

# Analysis of Oxidized Moiety of Partially Periodate-Oxidized Cellulose by NMR Spectroscopy

EIICHI MAEKAWA

Research Section of Wood Chemistry, Wood Research Institute, Kyoto University, Gokasho, Uji, Kyoto 611, Japan

## SYNOPSIS

Periodate-oxidized celluloses (S-100, S-70, S-50) with different degrees of oxidation were prepared. It is presented in this article that oxidized moieties of partially periodate-oxidized cellulose are determined from analyses of  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectra of those reduction products and the corresponding polytriacetates.

## INTRODUCTION

The selective cleavage of the carbon-carbon bond bearing vicinal hydroxyl groups is generally achieved by periodate oxidation.<sup>1</sup> In periodate oxidation of cellulose, the splitting reaction occurs at the C-2 and C-3 of glucose residue without causing cleavage of the glucosidic linkages, resulting in the product referred to as dialdehyde cellulose.<sup>2</sup>

The reaction has been widely applied as the useful technique for structural analysis of mono-, oligo-, and polysaccharides. Reduction of periodate-oxidized carbohydrates with sodium borohydride gave the corresponding polyhydric alcohols. The portions reduced with borohydride are easily degraded with dilute acid, and structural determination of the fragments produced by degradation is performed by using various analytical techniques.<sup>3</sup> Thus, this method known as the Smith degradation, affords useful information for characterization of the substitution pattern of the original carbohydrates.<sup>4</sup>

In this experiment, variable degrees of oxidized products were prepared from cellulose composed of a large number of potentially oxidizable units by using sodium metaperiodate of 0.5, 0.7, and 1.3 times as much as the theoretical amount for oxidation of cellulose. The products (referred to as S-50, S-70, S-100) composed of different degrees of oxidation were converted to the corresponding acyclic polyalcohols by reduction with sodium borohydride. Fur-

thermore, the corresponding polytriacetates were prepared by conventional peracetylation of the polyalcohols with pyridine and acetic anhydride. The resulting acyclic polyalcohols and those peracetates were characterized by using  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy, and oxidized moieties of partially periodate-oxidized cellulose were determined by detailed analyses of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra.

## EXPERIMENTAL

### Preparation of Periodate-Oxidized and Partially Oxidized Dialdehyde Celluloses

Commercial Schleicher & Schüll cellulose powder from wood pulp and sulfite pulp ( $\alpha$ -cellulose, 98% purity) were used. Periodate-oxidized celluloses were prepared by oxidizing 20 g cellulose powder suspended in water (1 L) with sodium metaperiodate at ambient temperature in the dark. For preparation of fully oxidized cellulose, sodium metaperiodate of 1.3 times as much as the theoretical amount (26.4 g) for oxidation of cellulose powder (20 g) was used. At the same time, partially oxidized celluloses were also prepared by addition of sodium metaperiodate of 0.7 and 0.5 times of theoretical amount (26.4 g). Periodate ( $\text{IO}_4^-$ ) concentration of each solution prepared was 0.160, 0.086, and 0.062 mol, respectively. Periodate oxidation was performed under shaking for at least 150 h until periodate consump-

tion reached a constant value.<sup>5</sup> After decomposition of the excess periodate with ethylene glycol, oxidized products were recovered by centrifugation and treated according to the method<sup>5</sup> described previously to give the oxidized products of S-100, S-70, S-50 with different degree of oxidation.

### Preparation of Polyalcoholic Products from Dialdehyde Celluloses

Periodate-oxidized product (5 g) was added to the aqueous solution containing sodium borohydride (1.0–2.0 g) enough for reduction. The reduction was allowed to proceed at room temperature with stirring overnight, and the excess borohydride was destroyed with acetic acid. The product of S-100 was dissolved completely, but other products of S-70 and S-50 still included undissolved portion. Each polyalcoholic product of S-70 and S-50 was separated through filtration into the supernatant solution and the undissolved portion. All complete solution was dialyzed against distilled water to remove inorganic materials and contaminants, and the dialyzed solution was concentrated to 100 mL volume and freeze-dried to give colorless, fluffy amorphous mass. On the other hand, the insoluble portion was suspended in 10% AcOH, and then filtrated through a glass filter and thoroughly washed with cold water and ethanol, successively, and was recovered as colorless amorphous products by drying in a vacuum.

