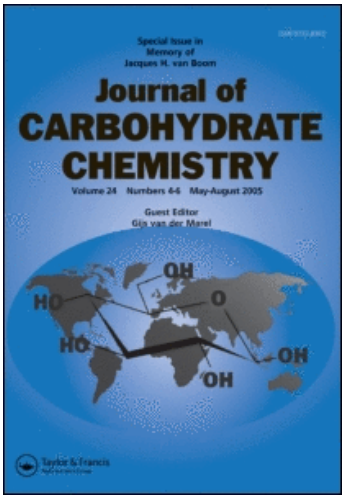

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Oxidation of Primary Alcohol Groups of Naturally Occurring Polysaccharides with 2,2,6,6-Tetramethyl-1-Piperidine Oxoammonium Ion

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OXIDATION OF PRIMARY ALCOHOL GROUPS OF NATURALLY OCCURRING POLYSACCHARIDES WITH 2,2,6,6-TETRAMETHYL-1-PIPERIDINE OXOAMMONIUM ION

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ABSTRACT

The primary alcohol groups of ten polysaccharides, with widely different structures and water solubilities, were oxidized to carboxyl groups using 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion (TEMPO) at pH 10.8 and 0°C. The yield and selectivity for the primary alcohol group were high for all ten of the polysaccharides. The oxidation greatly increased the water-solubility of the polysaccharides. Water-insoluble polysaccharides such as amylose, cellulose, and chitin became water-soluble to the extent of approximately 10% (w/v). The water-soluble polysaccharides had their degree of solubility doubled or tripled. The specific optical rotation, viscosity, and gelling properties with calcium ion were determined. The oxidized polysaccharides are new anionic polymers with unique structures that could have application as gums, gels, and films.

INTRODUCTION

Miyazawa *et al.*[1] reported the selective oxidation of alcohols by oxoammonium salts ($R_2N{=}O^+ X^-$). Anelli, *et al.*[2] expanded the scope of the reaction and showed that

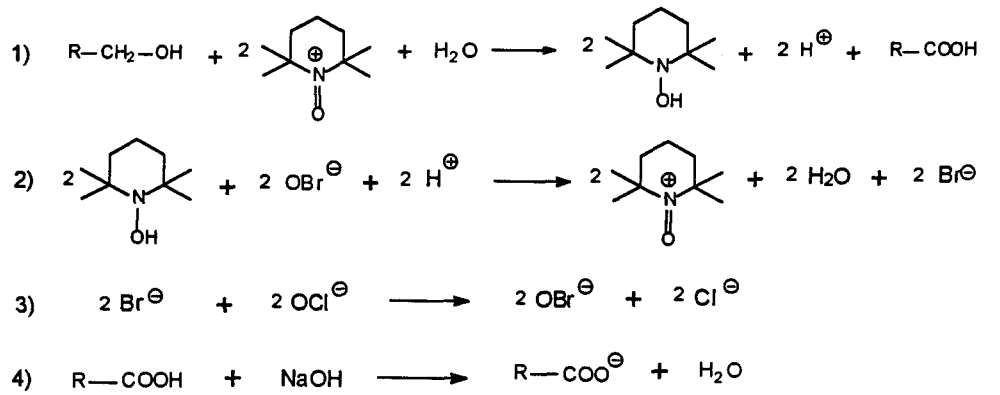
oxoammonium salts oxidized primary alcohols to aldehydes, which in turn were oxidized to carboxylic acids, and secondary alcohols were oxidized to ketones. The oxidation of aldehydes was slow, but could be increased by the addition of hypochlorite to give the rapid regeneration of the oxoammonium oxidant. De Nooy, *et al.*[3] reported high yields for the selective oxidation of the hydroxy methylene, primary alcohol group, of amylose using 2,2,6,6-tetramethyl-1-piperidine oxoammonium salt (TEMPO) with sodium hypochlorite and sodium bromide in an aqueous, alkaline solution of pH 10.5--11. De Nooy *et al.* showed by ^{13}C NMR and the stoichiometric consumption of base that the oxidation was 98% selective for the oxidation of the primary hydroxyl group. In a more recent study, De Nooy, *et al.*[4] reported the TEMPO oxidation of the primary alcohol group(s) of methyl-α-D-glucopyranoside, methyl-ß-D-glucopyranoside, α,α-trehalose, potato starch, pullulan, and Pharmacia B-512F dextran T-40.

