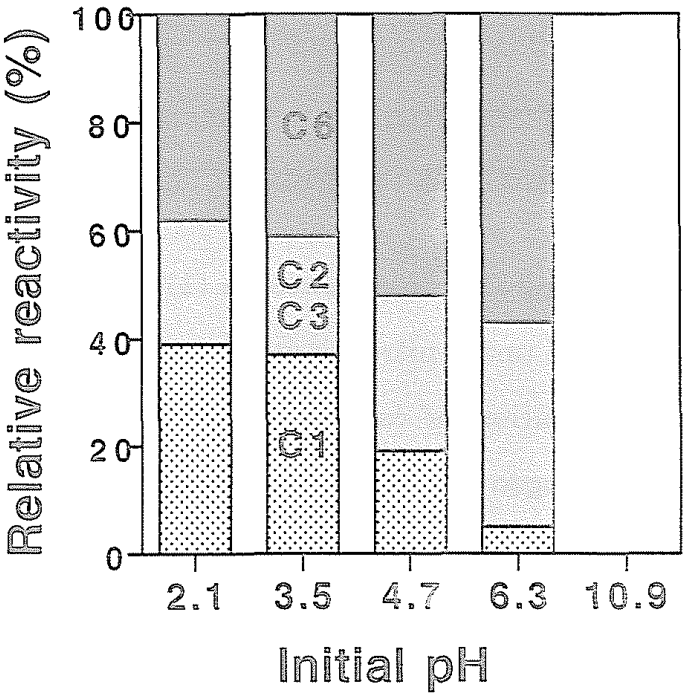


Figure 4.2 Effect of initial pH on the relative reactivities of carbon atoms at C1-C6 positions in methyl 4-*O*-ethyl-β-D-glucopyranoside toward Fenton's reagent under air. (Conditions: same as in Figure 4.1).

understood sufficiently. The selectivity of the abstraction of hydrogen atoms by hydroxyl radicals is probably independent on the pH of the reaction mixture. The higher selectivities at pHs 4.7 and 6.3 may be caused by reactive species other than hydroxyl radicals.

Consequently, hydroxyl radicals cause the degradation of carbohydrate with low selectivities at low pH under air, whereas higher selectivities was observed at higher pH: oxidation of primary hydroxyl groups at C6 is predominant reaction at higher pH.

4.4 Effect of oxygen on the oxidation of the model compound with Fenton's reagent

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was treated with Fenton's reagent at initial pH 3.5 under nitrogen or oxygen, to investigate the effect of oxygen on the degradation of carbohydrate. The degradation of model compound **1** with hydrogen peroxide in the presence of Fe^{3+} ions instead of Fe^{2+} ions was also investigated for comparison (hydroperoxyl radicals producing system; $Fe^{3+} + H_2O_2 \rightleftharpoons FeOOH^{2+} + H^+ \rightarrow Fe^{2+} + H^+ + {}^{\circ}OOH$, Walling 1975).

The pH of the reaction mixture fell both under nitrogen and oxygen, and reached to final pH 2.5 and 2.8, respectively. As shown in **Figure 4.3**, the degradation of model compound **1** was drastically enhanced under nitrogen: model compound **1** and initial reaction products disappeared completely after 2 h. On the other hand, oxygen drastically depressed the degradation of model compound **1**.

As shown in **Figure 4.4**, the relative reactivities of carbon atoms in model compound **1** were C1 (44%) > C6 (35%) > C2, C3 (21%) > C4, C5 under nitrogen, C6 (41%) > C1 (37%) > C2, C3 (22%) > C4, C5 under air, and C6 (63%) > C2, C3 (27%) > C1 (11%) > C4, C5 under oxygen. The 0.85 mM $FeSO_4$

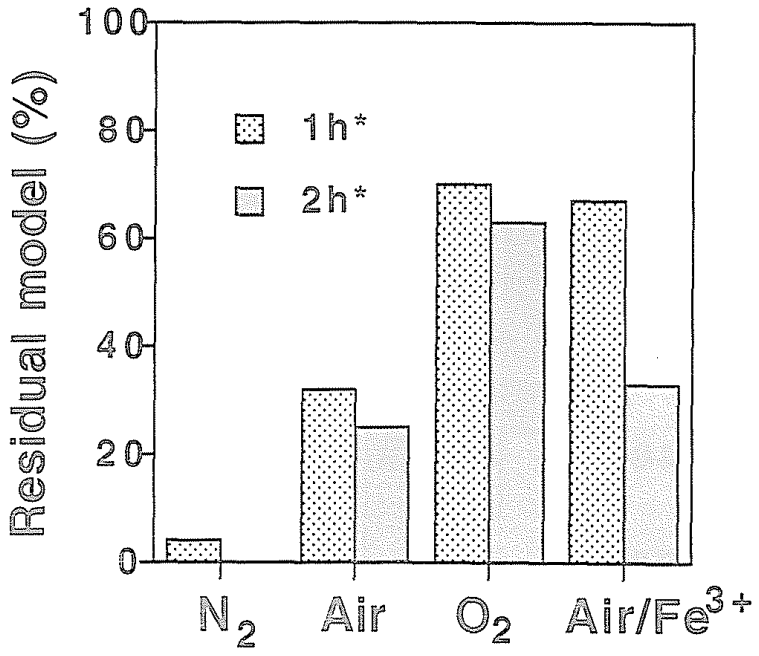


Figure. 4.3 Effect of oxygen on the oxidation of methyl 4-*O*-ethyl-β-D-glucopyranoside with Fenton's reagentat pH 3.5. (Conditions: See Figure 4.1, N_2: under N_2; Air: under air; O_2: under O_2; Air/Fe^{3+}: $FeCl_3$ instead of $FeSO_4$, under air). *Reaction time.

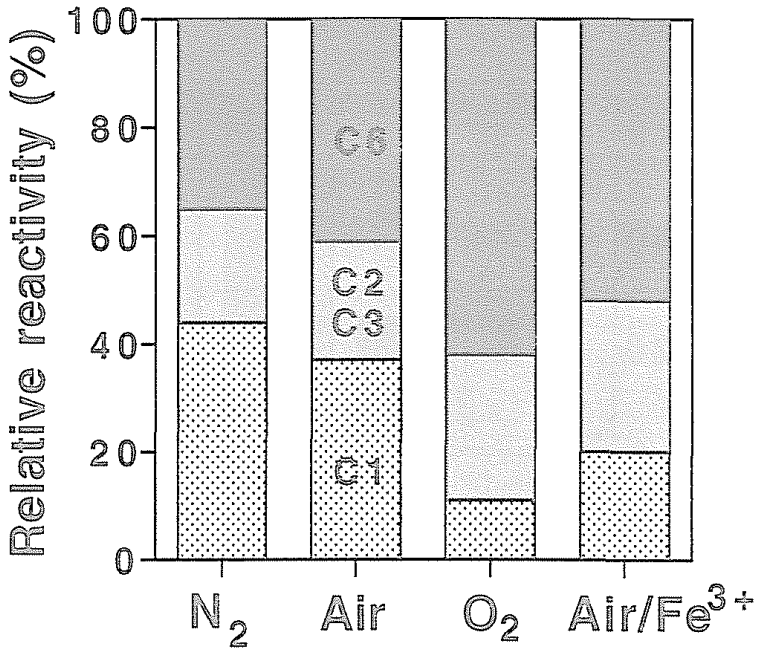


Figure. 4.4 Effect of oxygen on the relative reactivities of carbon atoms at C1-C6 positons in methyl 4-*O*-ethyl-β-D-glucopyranoside toward Fenton's reagent at pH 3.5. (Conditions: See Figure 4.3, N_2: 0.85 mM instead of 1.7mM $FeSO_4$).

solution was used under nitrogen, because the initial reaction products disappeared completely within 2h in the case of 1.7 mM $FeSO_4$ as described above. The low selectivity was observed under nitrogen, but the high selectivity was under oxygen: that is, the glycosidic bond cleavage at C1 was drastically inhibited by oxygen.

These experimental results may be explained as shown in **Figure 4.5**. In the first reaction stage, hydroxyl radicals produced by Fenton system ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$) abstract hydrogen atom from carbon-hydrogen bond in carbohydrate to result in primary carbohydrate radicals.

In the absence of oxygen, *i. e.*, under nitrogen (**Pathway A**), the primary radicals are oxidized to carbonyl compound with Fe^{3+} ions, accompanying the reduction of Fe^{3+} ions to Fe^{2+} ions (Snook 1974). The generated Fe^{2+} ions are further oxidized with H_2O_2 to give hydroxyl radicals, reproducing Fe^{3+} ions. Thus, the hydroxyl radicals are mainly concerned in the Fenton system under nitrogen resulting in the low selectivity.

In the presence of oxygen (**Pathway B**), the primary radical may be converted to carbonyl compound by the alternative pathway. That is, the primary radicals react preferentially with oxygen at a virtually diffusion-controlled rate to yield peroxyl radicals (Sonntag 1980), which are converted to carbonyl compound to release hydroperoxyl radicals. Thus, the regeneration of Fe^{2+} ions from Fe^{3+} ions by the primary radicals in **Pathway A** were depressed under oxygen in **Pathway B**. The formed Fe^{3+} ions are reduced with H_2O_2 to give hydroperoxyl radicals, which are less reactive, but has higher selectivities than hydroxyl radicals. Thus, the contribution of hydroperoxyl radicals probably increases under oxygen in the Fenton system, resulting in the high selectivity. Hydroperoxyl radicals are present predominantly as the $\cdot OOH$ species under these acidic conditions ($\cdot OOH \rightleftharpoons \cdot O_2^- + H^+$, pKa 4.8).