### Acetylation of Polyalcoholic Products

The dry polyalcoholic product (1 g) was swelled in formamide (20 mL) by stirring for 1 h, and then the

mixture of dry pyridine and acetic anhydride (2 : 1, v/v 60 mL) was added and stirred at room temperature for more than 72 h. The deep brown colored reaction mixture was poured into ice water to precipitate the product. After filtration through a glass filter, the precipitate was washed with cold water and methanol, successively, and then dissolved in chloroform. The chloroform solution was treated with HCl (1%), Na<sub>2</sub>CO<sub>3</sub> (1%), and cold water, respectively, and finally evaporated to dryness. The residue was redissolved in a small amount of chloroform (10 mL) and precipitated from the solution by addition of 10 volumes methanol. The precipitate was recovered by filtration, washed with methanol, and dried in a vacuum. The products yielded 0.17–0.35 g for precipitate from insoluble fraction and 0.50–0.80 g for precipitate from soluble fraction.

### NMR Measurement

The NMR spectra were recorded with a Varian XL-200 spectrometer (50.3 MHz for C-13) operating in the pulsed, Fourier transform mode system. Measurements of the spectra were performed on the solution in D<sub>2</sub>O (99.8% purity) for the polyalcoholic products and on the solution in CDCl<sub>3</sub> (99.8% purity) for the corresponding peracetates. Measurement temperature of the former was done at 30°C, and that of the latter at ambient temperature (16–17°C). All samples were prepared in the concentration of 1.0–1.2%. In <sup>1</sup>H chemical shifts, 3-(trimethylsilyl) propanesulfonic acid Na salt Me<sub>3</sub>Si(CH<sub>2</sub>)<sub>3</sub>SO<sub>3</sub>Na and Me<sub>4</sub>Si were used as internal reference for solution in D<sub>2</sub>O and CDCl<sub>3</sub>, respectively. C-13 chemical shifts in D<sub>2</sub>O are referred to internal methanol ( $\delta = 49.9$  ppm) relative external TMS.