Using 2,2,6,6-tetramethyl-1-piperidine oxoammonium salt/sodium hypochlorite/sodium bromide, we have oxidized the primary alcohol groups of ten naturally occurring polysaccharides, six that were water soluble (potato amylopectin, pullulan, alternan, regular comb dextran, carboxymethyl cellulose, and chitosan) and four that were water insoluble (wheat starch, potato amylose, cellulose, and chitin). We report here on some of the properties of the products, such as percent of primary alcohol group oxidized, water-solubility, intrinsic viscosity, specific optical rotation, and gel formation with calcium ion.

RESULTS AND DISCUSSION

Ten polysaccharides, with different structures, were oxidized using 2,2,6,6-tetramethyl-1-piperidine oxoammonium salt with sodium hypochlorite and sodium bromide. ^{13}C NMR of the oxidized polysaccharides gave a chemical shift at 175 ppm, indicating that the primary alcohol group had been oxidized to a carboxyl group. There were no chemical shifts in the range of 198--205 ppm, indicating the absence of keto groups that could have been formed by the oxidation of secondary alcohols. The pH during the reaction dropped due to the formation of the carboxylic acid. The pH was kept constant at 10.5 by the addition of sodium hydroxide. When each of the polysaccharide reactions had consumed 1 mmol of hydroxide/mmol of theoretical primary alcohol, the reaction was quenched by the addition of ethanol.

The oxidation, regeneration of the oxoammonium ion oxidant, and the titration of the resultant carboxylic acid with sodium hydroxide involves the following four distinct reactions:



The oxidation of a primary alcohol group requires two oxoammonium groups in an alkaline, aqueous solution (reaction 1). The reduced product, *N*-hydroxylamine-1-piperidine, is reoxidized to the oxoammonium salt by hypobromite (reaction 2). The hypobromite is reformed by reaction with hypochlorite (reaction 3). The carboxylic acid product is titrated with sodium hydroxide (reaction 4). The amount of hydroxide that is used is proportional to the amount of acid formed in the oxidation.

Table 1 lists the ten polysaccharides, the temperatures of the reactions, the time necessary for each reaction system to consume 1 mmol of hydroxide ion/mmol of primary alcohol, the yield of oxidized polysaccharide, the percent of the primary alcohol groups that were oxidized, and the selectivity of the oxidation reaction. The amount of uronic acid produced was determined by acid hydrolysis of the oxidized product, followed by the carbazole procedure, using D-glucuronic acid as a standard (hereinafter called the carbazole procedure or carbazole analysis). The yields and the selectivity for oxidizing the primary alcohol groups were uniformly high, with the exception of chitin. The carbazole analysis of the hydrolyzed, oxidized chitin was low due to a lower absorbance produced from *N*-acetyl-2-amino-deoxy-D-glucuronic acid compared with the absorbance obtained from D-glucuronic acid. The amount of sodium hydroxide consumed in the chitin reaction, however, indicated that nearly 100% of the *N*-acetyl-2-amino-2-deoxy-D-glucose residues had been oxidized (see Fig. 1).

The water-insoluble polysaccharides (wheat starch granules, potato amylose, cellulose, and chitin) were reacted in water in a heterogeneous suspension. They all became soluble as the oxidation proceeded. They were isolated by precipitation with 2 volumes of alcohol. The water-soluble polysaccharides were oxidized in a dissolved state in water and also were isolated by precipitation with 2 volumes of alcohol. All of the oxidized samples were worked-up by trituration with acetone and ethanol to give dry powders.