In fact the degradation of model compound **1** by hydrogen peroxide in the presence of Fe^{3+} ions under air at initial pH 3.5 ($Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ +$

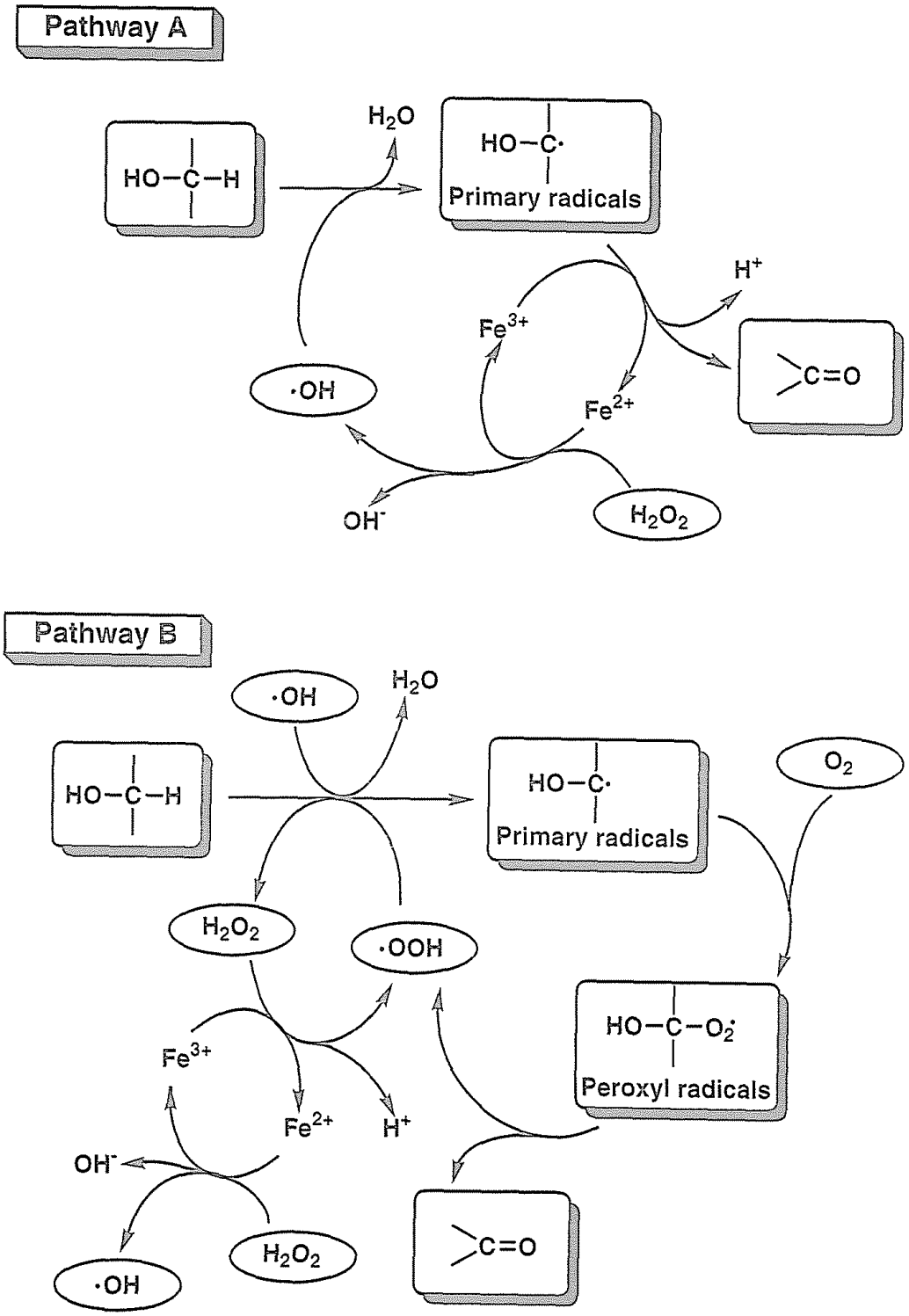


Figure 4.5 Oxidation of carbohydrate with Fenton's reagent in the absence (Pathway A) and presence of oxygen (Pathway B)

•OOH, Walling 1975) was slower than that in the presence of Fe^{2+} ions under air (**Figure 4.3**). The higher selectivity was observed in the presence of Fe^{3+} ions. The reactivity at C1 was lower than that in the presence of Fe^{2+} ions (**Figure 4.4**). These results also support that hydroperoxyl radicals are predominant under oxygen in acidic conditions and participate in the higher selectivity.

These experimental results suggest that hydroxyl radicals are predominant under nitrogen in acidic conditions and cause the degradation of carbohydrate with low selectivities. On the other hand, the degradation of carbohydrate is depressed under oxygen, that is, oxygen acts as inhibitor. The contribution of the less reactive hydroperoxyl radicals increases under oxygen. Hydroperoxyl radicals probably attack selectively at hydroxyl groups, especially primary hydroxyl groups, and does not cause significantly glycosidic bond cleavage.

Thus, oxygen may play an important role in the inhibition of the degradation of polysaccharide by radical species during hydrogen peroxide or peroxy acid bleaching.

4.5 Summary

Methyl 4-*O*-ethyl-β-D-glucopyranoside was used as a model for cellulose, and treated with Fenton's reagent at room temperature for 2 h, to investigate the free-radical reactions of polysaccharide. The reaction products were almost the same as those from ozonation of the model compound. These products were formed by the abstraction of hydrogen atoms from carbon-hydrogen bonds by radical species. The degradation of model compound and the relative reactivities of carbon atoms at C1-C6 positions in the model compound were affected very much by the initial pH and oxygen content. The initial higher pH inhibited the degradation of model compound. The reactivity

at C1, that is glycosidic bond cleavage decreased with an increase of initial pH. The higher selectivities at higher pH may be caused by reactive species other than hydroxyl radicals. Oxygen also inhibited drastically the degradation of model compound compared with that under nitrogen. The glycosidic bond cleavage was also inhibited by oxygen. These results may be explained as follows: Hydroxyl radicals are predominant under nitrogen, and cause the degradation of carbohydrate with low selectivities. Contribution of hydroperoxyl radicals increases under oxygen. Hydroperoxyl radicals probably cause the degradation of carbohydrate with high selectivities.

Chapter 5

Participation of Radical Species in Ozonation of Methyl 4-*O*-ethyl-β-D-glucopyranoside

5.1 Introduction

Ozone reacts not only with lignin but also with polysaccharides, impairing pulp viscosity and fiber strength during ozone bleaching. Insertion of ozone into carbon-hydrogen bonds in polysaccharide probably causes the degradation of polysaccharide during ozone bleaching. Non-selective radical species are also assumed to be responsible for the degradation of polysaccharide. Radical species abstract hydrogen atoms from carbon-hydrogen bonds in polysaccharide.

The formation of radical species during ozone bleaching has been explained by the hydroxide-anion-catalyzed decomposition of ozone, the transition metal-ion-catalyzed decomposition of ozone, the decomposition of hydroperoxidic intermediates, and one-electron oxidation of phenolic lignin units by ozone (Gierer and Zhang 1993). However, there is little experimental proof based on the reaction products for the participation of the radical species in the degradation of carbohydrate during ozonation.

The author described in Chapter 3 that the order of the relative reactivities of carbon atoms at C1-C6 positions in methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) during ozonation in distilled water is C1 > C6 > C2, C3 > C4, C5. The order was derived from the degradation products of model **1** with ozone itself and/or the radical species converted from ozone. However, the extent of contribution of ionic (ozone itself) and radical reactions was not

sufficiently elucidated. Then, in Chapter 4 the author described the reaction of model **1** with Fenton's reagents, selected as a typical hydroxyl radical generating system, to know only radical reaction of model **1**.

In this chapter the author describes the treatment of model **1** with ozone in anhydrous CH_2Cl_2, to elucidate the reactions of polysaccharide with ozone itself, because ozone is stable in CH_2Cl_2 without decomposition. Furthermore, the author discusses various factors affecting the formation of radical species from ozone reported previously, such as hydroxyl anion (pH), heavy metal ion, and phenolic lignin. We may understand polysaccharide reactions during ozonation in water in more detail, and obtain some information inhibiting the impairment of pulp viscosity and fiber strength during ozone bleaching.

5.2 Reactions of the model compound with ozone itself (Figure 5.1 A) or radical species (Figure 5.1 E)

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was treated with ozone in anhydrous CH_2Cl_2. Radical species probably do not participate in the ozonation of carbohydrate in anhydrous CH_2Cl_2. Model compound **1** with methyl group at 1-*O*-position and ethyl group at 4-*O*-position is slightly soluble in CH_2Cl_2, and its solubility is enough for the present experiments.

The composition of the reaction products were the same as those from ozonation in distilled water (Chapter 3), but the products distribution was largely different. The following products were determined: methyl β-D-glucopyranoside (**5**) (25.4%), 4-*O*-ethyl-gluconic acid (**18**) (5.5%), gluconic acid (**19**) (2.2%), methyl 4-*O*-ethyl-glucuronoside (**20**) (0.5%), methyl glucuronoside (**21**) (0.3%), methyl 4-*O*-ethyl-2-keto-glucoside (**22**), methyl 4-*O*-ethyl-3-keto-glucoside (**23**) (0.5%, total yields of **22** and **23**), methyl 4-*O*-ethyl-6-aldehyde-glucoside (**24**) (0.1%), methyl 6-aldehyde-glucoside (**25**) (0.4%), 4-*O*-ethyl-

glucose (**26**) (0.2%), glucose (**27**) (0.3%), and 3-*O*-ethyl-D-arabinose (**28**) (0.6%). The recovery of model **1** after ozonation in anhydrous CH_2Cl_2 for 2 h was 21.1 %.

As shown in **Figure 5.1 A**, the relative reactivities of carbon atoms in model **1** during ozonation in anhydrous CH_2Cl_2 were C1 (82%) > C6 (13%) > C2, C3 (5%) > C4, C5, which were determined from the total yields of the reaction products. In this case, the relative products-ratio did not change during ozonation as described in Chapter 3: ozonation in distilled water. These results indicate that the reaction of ozone itself (insertion of ozone into carbon-hydrogen bonds) occurs highly selectively at C1: ozone causes mainly the glycosidic bond cleavage (GC reaction).

On the other hand, the author already described in Chapter 4 that the relative reactivities of carbon atoms in model **1** toward Fenton's reagent are C1 (44%) > C6 (35%) > C2, C3 (21%) > C4, C5 under nitrogen at pH 3.5, and C6 (63%) > C2, C3 (27%) > C1 (11%) > C4, C5 under oxygen at pH 3.5 as shown in **Figure 5.1 E**. The author concluded that degradation of model **1** under nitrogen is mainly due to hydroxyl radicals (•OH), whereas contribution of hydroperoxyl radicals (•OOH) increases under oxygen in Fenton system.

Thus, the reactivities at C1 (GC reaction) are depend on the reactive species. The comparisons of relative reactivities at C1 make it possible for us to discuss the participation of radical species in the degradation of model **1** during ozonation under various conditions.