**Table I** Yields of Polyalcoholic Products from Dialdehyde Celluloses

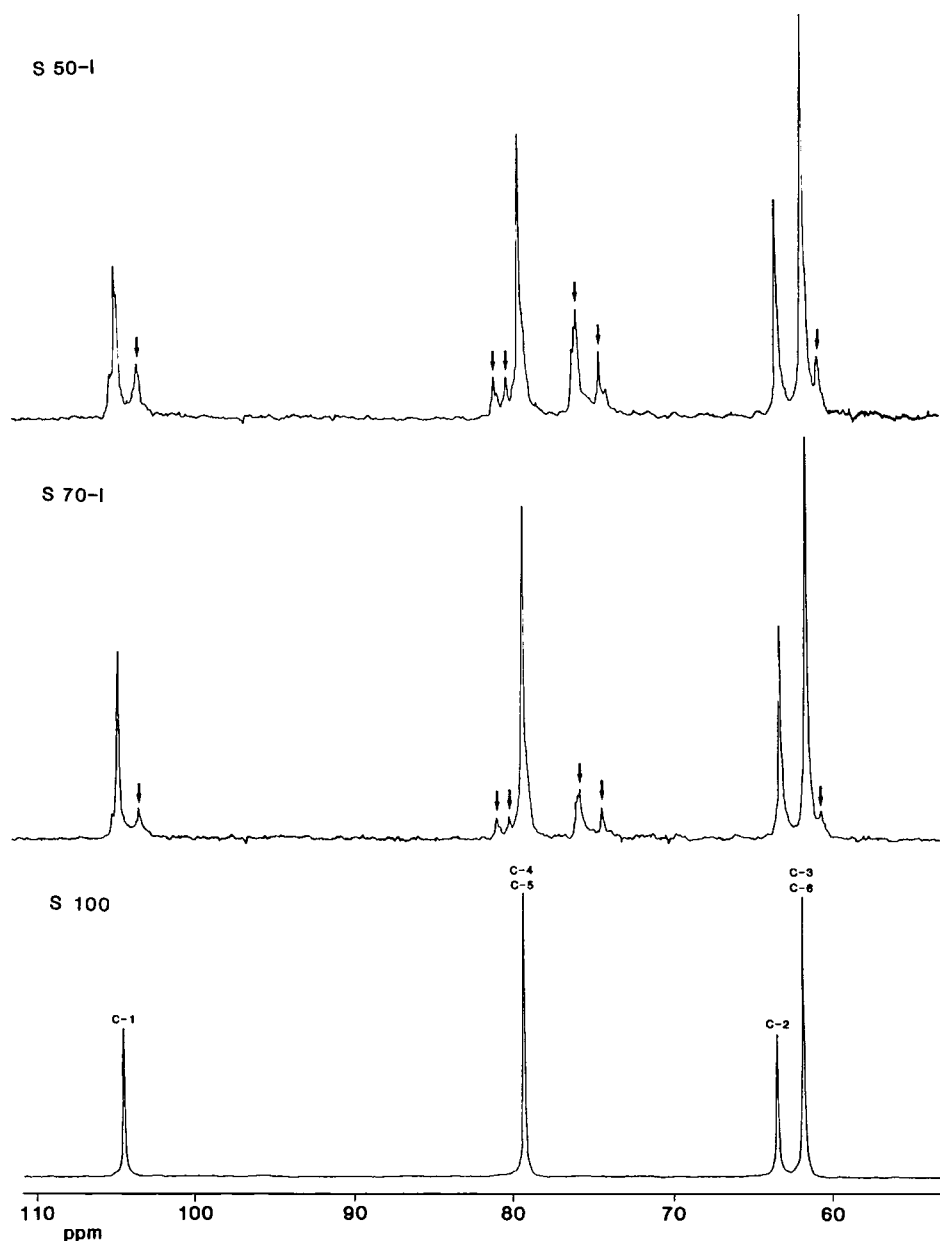
Sample Used	Actual Yield (g)		Recovery (%)		Total
	Insol.	Sol.	Insol.	Sol.	
<b>I</b>					
S-100	—	3.73	—	72.7	72.7
S-70	1.87	2.42	36.7	47.5	84.2
S-50	3.10	1.34	61.3	26.5	87.8
<b>II</b>					
S-100	—	3.41	—	66.5	66.5
S-70	2.80	1.67	55.0	32.8	87.8
S-50	2.55	1.48	50.4	29.2	79.6

I, Commercial Schleicher and Schüll cellulose powder; II, wood sulfite pulp. Recovery (%) = actual yield (g)/theoretical yield (g) × 100. Theoretical yield: S-100, 5.13 g; S-70, 5.09 g; S-50, 5.06 g.

## RESULTS AND DISCUSSION

The polyalcoholic products prepared by reduction of dialdehyde cellulose composed of different degree of oxidation were obtained in a yield of 67–89% as shown in Table I. The corresponding chloroform-soluble peracetates were recovered in a yield of 35–60%. As expected in advance, the yields of higher oxidized products gave low values and those of lower oxidized products were high.