Table 1. 2,2,6,6-Tetramethyl-1-piperidine oxoammonium Ion Mediated Oxidation of Different Polysaccharides

Polysaccharide	Temperature (°C)	Time[a] (min)	Yield[b] (%)	Oxidation yield[c] (%)	Selectivity (%)
Wheat starch	0	107	98	95	100
Potato amylose	0	79	97	96	96
Potato amylopectin	0	45	98	95	100
Chitin	0	170	88	42[e]	---
Chitosan	0	53	96	91	91
Pullulan	0	80	97	66	100
Alternan	0	150	89	43	100
Regular comb dextran	0	160	87	46	92
Cellulose (Avicel)	25	500	88	84	84
α-Cellulose	25	360	92	87	87
Carboxymethyl cellulose	25	300	89	72	90
Chitin	25	79	91	44[e]	---

a. The time necessary for the consumption of 1 mmol OH^-/mmol primary alcohol.

b. The yield was determined from the weight of the dried polysaccharide obtained by precipitation with two volumes of alcohol, corrected by using the anhydro molecular weight of the sodium salt of the uronic acid.

c. The oxidation yield is the percentage of anhydro monomer units converted to anhydro uronic acid units as determined by the carbazole procedure.

d. The selectivity yield is the percentage of uronic acid units produced from the monomer units with a primary alcohol group.

e. The oxidation yield for chitin was low as *N*-acetyl-D-glucosamine uronic acid gave low values by the carbazole procedure.

Pullulan is a glucan composed of repeating maltotriose units linked end-to-end by α-1→6 glycosidic bonds.[5,6] As such, pullulan has two out of three anhydro-D-glucose residues that have primary alcohol groups. Carbazole analysis of oxidized pullulan showed that 66% of the anhydro-D-glucose residues had been oxidized, indicating that two out of three D-glucose residues were oxidized. The selectivity factor is, thus, 100%. Alternan is a glucan with D-glucose residues linked alternately α-1→6 and α-

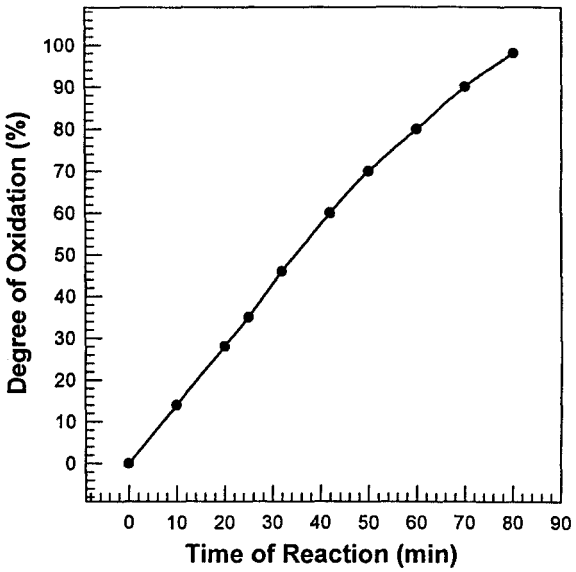


Figure 1. Reaction kinetics of the oxidation of chitin by 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion at pH 10.8 and 25 °C. The degree of oxidation is expressed as the number of mmol of sodium hydroxide consumed per mmol of primary alcohol groups present.

1→3 with 7--11% α-1→3 branch linkages.[7] It, therefore, would have 41--45% of its D-glucose residues that have a primary alcohol group that could be oxidized. The carbazole analysis showed that 43% of the anhydro-D-glucose residues had been oxidized, indicating a selectivity of 100%.