5.3 Effect of initial pH on the ozonation of the model compound (Figure 5.1 B)

It has been reported that ozone is unstable in water and hydroxyl radicals are formed by hydroxide-ion-catalyzed decomposition of ozone (Weiss 1935). The consumption of ozone in water has been reported to be

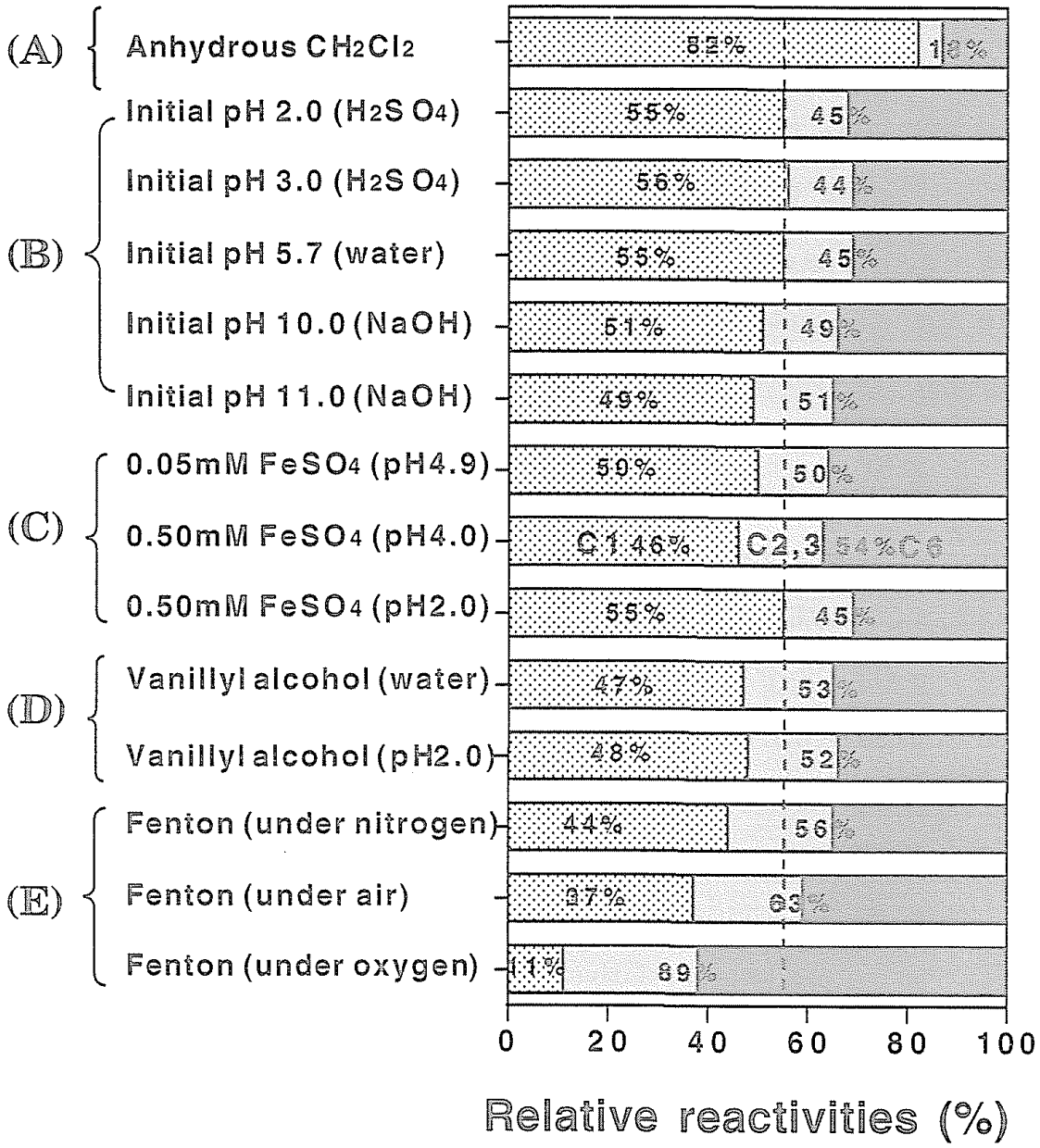


Figure 5.1 Relative reactivities of carbon atoms at C1-C6 positions in methyl 4-*O*-ethyl-β-D-glucopyranoside: ozonation in anhydrous CH_2Cl_2 (A), in aqueous solutions at initial pHs 2-11 (B), in aqueous $FeSO_4$ solutions (C), and in aqueous solutions with vanillyl alcohol (D); oxidation with Fenton's reagent (E).

accelerated by high pH in buffered solutions (Pan *et al.* 1984). However, it is not confirmed whether the degradation of carbohydrate during ozonation in aqueous solution is due to ozone itself or due to radical species. There have been conflicting discussions in literature concerning this subject (Schuchmann and Sonntag 1989, Eriksson and Reitbergar 1995). Thus, the author investigated the effect of pH on the degradation of model **1** and the participation of radical species in the degradation of carbohydrate in aqueous solutions.

Model **1** was treated with ozone at initial pHs 2-11 in aqueous solutions. The pH of the reaction mixture did not change during ozonation at initial pHs 2.0 and 3.0 (adjusted with H_2SO_4). The pHs fell during ozonation at initial pHs 5.7 (distilled water), 10.0 and 11.0 (adjusted with NaOH), and reached to final pHs 3.6, 3.9 and 7.6, respectively after 120 min. The amount of residual model **1** during ozonation in acidic or alkaline water is shown in **Figure 5.2**. At initial pH 2 and 3, the degradation of model **1** is almost the same as that in distilled water (initial pH 5.7). At initial pH 10, the degradation is enhanced within 30 min, but becomes parallel to that in distilled water after 30 min, when the pH of the reaction mixture became 6.0. At initial pH 11, the degradation was enhanced throughout the ozonation, where the final pH was 7.6 after 120 min. Thus, the degradation of model **1** is accelerated in alkaline conditions.

The same products were formed by ozonation both under acidic and alkaline conditions. **Figure 5.1 B** shows the relative reactivities of carbon atoms at C1-C6 positions in model **1** during ozonation at initial pHs 2-11. The reactivity at C1 by ozonation in distilled water (55%) is lower than that in anhydrous CH_2Cl_2 (82%), and is higher than those by oxidation with Fenton's reagent both under nitrogen (44%) and under oxygen (11%). These results indicate that the degradation of model **1** during ozonation in distilled water is partly due to radical species and partly due to ozone itself. Contribution of

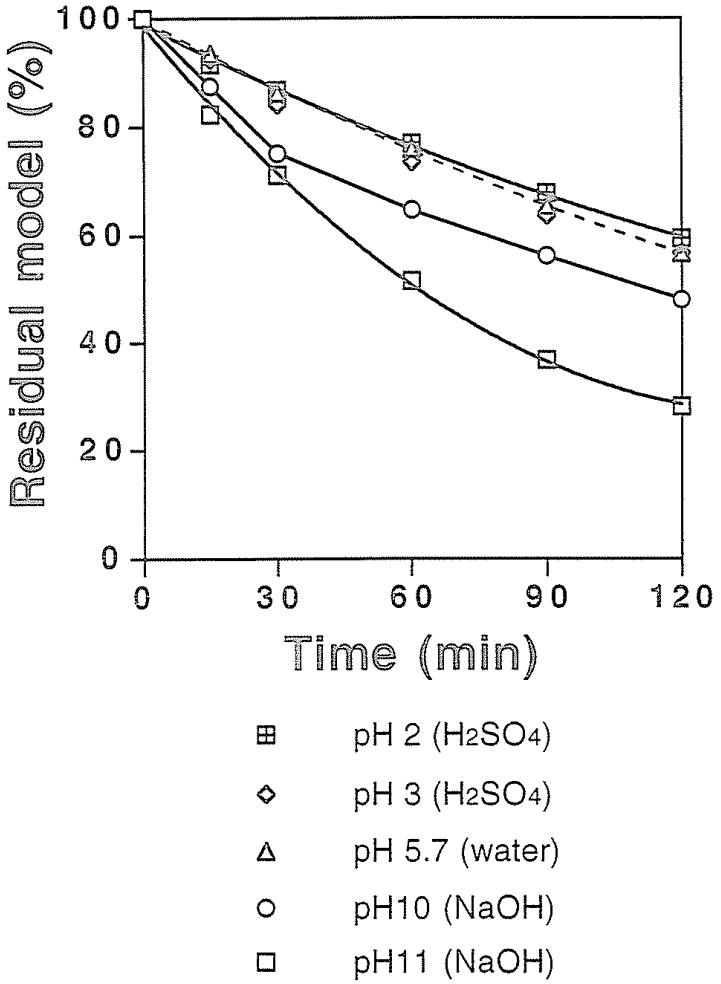


Figure 5.2 Effect of pH on the ozonation of methyl β-D-glucopyranoside (Conditions: model **1**: 100 mg (0.45 mmol) in 100 ml of distilled water, O_3 12 mg/min for 2 h)

radical species in distilled water (reactivity at C1: 55%, **Figure 5.1 B**) is estimated to be about 40-70%. Here, the relative reactivities at C1 toward ozone itself was assumed to be 82% (**Figure 5.1 A**), and that toward radical species was maximum 44% (**Figure 5.1 E**, nitrogen) to minimum 11% (**Figure 5.1 E**, oxygen): for example, the minimum value of 40% was obtained by the following equation: (82-55) / (82-11) x 100 = 38 = ca. 40.

Thus, it is concluded that glycosidic bond cleavage (GC reaction) is caused both by ozone itself and radical species in ozonation in distilled water, and oxidation of hydroxyl groups at C2, C3 and C6 positions to carbonyl groups (OX reaction) is mainly caused by radical species. Inhibition of the formation or reaction of radical species is effective to the prevention of viscosity drop in ozone bleaching of kraft pulp.

In acidic water the relative reactivities of carbon atoms at C1-C6 positions in model 1 are almost the same as those in distilled water. These results indicate that there is no fundamental difference between the reaction of carbohydrates in acidic water and in distilled water during ozonation at room temperature, partly because the pH of the reaction mixture falls during ozonation in distilled water owing to the formation of acids (Chapter 3).

In alkaline water (pH10: 51%, pH11: 49%), the relative reactivities at C1 is lower than that in distilled water (55%), and is higher than those by oxidation with Fenton's reagent both under nitrogen (44%) and oxygen (11%). These results support that radical species formed by hydroxide-ion-catalyzed decomposition of ozone participate in the additional degradation of carbohydrate in alkaline water.

5.4 Effect of Fe^{2+} ions on the ozonation of the model compound (Figure 5.1 E)

It has been reported that heavy metal ions such as Fe^{2+} and Co^{2+} ions enhance the decomposition of ozone (Pan *et al.* 1984). Gierer and Zhang (1993) reported that the relative yield of hydroxyl radicals increases with addition of different transition metal ions in the order of $Fe^{2+} > Mn^{2+} > Cu^{2+}$ during ozonation of fully-bleached softwood kraft pulps. However, it is not confirmed whether or not the radical species formed by the decomposition of ozone with heavy metal ions participate in the degradation of carbohydrate during ozonation.