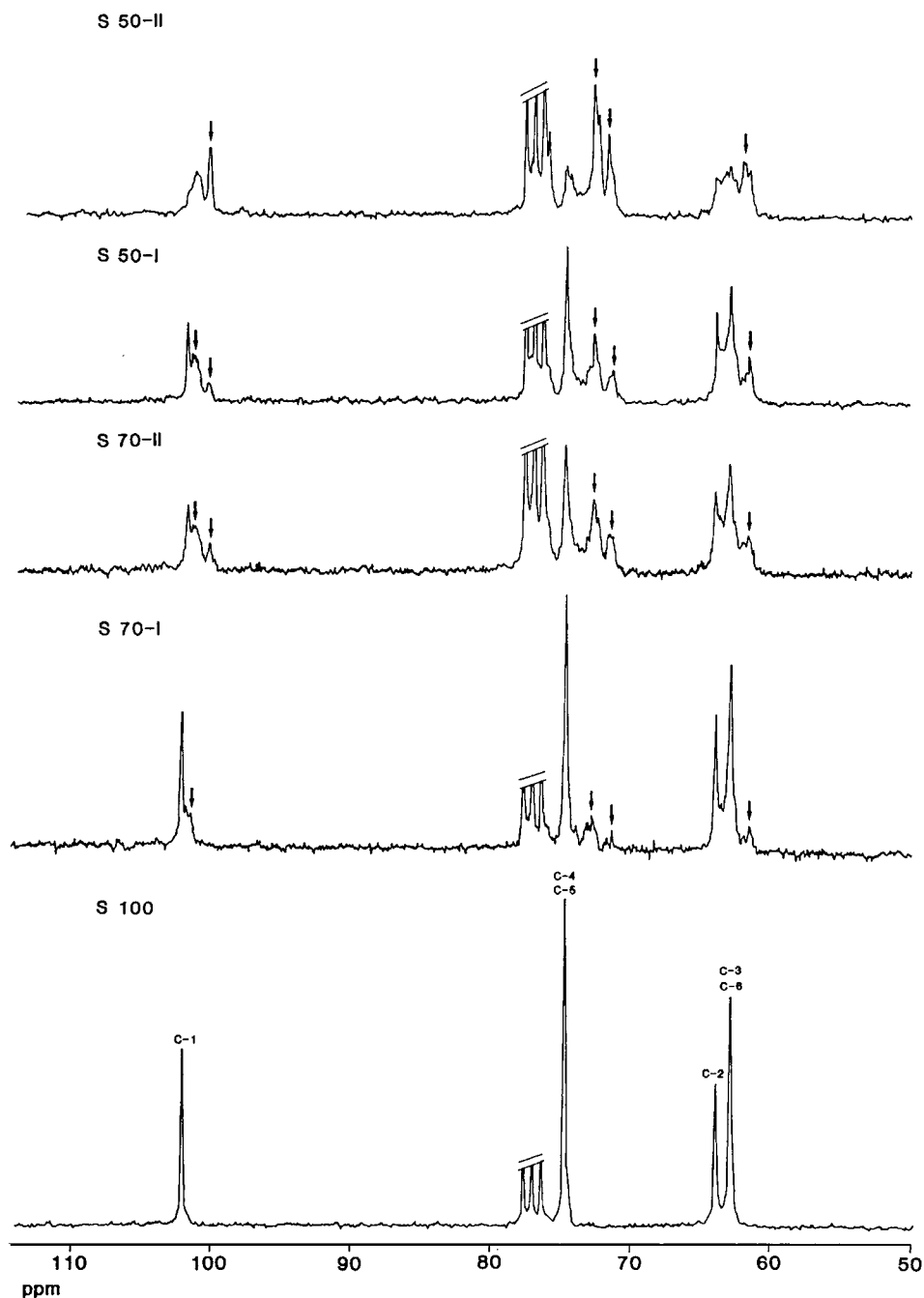
The typical C-13 NMR spectrum of the fully oxidized products (S-100) gives the expected four signals, two of which correspond to two equivalent carbons overlapping each other as shown in Figure 1, indicating a high degree of overall conversion from the original polymer. The NMR spectrum of polyalcohols suggests structurally regular simplicity as shown by Casu et al.<sup>6</sup> The spectrum also suggests that both carbon signals at the C-1 of the anomeric carbon and C-6 of the primary alcohol have retained