Regular comb dextran has α-1→6 linked D-glucose residues in the main chains with single D-glucose residues attached to every D-glucose residue in the main chains by an α-1→3 branch linkage.[8] Regular comb dextran, thus, would have 50% of its D-glucose residues (those residues attached to the main chain) with a primary alcohol group. Carbazole analysis showed that 46% of the anhydro-D-glucose residues had been oxidized, giving a selectivity factor of 92%. The carboxymethyl cellulose (CM-cellulose) had a d.s. of 0.2, or one out of five D-glucose residues have a carboxymethyl substituent at positions 2,3,6, which renders the unit resistant to the carbazole reaction. Carbazole analysis indicated that 72% of the D-glucose residues of CM-cellulose had been oxidized, giving a selectivity of 90%. Carbazole analysis of the two oxidized celluloses, Avicel and α-cellulose, showed that 84% and 87% of their primary alcohol groups had been oxidized, respectively.

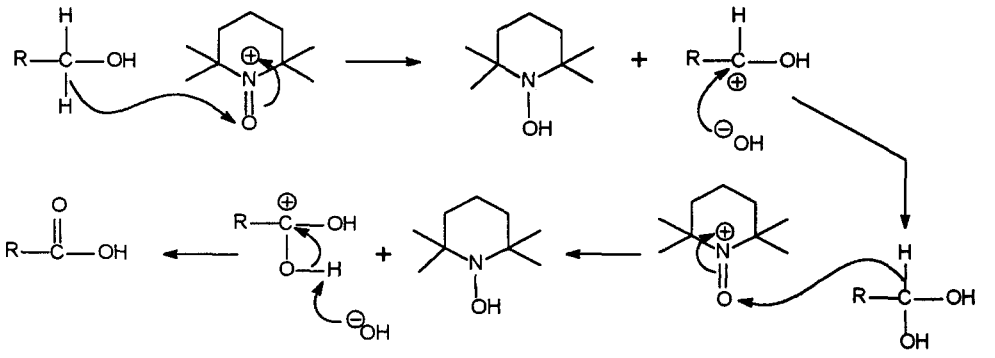


Figure 2 Mechanism for the oxidation of primary alcohol groups of carbohydrates to carboxylic acids by 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion.

A comparison of the water solubility of the starting polysaccharides with that of the oxidized polysaccharides (Table 2) showed that all of the oxidized polysaccharides had a higher water solubility than the native polysaccharides. Wheat starch and potato amylose both had about 11% (w/v) water solubility, giving dramatic increases of 55.5-fold and 109-fold over the starting polysaccharides, respectively. The water-soluble polysaccharides (alternan, regular comb dextran, chitosan, CM-cellulose, and pullulan) also had significant increases in their water solubilities.

In the examples where they could be compared, the water-soluble polysaccharides, the specific optical rotations of the oxidized polysaccharides decreased from the specific optical rotations of the unoxidized polysaccharides (see Table 3). The intrinsic viscosities of the unoxidized and the oxidized polysaccharides did not change significantly, except for oxidized chitosan and oxidized CM-cellulose in which there was a significant decrease in the intrinsic viscosities (see Table 4).

All of the oxidized polysaccharides have unique structures. Oxidized alternan has a structure in which every other monomer unit is a D-glucuronic acid residue linked α-1→6 to a D-glucose residue. Oxidized pullulan has a structure with two adjacent D-glucuronic acid residues linked α-1→4 to each other and attached to a D-glucose residue by an α-1→6 linkage. The structure is a repeating unit of α-D-glucopyranosyl (1→4)-α-D-glucuronopyranosyl (1→4)-α-D-glucuronopyranosyl (1→6). Oxidized regular comb dextran has main chains of D-glucopyranosyl residues linked α-1→6 to each other with D-glucuronopyranosyl residues linked by an α-1→3 branch linkage to every D-glucose residue in the main chains. Oxidized amylopectin is a 5% α-1→6 branched molecule of poly-(α-1→4)-linked D-glucuronopyranose residues.