Model 1 (0.45 mmol) was treated with ozone both in 0.05 mM (about 3 ppm, initial pH 4.9) and in 0.50 mM $FeSO_4$ aqueous solutions (about 30 ppm, initial pH 4.0) as shown in **Figure 5.3**. These data were compared with those in distilled water shown by a dotted line. The enhanced degradation during ozonation in 0.05 mM $FeSO_4$ was small, but large additional degradation was observed in 0.50 mM $FeSO_4$. The enhanced degradation of model 1 suggests that the radical species formed by decomposition of ozone catalyzed by Fe^{2+} ions participate in the degradation of model 1.

However, the degradation at pH 2 (H_2SO_4) is almost the same as that in distilled water without $FeSO_4$. The additional degradation of carbohydrates in the presence of Fe^{2+} ions can be inhibited completely by adjusting to low pH.

Figure 5.1 C shows the relative reactivities of carbon atoms at C1-C6 positions in model 1 during ozonation in aqueous $FeSO_4$ solutions. The relative reactivities at C1 during ozonation both in 0.05 mM (50%) and in 0.50 mM $FeSO_4$ (46%) are lower than that in distilled water without $FeSO_4$ (55%), and are higher than those by oxidation with Fenton's reagent (under nitrogen: 44%, under oxygen: 11%). The relative reactivities of carbon atoms in model 1 at pH 2 in 0.5 mM $FeSO_4$ are completely the same as those by ozonation in distilled water without $FeSO_4$. Thus, the additional degradation by adding Fe^{2+} ions to the reaction medium is apparently attributable to the

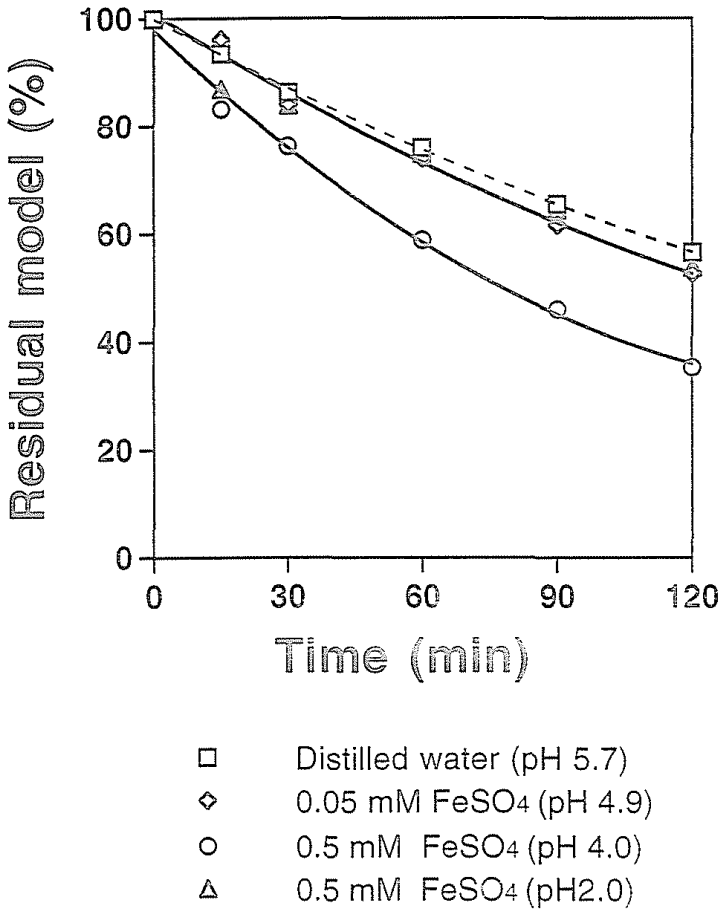


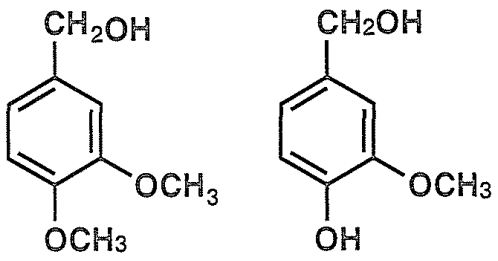
Figure 5.3 Effect of ferrous sulfate on the ozonation of methyl 4-*O*-ethyl-β-D-glucopyranoside. (Conditions: see Figure 5.2).

radical species formed by the decomposition of ozone catalyzed by Fe^{2+} ions, and it is completely inhibited by adjusting to low pH.

It is significant that the removal of Fe^{2+} ions is not necessary for inhibition of viscosity drop in ozone bleaching of pulp, if the bleaching is conducted at low pH. It is suggested that the improvement effect of low pH on the selectivities of ozone bleaching reported so far is partly due to the complete inhibition of the radical-formation catalyzed by heavy metal ions: the heavy metal-ion-catalyzed decomposition of ozone is completely inhibited by adjusting to low pH.

5.5 Effect of lignins on the ozonation of the model compound (Figure 5.1 D)

It has been reported that phenolic lignin model compounds enhance the degradation of carbohydrates during ozonation (Magara *et al.* 1994, Kang *et al.* 1995). The enhanced degradation of carbohydrates has been tentatively explained by the formation of hydroxyl radicals by one-electron reduction of ozone with a phenolic lignin unit (Gierer and Zhang 1993). The formation of radical species in the presence of lignin model compounds has been investigated (Eriksson and Reitbergar 1995). However, there is no direct proof supporting the radical degradation of carbohydrate. As described above, the product distribution obtained from ozonation of model **1** become a suitable guide to evaluate whether the reaction proceeds by ozone or by radical species. Hydroperoxidic intermediates, i. e. primary reaction products from unsaturated carbon-carbon bond in lignins are also assumed to have harmful effect on carbohydrate (Gierer and Zhang 1993). Then, the author investigated ozonation of model **1** in the presence of lignin model compounds for evaluating the radical reaction from the degradation products.



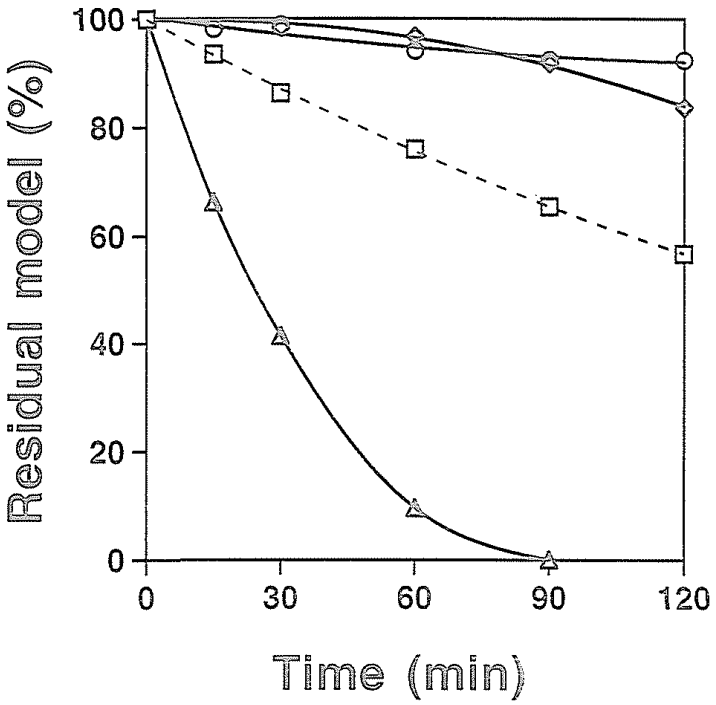
31 32

Figure 5.4 Lignin model compounds used in this experiment

Both additions of a non-phenolic lignin model, veratryl alcohol (**31**) and an ozonated products of vanillyl alcohol (**32**) obtained from ozonolysis of **32** for 90 min inhibit the degradation of model **1** during ozonation in distilled water as shown in **Figure 5.5**. These inhibitions by the non-phenolic structure and the ozonated products are probably due to their higher reactivities toward ozone than carbohydrates: in fact, Eriksson and Gierer (1985) reported that the order of relative reactivities toward ozone is phenolic structures > muconic acid intermediates (primary products from ozonation of vanillyl alcohol) > non-phenolic structures >> carbohydrates.

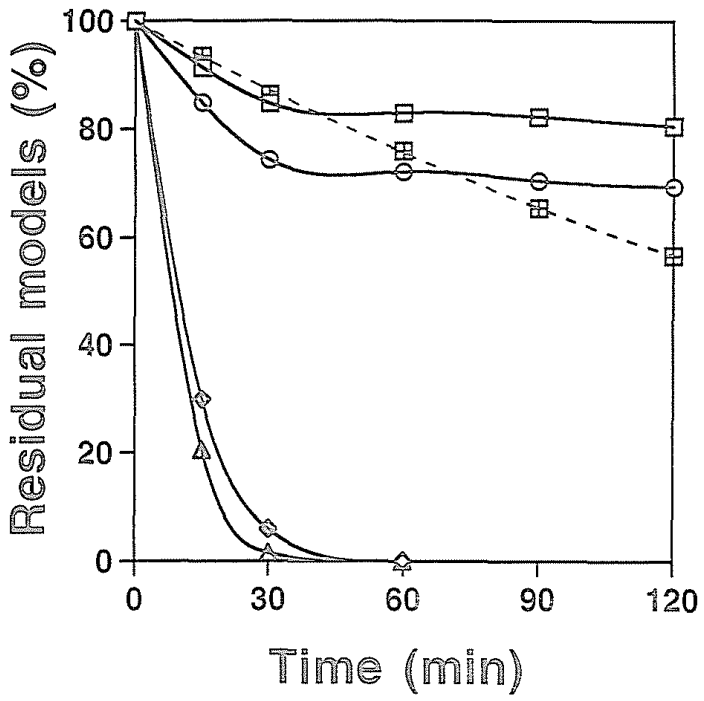
On the other hand, addition of a phenolic lignin model, vanillyl alcohol (**32**) (0.45 mmol) enhanced the degradation of model **1** within 30 min, but the degradation was retarded after the disappearance of vanillyl alcohol in the reaction mixture as shown in **Figure 5.6**, coinciding with those reported previously (Magara *et al.* 1994). The enhanced degradation of model **1** observed within 30 min suggests that radical species formed by the reaction of ozone with vanillyl alcohol caused the additional degradation of model **1**. The retardation of the degradation after 30 min is probably due to the formation of more reactive unsaturated carboxylic acid intermediates such as muconic acid than model **1**.