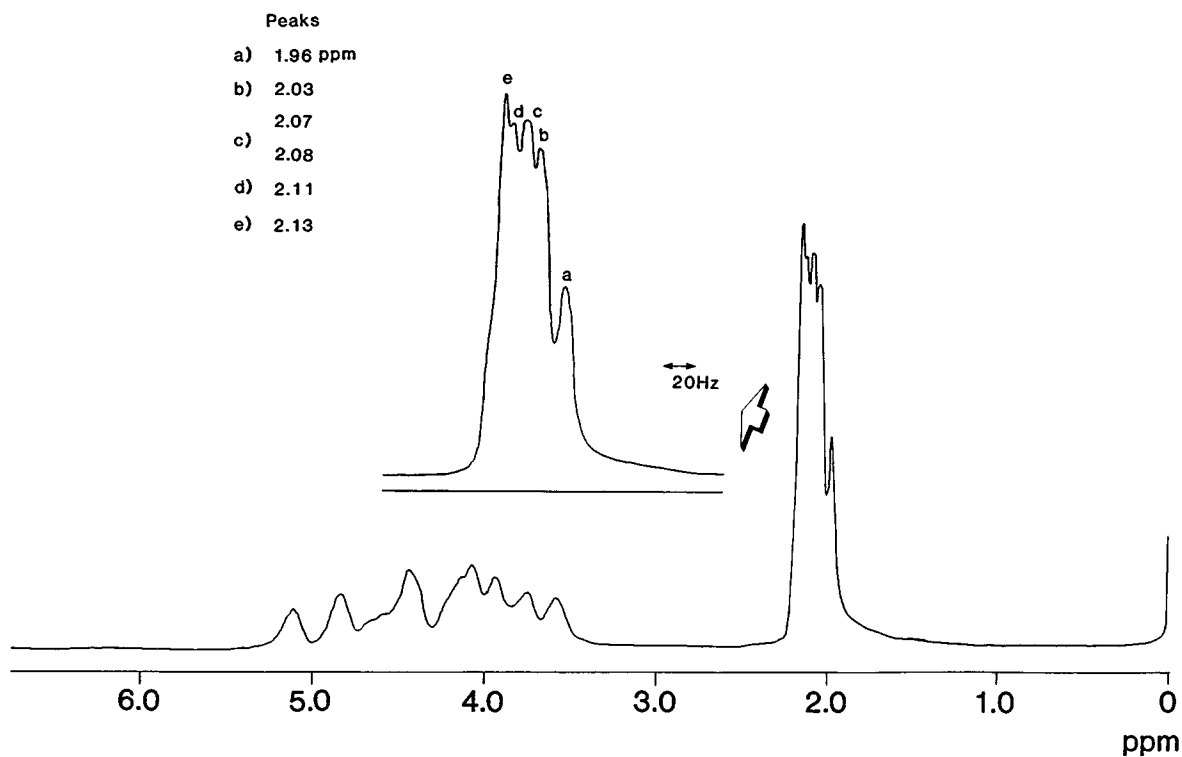
**Figure 1** Proton decoupled C-13 NMR spectra (50.3 MHz, D<sub>2</sub>O) of reduced products of periodate oxidized celluloses. Chemical shifts are referred to methyl carbon of internal methanol (49.9 ppm) relative external TMS.

the individual configuration of the glucose residue in the original cellulose since both carbon atoms resonate at the similar region as observed in the glucose residue. On the contrary, in C-13 NMR spectra of lower oxidized products, with lowering of degree of oxidation, minor broad signals arising from unoxidized glucose residues and incomplete reduced