Table 2. Solubilities of Unoxidized and Oxidized Polysaccharides

Polysaccharide	Solubility of unoxidized polysaccharide (% w/v)	Solubility of oxidized polysaccharide (% w/v)
Wheat starch	0.2	11.1
Potato amylose	0.1	10.9
Potato amylopectin	14.8	41.9
Chitin	0.0	7.9
Chitosan	29.7	33.4
Pullulan	12.6	34.2
Alternan	5.4	16.2
Regular comb dextran	7.8	20.7
Cellulose (Avicel)	0.0	11.2
α-Cellulose	0.0	9.4
Carboxymethyl cellulose	18.1	35.1

Table 3. Specific Optical Rotations of Unoxidized and Oxidized Polysaccharides

Polysaccharide	Specific optical rotation ($[\alpha]_D^{20}$)	
	Unoxidized	Oxidized
Wheat starch	+200±1°	+173.30±1.05°
Potato amylose	+220±1°	+174.15±0.42°
Potato amylopectin	+198±1°	+188.08±0.36°
Chitin	---[a]	-199.09±0.17°
Chitosan	-13.34±0.19°	-14.96±0.30°
Pullulan	+142.18±0.22°	+135.63±0.21°
Alternan	+173.98±1.40°	+60.80±0.21°
Regular comb dextran	+148.43±0.57°	+121.32±0.31°
Cellulose (Avicel)	---[a]	-70.10±1.46°
α-Cellulose	---[a]	-44.93±0.68°
Carboxymethyl cellulose	+137.42±0.75°	-7.36±0.14°

a. The specific optical rotation could not be determined because of water-insolubility of the unoxidized polysaccharide.

Table 4. Intrinsic Viscosities of Unoxidized and Oxidized Polysaccharides at 25 °C

Polysaccharide	Intrinsic viscosity (millipoise) Unoxidized	Oxidized
Wheat starch	10.02±0.01	9.95±0.05
Potato amylose	9.59±0.03	9.42±0.03
Potato amylopectin	10.72±0.01	10.62±0.01
Chitin	---[a]	10.52±0.01
Chitosan	67.58±0.48	9.32±0.02
Pullulan	9.95±0.04	9.82±0.10
Alternan	9.62±0.07	10.98±0.04
Regular comb dextran	9.67±0.07	9.74±0.10
Cellulose (Avicel)	---[a]	11.11±0.01
α-Cellulose	---[a]	10.59±0.06
Carboxymethyl cellulose	27.82±0.05	9.79±0.05
Sodium alginate	98.01±0.62	---

a. Intrinsic viscosity could not be measured because of water-insolubility.

Oxidation of the primary alcohol groups of cellulose gives poly-(ß-1→4)-D-glucuronopyranose. This structure is very similar to the poly-(ß-1→4)-D-mannuronic acid structure of the precursor of algin with only structural difference in the configuration at C-2. Oxidized amylose is a poly-(α-1→4)-D-glucuronopyranose with structural difference of the configuration at C-2 and of the α-configuration of the glycosidic linkage instead of the ß-configuration.

Oxidized CM-cellulose (d.s. = 0.2) is very similar to oxidized cellulose with the exception that 1 out of 5 monosaccharide residues have a carboxymethyl substituent. Oxidized chitin and oxidized chitosan also have structures similar to oxidized cellulose with the substitution of a *N*-acetyl amino-group and an amino group, respectively, for a hydroxyl group at C-2. Oxidized chitosan has the additional feature of having both an anionic and a cationic group that gives the monomer units a net zero charge.

The selectivity for the primary alcohol of carbohydrates involves the reaction of the primary alcohol on a pyranose ring with a bulky sterically hindered 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion, which is similar to the selectivity of the reaction of carbohydrate primary alcohol groups with the sterically hindered tosyl chloride or trityl chloride. The mechanism of the oxidation reaction has similarities to the enzyme catalyzed reaction of alcohol dehydrogenase that uses the pyridinium salt