The additional degradation of model **1** in the presence of vanillyl alcohol was inhibited at low pH (pH 2, H_2SO_4) as reported previously (Kang *et al.*



□ Remaining 1 (ozonation of 1)
○ Remaining 1 (ozonation of 1 and 31 mixture)
△ Remaining 31 (ozonation of 1 and 31 mixture)
◇ Remaining 1 (ozonation of 1 and ozonated Va mixture)

Figure 5.5 Effect of veratryl alcohol (**31**) and ozonated vanillyl alcohol (ozonated Va) on the ozonation of methyl 4-*O*-ethyl-β-D-glucopyranoside in distilled water. [Conditions: see Figure 5.2, Ozonated Va: ozonation products of vanillyl alcohol (0.45 mmol) for 90 min. Veratryl alcohol: 0.45 mmol]



⊞ Remaining 1 (ozonation of 1, distilled water)

○ Remaining 1 (ozonation of 1 and 32 mixture, water)

□ Remaining 1 (ozonation of 1 and 32 mixture, pH2)

△ Remaining 32 (ozonation of 1 and 32 mixture, water)

◇ Remaining 32 (ozonation of 1 and 32 mixture, pH2)

Figure 5.6 Effect of vanillyl alcohol (32) on the ozonation of methyl 4-*O*-ethyl-β-D-glucopyranoside (1) in aqueous solutions (Conditions: see Figure 5.2, Vanillyl alcohol: 0.45 mmol)

1995). Thus, the improvement effect of low pH on the selectivities of ozone bleaching is partly due to the inhibition of the degradation of carbohydrate in the presence of phenolic lignins.

Figure 5.1 D shows the relative reactivities of carbon atoms at C1-C6 positions in model **1** during ozonation in aqueous solutions with vanillyl alcohol. The relative reactivity at C1 by ozonation with vanillyl alcohol (47%) is lower than that without vanillyl alcohol (55%), but is higher than those by oxidation with Fenton's reagent (under nitrogen: 44%, under oxygen: 11%). These results support that radical species formed by phenolic lignin participate in the additional degradation of model **1** during ozonation. Thus, phenolic lignins probably act as radical initiator as proposed previously (Gierer and Zhang 1993).

On the other hand, the relative reactivity at C1 by ozonation with vanillyl alcohol at pH 2 (48%) is only a little higher than that with vanillyl alcohol in distilled water (47%), and is lower than that without vanillyl alcohol in distilled water (55%). These results suggest that radical species formed by vanillyl alcohol participate in the degradation of model **1** during ozonation even at pH 2. The low pH cannot inhibit selectively the formation of radical species by phenolic lignins in contrast to the case of Fe^{2+} ions. The inhibition mechanism of the degradation of carbohydrates by the low pH in the presence of phenolic structure may be different from that in the presence of Fe^{2+} ions.

5.6 Summary

Methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) was treated with ozone both in anhydrous CH_2Cl_2 and in aqueous solutions, to investigate the participation of radical species in the degradation of polysaccharide during ozone bleaching. The relative reactivities of carbon atom at C1 position in

model 1 during ozonation were compared with those by oxidation with Fenton's reagent. As a result, it was found that ozone itself highly selectively causes GC reaction. The degradation of model 1 during ozonation in distilled water is partly due to radical species, and partly due to ozone itself. Contribution of radical species is about 40-70% during ozonation in distilled water. The initial high pH and Fe^{2+} ions enhanced the degradation of model 1, and the contribution of radical species increased. However, the formation of radical species by Fe^{2+} ions can be inhibited by adjusting to low pH without the removal of Fe^{2+} ions. Vanillyl alcohol also enhanced the degradation of model 1 during ozonation. Radical species formed by vanillyl alcohol participate in the degradation of model 1 even at pH 2, although the additional degradation of model 1 was inhibited by the low pH. Thus, inhibition of the degradation of carbohydrates by radical species is one of the key to the prevention of the viscosity drop in ozone bleaching of pulp.

Conclusion

Ozone reacts not only with lignin but also with polysaccharide, impairing pulp viscosity and fiber strength. Several undesirable ozone-polysaccharide reactions have been suggested to occur during ozone bleaching. The reactive radical species are also assumed to play an important role in the degradation of polysaccharide. However, it is not known what reactions are the most harmful to polysaccharide, and the participation of radical species in the degradation of polysaccharide during ozone bleaching is not confirmed by the experimental results based on the analyses of the reaction products.

In the present investigations, an oxygen-bleached kraft pulp and a cellulose model compound were treated with ozone, to elucidate the degradation of polysaccharide during ozone bleaching in more detail.

In Chapter 1, evaluation of polysaccharide reactions in ozone bleaching of kraft pulp was described. The polysaccharide reactions during ozone bleaching were divide into two groups: glycosidic bond cleavage (GC reaction) and oxidation of hydroxyl groups to carbonyl groups (OX reaction). These two reactions were evaluated separately by viscosities measured before and after borohydride treatment. It is found that the viscosity drop caused by GC reaction is greater than that by OX reaction. The inhibition of the viscosity drop by methanol or acidic water is attributed to the inhibition of GC reaction.

In Chapter 2, preparation and analyses of a cellulose model compound: methyl 4-*O*-ethyl-β-D-glucopyranoside (1) and its acetylated carbonyl sugars were described, to elucidate in more detail the reaction of polysaccharide based on the analyses of reaction products. These acetylated carbonyl sugars were converted into *O*-methyloximes and analyzed by GC-MS. Thus, the carbonyl

sugars which would be formed by ozonation of model **1** can be analyzed by gas chromatography after *O*-methyloximation and subsequent acetylation. The reactivities of inner glucose repeating unit of cellulose during ozonation can be elucidated by use of 1,4-substituted model **1**.

In Chapter 3, ozonation of model compound **1** in distilled water and analyses of the reaction products by NMR spectroscopy and gas chromatography were described. The broadening and multiplicity of the ^{1}H and ^{13}C-NMR signals indicate the degradation of model compound **1**, but the more detailed discussion could not be done. The gas chromatographic analyses showed that carbonyl groups are introduced at C2, C3 and C6-positions, and carboxyl groups are at C1 and C6-positions during ozonation. The carbonyl and carboxyl groups formation could be explained by the insertion of ozone and/or the radical attack at the carbon-hydrogen bonds. The order of relative reactivities of carbon atoms at C1-C6 positions in model compound **1** during ozonation in distilled water at room temperature was determined: C1 (acetal) > C6 (primary alcohol) > C2, C3 (secondary alcohols) > C4, C5 (ethers). The highest reactivity at C1-position supports that GC reaction is one of the predominant reactions of polysaccharide during ozone treatment.

In Chapter 4, free-radical reactions of model **1** with Fenton's reagent were described. The reaction products were formed by abstraction of hydrogen atoms from carbon-hydrogen bonds by radical species, and were almost the same as those from ozonation of model **1**. The degradation of model **1** was inhibited at initial higher pH. The glycosidic bond cleavage decreased with an increase of initial pH. Reactive species other than hydroxyl radicals may participate at higher pH. Oxygen also inhibited drastically the degradation of model **1**. The glycosidic bond cleavage was also inhibited by oxygen. These results may be explained as follows: Hydroxyl radicals are predominant under nitrogen, and cause the degradation of carbohydrate with low selectivities, whereas under oxygen contribution of hydroperoxyl radicals increases.

Hydroperoxyl radicals probably cause the degradation of carbohydrate with high selectivities and do not cause significantly glycosidic bond cleavage.

In Chapter 5, participation of radical species in the ozonation of model 1 was described. Model compound 1 was ozonated both in anhydrous CH_2Cl_2 and in aqueous solutions. The ozonation in anhydrous CH_2Cl_2 shows that ozone itself selectively attacks glycosidic carbon-hydrogen bond at C1 (GC reaction). Thus, the comparisons of the reactivities at C1 make it possible for us to discuss the participation of radical species in the degradation of model 1 during ozonation in aqueous solutions. As a result, both ozone itself and radical species participate in the degradation of carbohydrate during ozonation in aqueous solutions. The contribution of radical species is about 40-70% during ozonation in distilled water. The high pH, Fe^{2+} ions and vanillyl alcohol enhance the degradation of model 1, and the contribution of radical species increased. The additional degradation can be inhibited by low pH. The formation of radical species by Fe^{2+} ions can be inhibited by the low pH without the removal of Fe^{2+} ions. However, the formation of radical species by phenolic lignins cannot be inhibited selectively by low pH in contrast to the case of Fe^{2+} ions. The inhibition mechanism of the degradation of carbohydrates by low pH may be different.

Consequently, it is concluded that GC reaction is the most predominant reactions of polysaccharide in ozone bleaching of kraft pulp, and is caused both by ozone itself and radical species. OX reaction is mainly caused by radical species. Inhibition of these undesirable reactions of polysaccharide is one of the key to the prevention of the viscosity drop in ozone bleaching of kraft pulp. For this purpose, inhibition of the formation or reaction of radical species is extremely effective, because contribution of radical species is about 40-70%. Ozone bleaching performed at low pH is desirable to inhibit the undesirable polysaccharide reactions, although the inhibition mechanism is complicated.

Experimental

General All melting points (m.p.) are uncorrected. ^{1}H-NMR spectra and ^{13}C-NMR spectra were recorded with a Bruker AC300 FT-NMR (300MHz) spectrometer, a Varian XL-200 FT-NMR (200MHz) spectrometer, or a JEOL FX-90 FT-NMR (22.5MHz) spectrometer in chloroform-*d* with tetramethylsilane (TMS), or in D_2O with 3-(trimethylsilyl) propinic-2,2,3,3-d_4-acid sodium salt (TSP) as an internal standard. Chemical sifts (δ) and coupling constants (*J*) are given in δ-values (ppm) and Hz, respectively. Some chemical sift assignments were made by using a decoupling method; others were made by analogy with model compounds. Optical rotations were measured using a JASCO Dip-4 digital polarimeter. IR spectra were recorded with a Shimazu FTIR-4000 spectrophotometer. Preparative thin layer chromatography (PTLC) was performed on silica-gel plates (Kieselgel 60 F254, Merck). The standard work-up procedure included diluting with an ethyl acetate, washing with aq. $NaHCO_3$, and a brine, drying over Na_2SO_4, and evaporating in *vacuo*. Trimethylsilylation (10 mg of sample) was performed with dried pyridine (0.5 ml), bis(trimethylsilyl) trifluoroacetamide (BSTFA) (0.2 ml) and trimethylchlorosilane (TMCS) (0.1 ml) at room temperature for 2 h.

Gas chromatography and mass spectrometry

The Shimadzu gas chromatograph GC 14A equipped with hydrogen flame detector was used. Acetylated neutral sugars were injected into a sp 2330 fused silica gel capillary column (0.25 mm x 15 m). The temperature program was 180-220 °C at 2 °C/min. The injection zone temperature was 240 °C and the detector bath was at 250 °C. Trimethylsilylated acidic sugars were

injected into a High Cap-17 fused silica gel capillary column (0.25 mm x 30 m). The temperature program was 180-200 °C at 1 °C/min. The injection zone temperature was 270 °C and the detector bath temperature was 270 °C. The typical gas pressures were: helium (carrier gas) 0.9 kg/cm^2; nitrogen 5.0 kg/cm^2; hydrogen 0.6 kg/cm^2; and air 0.5 kg/cm^2. Mass spectrometric analyses were performed with Shimadzu GC MS QP-1000 mass spectrometer, using chemical ionization method.