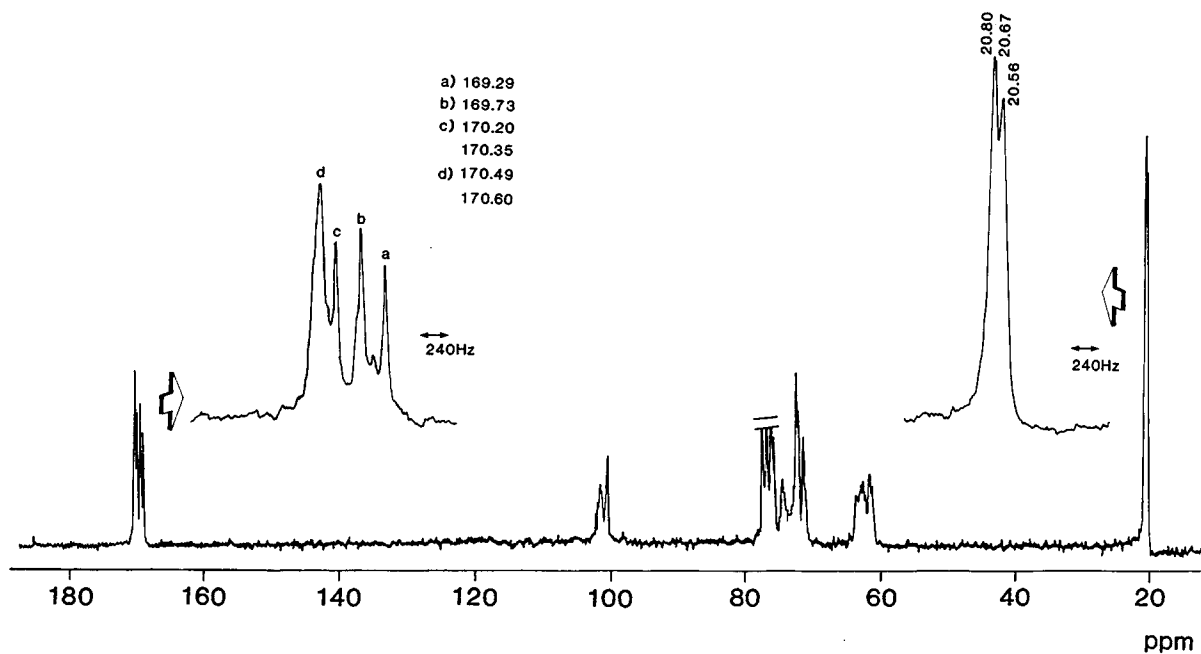
products are observed, as shown by the arrows in Figure 1, suggesting the heterogeneity of the oxidized products.<sup>7</sup> As observed in C-13 NMR spectrum of S-50 with low degree of oxidation, the signals due to unoxidized glucose residues are easily distinguishable, as shown by the arrows, and the strengths of signals originated from oxidized units are de-



**Figure 2** Proton decoupled C-13 NMR spectra (50.3 MHz,  $\text{CDCl}_3$ ) of reduced polyhydric alcohol peracetates of periodate-oxidized celluloses. S 70-I and S 50-I were recovered from the soluble fraction after reduction of periodate oxidized celluloses, and S 70-II and S 50-II were recovered from the insoluble fraction.



**Figure 3** Proton NMR spectra of partially periodate oxidized and reduced polyalcohol peracetate of S-50 prepared from cellulose. Note: (a), (b), (e), methyl protons of acetyl group at the C-3, C-2, and C-6, respectively, of unoxidized glucose residue; (c), (d), methyl protons of acetyl group at the C-3, C-2, and C-6, respectively, of oxidized glucose residue.