Chapter 1

Pulp

An industrially oxygen-bleached hardwood kraft pulp (kappa number: 11.6, viscosity before and after sodium borohydride reduction: 36.4 and 37.6 cP, respectively) was used in the experiment. The pulp was washed with ion-exchanged water before bleaching.

Ozone bleaching

Ozone bleaching was performed at 25°C at low consistency (1%) in a 500 ml Erlenmeyer flask equipped with gas inlet tubes. Ozone was produced from oxygen using a Nippon Ozone O-3-2 ozonizer.

To stirred suspensions of three grams of oxygen-bleached hardwood kraft pulp in 300 ml of reaction medium, streams of oxygen gas containing 4.5 w% of ozone were bubbled for 30, 60, and 90 mins. The dosages of ozone were 36 mg/min. As reaction media, distilled water, water acidified to pH 2.0 with sulfuric or oxalic acid, and methanol were used. After bleaching for the fixed times, excess ozone was removed by passing streams of nitrogen through the suspensions which then were filtered. Pulps were washed successively with ion-exchanged water and methanol, and dried over P_2O_5 in a vacuum-desiccator, before measuring viscosities and kappa numbers.

Treatment with sodium borohydride

To a stirred suspension of 600 mg of ozone-bleached pulp in 20 ml of water, 30 mg of sodium borohydride was added. The mixture was kept at room temperature for 30 min, filtered, and dried in the same manner as described above.

Treatment with hydroxylamine hydrochloride

To a stirred suspension of 600 mg of ozone-bleached pulp in 40 ml of methanol, 350 mg of hydroxylamine hydrochloride and 600 mg of sodium carbonate were added. The mixture was heated under reflux with stirring for 3 hr, and filtered. The pulp was washed and dried in the same manner described above.

Pulp properties

Kappa numbers and viscosities were determined according to TAPPI UM246 and TAPPI T230, respectively. Cupriethylenediamine was added to the pulp specimens, and after 30 min the viscosities were measured to bring the viscosity drops derived from carbonyl groups during the viscosity measurements to completion. When the cupriethylenediamine treatment was prolonged over 30 min, a slight additional viscosity drop was observed. The extent of this additional viscosity drop, however, was the same as those often observed even for pulp without any carbonyl groups when subjected to alkali treatment. Therefore, the viscosity drop caused by carbonyl groups can be regarded as proceeding quite rapidly and completely within 30 min of the cupriethylenediamine treatment.

Chapter 2

Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (6) To a solution of methyl β-D-glucopyranoside (5.02 g, 24.7 mmol) in *N*,*N*-dimethylformamide

(DMF) (30 ml), benzaldehyde dimethylacetal (7.4 ml, 49.4 mol) and *p*-toluenesulfonic acid (640 mg, 3.7 mmol) were added. The solution was kept at 50 °C under 15 mmHg for 50 min. Solid $NaHCO_3$ (1.7 g) was added to the mixture. The solution was concentrated to a syrup. The syrup was worked-up by the standard method, and then triturated from *n*-hexane to colorless crystals (6.35 g, 91 %), m.p. 202-205°, $[\alpha]_D$ +65.2° (c 0.66, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 3.58 (s (singlet), 3H (protons), -OCH_3), 4.33 (d (doublet), 1H, $J_{1,2}$=7.5, C_1-H), 5.54 (s, 1H, CHC_6H_5).

Anal. Calc. for $C_{14}H_{18}O_6$: C, 59.56; H, 6.43. Found: C, 59.30; H, 6.41.

Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (7) To a stirred solution of compound **6** (14.88 g, 52.8 mmol) in DMF (100 ml), sodium hydride (6.32 g, 0.158 mol, 60 % in mineral oil), tetra-*n*-butyl ammonium iodide (1.95 g, 5.28 mmol), and benzyl bromide (18.8 ml, 0.158 mol) were added at 0 °C. The reaction mixture was kept at room temperature for 1.5 h, and then methanol (10 ml) was added for the decomposition of excess benzyl bromide. The reaction mixture was worked-up by the standard method to afford a colorless syrup. The syrup was triturated from *n*-hexane to afford colorless crystals (21.2 g, 87 %), m.p. 120-122° $[\alpha]_D$ -32.4° (c 1.02, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 3.58 (s, 3H, -OCH_3), 4.42 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.75, 4.79, 4.87, 4.91 (d, 4H, $CH_2C_6H_5$), 5.57 (s, 1H, -CHC_6H_5).

Anal. Calc. for $C_{28}H_{30}O_6$: C, 72.71; H, 6.54. Found: C, 72.85; H, 6.52.

Methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8) To a stirred solution of compound **7** (0.924 g, 2 mmol) in acetonitrile (10 ml), powdered Molecular Sieves 4A (MS4A) (1 g) and sodium cyanoborohydride (0.529 g, 8 mmol) were added. Trimethylchlorosilane (2.5 ml, 19.7 mmol) was added dropwise over a period of 2 h to the reaction mixture at room temperature. The reaction mixture was filtered by the use of Celite 535 and the residue was washed with ethyl acetate. The combined filtrate and washing was worked-up by the standard method to afford a syrup. The product was purified on a silica

gel column (Wacogel C-200) with ethyl acetate/*n*-hexane (1/4, *v/v*) to give colorless crystals (867 mg, 93 %), m.p. 69-70° $[\alpha]_D$ -14.0° (c 0.93, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 3.59 (s, 3H, -OCH_3), 4.32 (d, 1H, $J_{1,2}$=7.0, C_1-H), 4.58 (s, 2H, $CH_2C_6H_5$), 4.71, 4.93 (dd (double doublet), 4H, -$CH_2C_6H_5$).

Anal. Calc. for $C_{28}H_{32}O_6$: C, 72.39; H, 6.94. Found: C, 72.61; H, 6.85.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-ethyl-β-D-glucopyranoside (9) To a stirred solution of compound 8 (7.3 g, 15.7 mmol) in DMF (70 ml), sodium hydride (1.26 g, 31.4 mmol) and ethyl iodide (2.52 ml, 31.4 mmol) were added. The reaction mixture was kept at room temperature for 1 h, and then worked-up by the standard method to afford a colorless syrup. The syrup was triturated from *n*-hexane to afford colorless crystals (7.6 g, 97 %), m.p. 61-62°, $[\alpha]_D$ +30.0° (c 0.80, $CHCl_3$), ^{1}H-NMR ($CDCl_3$): δ 1.27 (t (triplet), 3H, *J*=7.0, -$OCH_2C\underline{H}_3$), 3.76 (s, 3H, -OCH_3), 4.46 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.73, 4.82, 4.85, 4.93, 5.04, 5.06 (d, 6H, -$CH_2C_6H_5$).

Anal. Calc. for $C_{30}H_{36}O_6$: C, 73.14; H, 7.37. Found: C, 73.12; H, 7.43.

Methyl 4-*O*-ethyl-β-D-glucopyranoside (1) A stirred solution of compound 9 (1.979 g, 4.02 mmol) in ethanol / acetic acid (1/1, *v/v*) (20 ml) was treated with 10 % palladium carbon (2 g) under H_2 at 50 °C for 4 h. The reaction mixture was filtered and concentrated. The product was purified on a silica gel column with methanol/dichloromethane (1/9, *v/v*) to afford colorless crystals (891 mg, 99.8 %). For ozonation compound 1 was recrystallized from ethanol (737 mg, 83 %), m.p. 138-139°, $[\alpha]_D$ -10.1° (c 1.09, CH_3OH), ^{1}H-NMR (D_2O): δ 1.21 (t, 3H, *J*=7.0, -$OCH_2C\underline{H}_3$), 3.26 (dd, 1H, $J_{2,3}$=9.7, C_2-H), 3.27 (t, 1H, *J*4,5=9.6, C4-H), 3.44 (m, 1H, C5-H), 3.55 (t, 1H, $J_{3,4}$=9.3, C_3-H), 3.57 (s, 3H, -OCH_3), 3.74 (dd, 1H, J_{gem}=12.2, $J_{6a,5}$=5.2, C_6-H_a), 3.92 (dd, 1H, $J_{6b,5}$=2.1, C_6-H_b), 4.35 (d, 1H, $J_{1,2}$=8.0, C_1-H), ^{13}C-NMR (D_2O): 15.0 (-$OCH_2\underline{C}H_3$), 103.5 (C_1), 57.4, 60.7, 69.0, 73.4, 75.4, 75.9, 78.1.

Anal. Calc. for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 48.37; H, 8.06.

Methyl 2,3,6-tri-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (10), methyl 3,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (11), and methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (12) To a stirred solution of compound **1** (0.5 g, 2.25 mmol) in ethyl acetate (40 ml), acetyl chloride (1.36 ml, 19.2 mmol) and 2,6-lutidine (2.24 ml, 19.2 mmol) were added. The reaction mixture was kept under reflux for 12 h, and then worked-up by the standard method to afford a colorless syrup. The products were purified on a silica gel column with ethyl acetate/*n*-hexane (1/2, *v/v*) to afford **10** (231 mg, 30 %), **11** (145 mg, 20 %), and **12** (341 mg, 50 %), respectively.

10: m.p. 92-94°, $[\alpha]_D$ -22.4° (c 0.49, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1747, 1754 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ 1.16 (t, 3H, J=7, $-OCH_2C\underline{H}_3$), 2.08, 2.09, 2.14 (s, 9H, $-OCOCH_3$), 3.52 (s, 3H, $-OCH_3$), 4.24 (dd, 1H, J_{gem}=12, $J_{6a,5}$=4.5, C_6-H_a), 4.38 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.39 (dd, 1H, $J_{6b,5}$=2, C_6-H_b), 4.87 (dd, 1H, $J_{2,3}$=9.5, C_2-H), 5.16 (dd, 1H, $J_{3,4}$=8.5, C_3-H).

Anal. Calc. for $C_{15}H_{24}O_9$: C, 51.72; H, 6.94. Found: C, 51.22; H, 6.89.

11: m.p. 85-87°, $[\alpha]_D$ +5.1° (c 0.59, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1721, 1753 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ 1.17 (t, 3H, J=7, $-OCH_2C\underline{H}_3$), 2.14, 2.18 (s, 6H, $-OCOCH_3$), 3.39 (t, 1H, $J_{4,5}$=9.5, C_4-H), 3.44 (dd, 1H, $J_{2,3}$=9.5, C_2-H), 3.59 (s, 3H, $-OCH_3$), 4.24 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.25 (dd, 1H, J_{gem}=12, $J_{6a,5}$=4.5, C_6-H_a), 4.37 (dd, 1H, $J_{6b,5}$=2.5, C_6-H_b), 5.06 (t, 1H, $J_{3,4}$=9.5, C_3-H).

Anal. Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.71; H, 7.35.

12: syrup, $[\alpha]_D$ -29.4° (c 1.36, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1747 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ 1.11 (t, 3H, J=7, $-OCH_2C\underline{H}_3$), 2.05, 2.08 (s, 6H, $-OCOCH_3$), 3.31 (t, 1H, $J_{4,5}$=9.5, C_4-H), 3.46 (m (multiplet), 1H, C_5-H), 3.47 (s, 3H, $-OCH_3$), 3.63, 3.86 (m, 2H, $-OC\underline{H}_2CH_3$), 3.69 (t, 1H, $J_{3,4}$=9.5, C_3-H), 4.23 (dd, 1H, J_{gem}=12, $J_{6a,5}$=5.0, C_6-H_a), 4.32 (d, 1H, $J_{1,2}$=7.5, C_1-H), 4.39 (dd, 1H, $J_{6b,5}$=2.0, C_6-H_b), 4.77 (dd, 1H, $J_{2,3}$=9.5, C_2-H).

Anal. Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.45; H, 7.48.

Methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-glucopyranoside (13) To a stirred solution of compound 14 (165 mg, 0.23 mmol) in CH_3OH/CH_2Cl_2 (3 ml, 1:4, *v/v*), *p*-toluenesulfonic acid (62 mg, 0.361 mmol) was added. The reaction mixture was kept at room temperature for 2 h, and then worked-up by the standard method to afford a syrup. The products were purified on a silica gel column with CH_2Cl_2 to afford colorless crystals (78 mg, 85 %), $[\alpha]_D$ -39.5° (c 1.57, $CHCl_3$), IR ν_{max} cm^{-1} (KBr): 1754 (ester C=O), ^{1}H-NMR ($CDCl_3$): δ 1.16 (t, 3H, *J*=7, $-OCH_2C\underline{H}_3$), 2.07, 2.08 (s, 6H, $-OCOCH_3$), 3.39 (m, 1H, C_5-H), 3.52 (s, 3H, $-OCH_3$), 3.53 (t, $J_{4,5}$=9, C_4-H), 3.64 (q (quartet), 2H, $-OC\underline{H}_2CH_3$), 3.76 (dd, 1H, J_{gem}=12, $J_{6a,5}$=4, C_6-H_a), 3.93 (dd, 1H, $J_{6b,5}$=2, C_6-H_b), 4.42 (d, 1H, $J_{1,2}$=8, C_1-H), 4.85 (dd, 1H, $J_{2,3}$=10, C_2-H), 5.16 (dd, 1H, $J_{3,4}$=9, C_3-H). *Anal.* Calc. for $C_{13}H_{22}O_8$: C, 50.97; H, 7.24. Found: C, 50.80; H, 7.33.

Methyl 3, 6- di- *O*- acetyl- 4- *O*- ethyl- β-D-*arabino*-hexopyranosid-ulose (2) To a stirred solution of compound 11 (21.9 mg, 0.072 mmol) in CH_2Cl_2 (2 ml), MS4A (160 mg) and PCC (157 mg, 0.72 mmol) were added. The reaction mixture was kept at room temperature for 32 h. Ether was added, the mixture was filtered by the use of Celite 535, and the residue was washed with ether. The combined filtrate and washing was concentrated. The unreacted starting material was removed by PTLC to afford a syrup (14.0 mg, 64 %).

Methyl 2,6-di-*O*-acetyl-4-*O*-ethyl-β-D-*ribo*-hexopyranoside-3-ulose (3) To a stirred solution of compound 12 (21.3 mg, 0.070 mmol) in CH_2Cl_2 (4 ml), MS4A (100 mg) and PCC (92 mg, 0.42 mmol) were added. The reaction mixture was kept at room temperature for 35 h, and then filtered and concentrated. The products were purified by PTLC to afford a colorless syrup (16.7 mg, 79 %).

Methyl 2, 3- di- *O*- acetyl- 4- *O*- ethyl- β- D- *gluco*- hexodialdo- 1, 5-pyranoside (4) To a stirred solution of compound 13 (16.2 mg, 0.053 mmol) in CH_2Cl_2 (2 ml), MS4A (100 mg) and PCC (35 mg, 0.16 mmol) were added. The reaction mixture was kept at room temperature for 2.5 h, and then filtered

and concentrated. The products were purified by PTLC (CH_3OH/CH_2Cl_2, 2/98, *v/v*) to afford a colorless syrup (10.0 mg, 62 %).

The ^1H- and ^{13}C-NMR spectral data of carbonyl sugars (**2-4**) are shown in **Table 2.1**.

O-Methyloximation procedure

In typical experiment, to a stirred solution of compound **3** (16.0 mg, 0.052 mmol) in methanol (2 ml), methoxylamine hydrochloride (13.2 mg, 0.16 mmol) and pyridine (21 ml, 0.26 mmol) were added. The reaction mixture was kept at room temperature for 2.5 h, and then worked-up by the standard method. The products were purified by PTLC (1:2, *v/v* ethyl acetate / *n*-hexane) to afford *O*-methyloxime **16** (*syn* and *anti* mixture) as a syrup (15.2 mg, 87 %). The ^1H-NMR spectral data of *O*-methyloximes (**15-17**) are shown in **Table 2.2**.

Chapter 3

4-*O*-Ethyl-D-gluconic acid (18) To a stirred solution of 4-*O*-ethyl-D-glucose (**26**) (10.3 mg, 0.05 mmol) in water (10 ml), bromine (20 μl) was added. The pH of the solution was adjusted to 5.0. The reaction mixture was kept at room temperature for 12 h, and neutralized and concentrated to dryness to afford a syrup (17.6 mg). The product was characterized as its per-acetylated methyl-ester derivatives after successive treatments of the dried reaction mixture with methanolic hydrogen chloride under reflux, followed by pyridine-acetic anhydride acetylation and purification by PTLC; ^1H-NMR ($CDCl_3$): δ 1.18 (t, 3H, J=7.0, $-OCH_2CH_3$), 2.06, 2.09, 2.09, 2.20 (s, 12H, $-OCOCH_3$), 3.65 (q, 2H, J=7.0, $-OCH_2CH_3$), 3.75 (s, 3H, $-OCH_3$), 3.82 (t, 1H, $J_{4,5}$=4.3, C4-H), 4.15 (dd, 1H, J_{gem}=12.4, $J_{6a,5}$=5.6, C6-H_a), 4.49 (dd, 1H, $J_{6b,5}$=3.7, C6-H_b), 5.33 (d, 1H, $J_{2,3}$=4.3, C2-H), 5.45 (t, 1H, $J_{3,4}$=4.3, C3-H).

Methyl 4-*O*-ethyl-β-D-glucuronoside (20) To a stirred solution of methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-glucoside (**13**) (2.3 mg) (Kishimoto *et al.* 1995) in acetone (2 ml) a 50 μ l of the chromium oxide (VI) solution (chromium oxide (2.67 g) and sulfuric acid (2.3 ml) in water (8 ml)) was added. The reaction mixture was kept at room temperature for 12 h, and then methanol was added for decomposition of an excess CrO_3 and worked up by the standard method, to afford methyl 2,3-di-*O*-acetyl-4-*O*-ethyl-β-D-glucuronoside (2.5 mg). ^{1}H-NMR ($CDCl_3$): δ 1.14 (t, 3H, J=7.0, -OCH_2CH_3), 2.06, 2.07, (s, 6H, -$OCOCH_3$), 3.52 (s, 3H, -OCH_3), 4.04 (d, 1H, $J_{4,5}$=8.8, C5-H), 4.53 (d, 1H, $J_{1,2}$=7.5, C1-H), 4.92 (t, 1H, $J_{2,3}$=7.5, C2-H), 5.17 (t, 1H, $J_{3,4}$=7.5, C3-H).

Methyl β-D-glucuronoside (21) To a stirred solution of methyl 2,3,4-tri-*O*-acetyl-β-D-glucoside (20 mg) in acetone (2 ml), a 100 μl of chromium oxide (VI) solution described above was added. The reaction mixture was kept at room temperature for 12 h to afford a syrup (24 mg). The product was purified by PTLC developed with 20 % MeOH / CH_2Cl_2 to afford methyl 2,3,4-tri-*O*-acetyl-β-D-glucuronoside (**29**) (18.4 mg); ^{1}H-NMR ($CDCl_3$): δ 2.00, 2.03, 2.05 (s, 9H, -$OCOCH_3$), 3.54 (s, 3H, -OCH_3), 3.87 (d, 1H, $J_{4,5}$=9.5, C5-H), 4.46 (d, 1H, $J_{1,2}$=8.0, C1-H), 4.95 (t, 1H, C2-H), 5.20 (t, 1H, C4-H), 5.28 (t, 1H, C3-H).

To a solution of compound **29** (6 mg) in methanol (2 ml), 28%-NaOMe in methanol (6 μl) was added. The reaction mixture was kept at room temperature for 2 h to afford compound **21** (4 mg).

Methyl β-D-gluco-hexodialdo-1,5-pyranoside (25) Methyl β-D-glucoside (500 mg) in distilled water (20 ml) was treated with 3 wt% of ozone for 3 h. The product was characterized as a per-acetylated *O*-methyloxime derivative after treatment with *O*-methylhydroxylamine hydrochloride and pyridine-acetic anhydride and PTLC purification, ^{1}H-NMR ($CDCl_3$); δ 2.4, 2.9 (s, -$OCOCH_3$), 3.54 (s, C-OCH_3), 3.89 (s, N-OCH_3), 4.03 (dd, $J_{5,6}$=7.5, C5-H),

4.47 (d, $J_{1,2}$=8.0, C1-H), 4.98 (dd, $J_{2,3}$=9.5, C2-H), 5.06 (t, $J_{3,4}$=9.5, C3-H), 5.26 (t, $J_{4,5}$=9.5, C4-H), 7.30 (d, C6-H).

4-*O*-Ethyl-D-glucose (26) To a stirred solution of compound **1** (100 mg, 0.45 mmol) in acetic anhydride (2 ml) and acetic acid (2 ml), sulfuric acid (40 μl) was added. The reaction mixture was kept at room temperature for 12 h, and then worked-up by the standard method to afford a colorless syrup: 1,2,3,6-tetra-*O*-acetyl-4-*O*-ethyl-D-glucose (**30**) (170 mg) as a mixture of α/β-anomer (6:1), ^{1}H-NMR ($CDCl_3$): α-anomer: δ 1.16 (t, J=7.0, -OCH_2CH_3), 2.01, 2.10, 2.12, 2.16 (s, -$OCOCH_3$), 3.49 (t, $J_{4,5}$=9.9, C4-H), 3.97 (m, C5-H), 4.26 (dd, J_{gem}=12.1, $J_{6a,5}$=4.0, C6-H_a), 4.34 (dd, $J_{6b,5}$=2.3, C6-H_b), 5.01 (dd, $J_{2,3}$=10.3, C2-H), 5.47 (t, $J_{3,4}$=10.2, C3-H), 6.26 (d, $J_{1,2}$=3.6, C1-H), β-anomer: 5.21 (t, $J_{2,3}$=9.8, C2-H), 5.69 (d, $J_{1,2}$=9.4, C1-H).