**Figure 4** Proton decoupled C-13 NMR spectrum of partially periodate oxidized and reduced polyalcohol peracetate of S-50 prepared from cellulose. Note: (a), (b), (c), carbonyl signal of acetyl group at the C-2, C-3, C-6, respectively, of unoxidized glucose residue; (d), carbonyl signal of acetyl group at the C-2, C-3, and C-6, of oxidized glucose residue.

creasing and those from unoxidized units increasing with lowering of degree of oxidation. All signal assignments were made by reference to the already established chemical shifts for the corresponding polysaccharides.<sup>8</sup> The C-13 NMR spectra of the peracetates were similar to those of the corresponding polyalcohols as shown in Figure 2, except for substituent-induced shifts and the additional signals attributable to the acetyl groups. It is also observed from Figure 2 that, with lowering of degree of oxidation, sharp signals assigned to the carbon nuclei of oxidized glucose residue decrease, and signals arising from the carbon nuclei of unoxidized glucose residue increase in the near region as shown by arrows. In <sup>1</sup>H and <sup>13</sup>C NMR spectra of peracetates of partially oxidized and reduced polyhydric alcohol prepared from insoluble portion of S-50 (Figs. 3 and 4), detailed analyses of signals assigned to methyl and carbonyl groups in acetyl group afforded evidence distinguishable by identification of the corresponding signals for periodate oxidized and unoxidized glucose residues. In Figure 3, signals of (a), (b), and (e) correspond to methyl protons of acetyl group at the C-3, C-2, and C-6, respectively, of unoxidized glucose residue, and signals of (c) and (d) correspond to those at the C-3, C-2, and C-6, respectively, of oxidized residue. Furthermore, from proton intensities based on signals arising from oxidized and unoxidized glucose residues, the oxidation ratio of periodate oxidized cellulose was estimated to be 54.06%. This value is in good agreement with the result of the actual measurement (52.7%). At the same time, as shown in Figure 4, the corresponding carbon signals based on the C-2, C-3, and C-6 of unoxidized and oxidized residues are determined by analysis of signals attributable to carbonyl carbon of acetyl group.

Thus, chemical analysis of the polyalcohols derived from periodate oxidation of polysaccharides and the followed reduction has already been established for determination of the average chain length of polysaccharides.<sup>9</sup> However, this analytical method by using NMR spectroscopy may be more simple and convenient for characterization of periodate oxidized and unoxidized moiety of polysaccharides.

## REFERENCES

1. A. S. Perlin, in *The Carbohydrates, Chemistry Biochemistry*, W. Pigman and D. Horton, Eds., Vol. 1B, Academic Press, New York, 1980, pp. 1167-1215.
2. T. P. Nevel, in *Cellulose Chemistry and its Application*, T. P. Nevell and S. H. Zeronian, Eds., Ellis Horwood, Chichester, 1985, pp. 243-265.
3. I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith, in *Methods in Carbohydrate Chemistry*, Roy L. Whistler, J. N. BeMiller, and M. L. Wolfrom, Eds., Vol. V, Academic Press, New York, 1965, pp. 361-370.
4. G. O. Aspinall, in *The Polysaccharides*, Vol. 1, G. O. Aspinall, Ed., Academic Press, New York, 1982, pp. 36-131.
5. E. Maekawa and T. Koshijima, *J. Appl. Polym. Sci.*, **29**, 2289-2297 (1984).
6. B. Casu, A. Naggi, G. Torri, G. Allegra, S. V. Meille, A. Cosani, and M. Terbojevich, *Macromolecules*, **18**, 2762-2767 (1985).
7. T. J. Painter, *Carbohydr. Res.*, **179**, 259-268 (1988).
8. A. S. Perlin, in *Spectroscopic Methods in the Polysaccharides*, G. O. Aspinall, Ed., Vol. 1, Academic Press, New York, 1982, pp. 133-193.
9. G. W. Hay, B. A. Lewis, and F. Smith, in *Methods in Carbohydrate Chemistry*, R. L. Whistler and J. N. BeMiller, Eds., Vol. V, 1965, pp. 377-380.

Received September 21, 1990

Accepted November 26, 1990