To a stirred solution of compound **30** (150 mg, 0.40 mmol) in methanol (4 ml), 28%-NaOMe in methanol (16 μl) was added. The reaction mixture was kept at room temperature for 2.5 h, and treated with cation-exchange resin (Amberlyst 15) for neutralization, and then the resin was filtered off. The filtrate was concentrated to dryness. The products were purified on a silica gel column, eluted with methanol/CH_2Cl_2 (1/10 *v/v*) to afford a colorless syrup: 4-*O*-ethyl-D-glucose (**26**) (72 mg).

4-*O*-Ethyl-D-glucose (**26**) was characterized as a peracetylated *O*-methyloxime derivative prepared by the following procedure. To a stirred solution of compound **26** (15.9 mg) in methanol (2 ml), *O*-methylhydroxylamine hydrochloride (19.3 mg) and pyridine (260 μl) were added. The reaction mixture was kept at room temperature for 12 h, and concentrated to dryness. Acetic anhydride (2 ml) and pyridine (2 ml) were added to the mixture and kept at room temperature, and then worked-up by the standard method. The products were purified on a silica gel column with ethyl acetate/*n*-hexane (1/4, *v/v*) to afford a syrup (21.4 mg), ^{1}H-NMR ($CDCl_3$): *anti*-form: δ 1.20 (t, J=7.0, -OCH_2CH_3), 2.04, 2.08 (s, -$OCOCH_3$), 3.64 (m, -OCH_2CH_3), 3.78 (dd,

$J_{4,5}$=7.6, C4-H), 3.89 (s, N-OCH_3), 4.11 (dd, J_{gem}=12.4, $J_{6a,5}$=5.1, C6-H_a), 4.51 (dd, $J_{6b,5}$=2.7, C6-H_b), 5.05 (m, C5-H), 5.35 (dd, $J_{3,4}$=2.9, C3-H), 5.66 (dd, $J_{2,3}$=7.9, C2-H), 7.36 (d, $J_{1,2}$=6.2, C1-H), *syn*-form: δ 3.91 (s, N-OCH_3), 5.40 (t, $J_{3,4}$=5.0, C3-H), 6.05 (t, $J_{2,3}$=5.6, C2-H), 6.62 (d, $J_{1,2}$=5.7, C1-H).

Ozonation procedure

Ozonation was performed at 25 °C in a 50 ml three-necked flask equipped with gas inlet and outlet tubes. To a stirred solution of methyl 4-*O*-ethyl-β-D-glucopyranoside (30 mg) in distilled water (30 ml, pH 5.7), streams of oxygen gas containing 3 wt% of ozone were bubbled for the fixed time. The dosage of ozone was 12 mg/min, determined by iodometric titration. After ozonation for the prescribed time, excess ozone was removed by bubbling nitrogen into the reaction mixture. The pH of the reaction mixture was adjusted to 7.0 with 0.1 M sodium hydroxide, using a HITACHI-HORIBA pH-meter H-7 SD.

Analysis of reaction products

The ozonated reaction mixture was divided into two fractions for separately analyzing neutral and acidic sugars as shown in **Figure 3.6**.

Analysis of neutral sugars To a reaction mixture (10 ml) was added *O*-methylhydroxylamine hydrochloride (20 mg). The reaction mixture was kept at 50 °C and the pH value was maintained at 4.0 by addition of 0.1 M sodium hydroxide. After 2.5 h, the pH was adjusted to 7.0 with 0.1 M sodium hydroxide and 0.5 ml of *myo*-inositol stock solution (200 mg in 50 ml of water) was added as an internal standard. The reaction mixture was concentrated to dryness in *vacuo* at 30 °C and the residue was dissolved in pyridine (3 ml) and acetic anhydride (3 ml), and kept at room temperature for 12 h. The acetylated *O*-methyloxime derivatives were quantitatively analyzed by gas chromatography.

Analysis of acidic sugars A reaction mixture (20 ml) was adjusted to pH 10.0 by addition of 0.1 M sodium hydroxide and maintained for 30 min, and was allowed to stand at room temperature for 3.5 h. The pH was adjusted to 7.0. An aliquot (10 ml) of the reaction mixture was concentrated to dryness at 30 °C, and then trimethylsilylated and analyzed by gas chromatography.

Another aliquot (10 ml) was applied on a strongly basic anion-exchange column (2 ml of Amberlite IRA-400, acetate form). The column was washed with 30 ml of distilled water. Combined effluent and washings were concentrated to dryness at 30 °C to afford neutral sugars. The above column was eluted with acetic acid (2 M, 150 ml) and the effluent was evaporated to dryness at 30 °C to afford acidic sugars. Both neutral and acidic sugars were trimethylsilylated, and analyzed by gas chromatography.

Chapter 4

Treatment with Fenton's reagent

Unless otherwise noted, the reaction was run under air. To a stirred solution of methyl 4-*O*-ethyl-β-D-glucopyranoside (1) (30 mg, 0.135 mmol) in distilled water (30 ml) was added 1 ml of freshly prepared 50 mM $FeSO_4$ solution acidified to pH 2 with 1 N H_2SO_4 to avoid a precipitate. The pH of the reaction mixture was 3.5, when it was not adjusted. The addition of solid $FeSO_4$ instead of the $FeSO_4/H_2SO_4$ solution resulted in pH 4.7. The pH 2.1 was adjusted by adding 0.4 ml of 1N H_2SO_4. The pHs 6.3 and 10.9 were adjusted by adding 0.1 and 0.15 ml of 1N NaOH, respectively. Then, the reaction was started finally by adding hydrogen peroxide. The solution was kept at 25 °C for 2 h, and then sodium thiosulfate was added for decomposition of an excess hydrogen peroxide. The reaction products were analyzed by gas chromatography as described in Chapter 3.

Chapter 5

Veratryl alcohol (31) To a stirred solution of vanillin (5.0 g) in DMF (20 ml), finely powered potassium carbonate (5.5 g) and methyl iodide (2.3 ml) were added. The reaction mixture was kept at room temperature for 12 h, and then worked-up by the standard method to afford 3,4-dimethoxybezaldehyde (4.6 g). To a stirred solution of 3,4-dimethoxybezaldehyde (3 g) in methanol (20 ml), sodium borohydride (0.7 g) was added. The solution was kept at room temperature for 2.5 h. The reaction mixture was worked-up by the standard method to afford a syrup. The product was purified on a slica gel column (Wacogel C-200) with $CHCl_3$ to give a colorless syrup.

Vanillyl alcohol (32) To a stirred solution of vanillin (5.02 g) in methanol (30 ml), sodium borohydride (1.25 g) was added. The solution was kept at room temperature for 2.5 h, and then worked-up by the standard method to afford colorless crystals. The crystals were recrystallized from an ethyl acetate to afford vanillyl alcohol, m.p. 113-114°; lit. m.p. 113-115° (Aldrich Catalog / Handbook).

Ozonation procedure

Ozonation in anhydrous CH_2Cl_2 Ozonation was performed at 25 °C in a 50 ml three-necked flask equipped with a gas inlet tube, a drying tube containing anhydrous calcium sulfate (Alldrich Co., Drierite) and a glass stopper. Ozone was produced from dry oxygen. To a stirred solution of methyl 4-*O*-ethyl-β-D-glucopyranoside (**1**) (30 mg) in anhydrous CH_2Cl_2 (30 ml), streams of oxygen gas containing 3 wt% of ozone were bubbled for 30 min. Anhydrous CH_2Cl_2 was ddistilled from P_2O_5. The dosage of ozone was 12 mg/min. The reaction mixture was kept at 25 °C for another 1.5 h. After ozonation for 2 h, excess ozone was removed by bubbling nitrogen into the reaction mixture. The reaction mixture was extracted with water (15 ml x 2),

and then reaction products were analyzed by gas chromatography as described in Chapter 3.

Ozonation in aqueous solutions To a stirred solution of methyl 4-*O*-ethyl-β-D-glucopyranoside (1) (100 mg, 0.45 mM) in distilled water (100 ml) in a 100 ml three-necked flask, streams of oxygen gas containing 3 wt% of ozone were bubbled for 120 min at 25 °C. The dosage of ozone was 12 mg/min. The pH of the reaction mixture was adjusted with 0.1 N H_2SO_4 and 0.1 N NaOH. The fixed $FeSO_4$ solutions were prepared by addition of 50 mM $FeSO_4$ solution acidified to pH 2 with H_2SO_4. Vanillyl alcohol (0.45 mmol) and veratryl alcohol (0.45 mmol) were used as lignin model compounds. The ozonation products of vanillyl alcohol was prepared from ozonation of vanillyl alcohol (0.45 mmol) in distilled water (100 ml) at 25 °C for 90 min.

At intervals of the prescribed time, 1 ml of the reaction mixture was withdrawn and sodium thiosulfate was added. The reaction mixture was concentrated to dryness at 35 °C. The residues were acetylated with acetic anhydride (2 ml) and pyridine (2 ml) at room temperature, for analyzing the residual cellulose and lignin model compounds by gas chromatography.

After ozonation for 120 min, excess ozone was removed by bubbling nitrogen into the reaction mixture. The reaction products were analyzed quantitatively by gas chromatography.

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Acknowledgments

The author wishes to express his sincerest thanks to Professor Dr. Fumiaki Nakatsubo and Emeritus Professor Dr. Koji Murakami, Faculty of Agriculture, Kyoto University, for their kindest guidance and encouragement during the entire course of this work.

The author is deeply grateful to Professor Dr. Nobuo Shiraishi, Faculty of Agriculture, Kyoto University, and to Professor Dr. Mikio Shimada, Wood Research Institute, Kyoto University, for their valuable suggestions and critical readings of the manuscript.

The author gratefully appreciate to Associate Professor Dr. Toshiaki Umezawa, Wood Research Institute, Kyoto University, for the GC-MS measurements.

The author gratefully acknowledges Associate Professor Dr. Haruo Kawamoto, Faculty of Energy Science, Kyoto University, for his helpful suggestions and discussions.

Finally, thanks are also due to the members of Laboratory of Chemical Processing of Wood, Department of Wood Science and Technology, Faculty of Agriculture, Kyoto University for their kind help and discussions.