Table 5. Gel Formation with Ca^{+2} for the Oxidized Polysaccharides

Oxidized polysaccharide	Gel formation
Wheat starch	Yes (good)
Potato amylose	Yes (good)
Potato amylopectin	Yes (good)
Chitin	No
Chitosan	No
Pullulan	No
Alternan	No
Regular comb dextran	No
Cellulose (Avicel)	Yes (moderate)
α-Cellulose	Yes (moderate)
Carboxymethyl cellulose	Yes (moderate)

nicotinamide adenine dinucleotide (NAD^+) as a coenzyme oxidant. In the oxidation of the primary alcohol group with the piperidine oxoammonium ion, the oxoammonium ion abstracts a hydride ion from the methylene carbon. Under alkaline conditions, the resulting carbonium ion rapidly reacts with a hydroxide ion. The resulting hydrated aldehyde product rapidly reacts with a second piperidine oxoammonium ion to give a carbonium ion that reacts with one of the hydroxyl groups to give a carboxylic acid (see Fig. 2).

Because the highly oxidized polysaccharide (poly-ß-D-mannuronic acid/poly-α-L-guluronic acid), sodium alginate, forms gels when added to calcium chloride solutions, the oxidized polysaccharides (polyuronic acids) produced in this study were tested for gel formation with calcium chloride. A 3% (w/v) solution of the sodium salts of the oxidized polysaccharides were added dropwise to a 4% (w/v) solution of calcium chloride. The α-1→4 linked oxidized polysaccharides formed gels. Oxidized cellulose and CM-cellulose formed intermediate gels, while oxidized chitin, chitiosan, pullulan, alternan, and regular comb dextran did not form gels with calcium (see Table 5). None of the gels that formed, however, were of the quality formed by sodium alginate.

There are a number of naturally occurring anionic polysaccharides, such as the carrageenans that are sulfo-substituted poly-(ß-1→3) and poly-(ß-1→4)-D-galactans,[9] heparin that is a sulfo-substituted α-1→4-D-glucan, and the algins that are heteropolysaccharides of ß-1→4-D-mannuronic acid and α-1→4-L-guluronic acid.[10] A number of other anionic polysaccharides are produced by bacterial fermentation such as xanthan,

gellan, wellan, and rhamsan in which the principal anionic group is the carboxylate group of D-glucuronic acid in varying combination with other hexose residues.[11] The oxidation of the ten naturally occurring polysaccharides in this study give new anionic polysaccharides with unique structures. These oxidized polysaccharides could have new uses and applications as gums, gels, and films.

EXPERIMENTAL

Polysaccharides and Chemicals: Amylose and amylopectin were separated from potato starch that was obtained from National Starch and Chemical Co., Bridgewater, NJ, using the 1-butanol precipitation method for the amylose fraction and the precipitation of the amylopectin fraction with 2 volumes of ethanol.[12] Pullulan was obtained from Hayashibara Co. (Okayama, Japan). Microcrystalline cellulose (Avicel) was obtained from American Viscose Corp. (Marcus Hook, PA). Fibrous cellulose (α-cellulose), wheat starch, and practical grade, crab shell chitin were obtained from Sigma Chemical Co. (St. Louis, MO). The chitin was purified according to the procedures in reference 13. Alternan was synthesized from sucrose by *Leuconostoc mesenteroides* B-1355 alternansucrase[14] and purified by differential alcohol precipitation.[15] Regular comb dextran was synthesized from sucrose by *Leuc. mesenteroides* B-742 regular comb dextransucrase[16] and purified by differential alcohol precipitation.[15]

2,2,6,6-Tetramethyl-1-piperidine oxoammonium salt was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were of commercial analytical grade.

Oxidation Procedures: Potato amylopectin, pullulan, alternan, and regular comb dextran (30.9 mmol, 5.00 g) and carboxymethyl cellulose (27.8 mmol, 5 g) were dissolved in 200 mL of water. The water-insoluble polysaccharides (5.0 g of wheat starch granules, potato amylose, and cellulose, and 6.8 g of chitin) were suspended in 200 mL of water and stirred. 2,2,6,6-Tetramethyl-1-piperidine oxoammonium salt (0.31 mmol, 50 mg) was added to the solutions or suspensions with 1.6 g of sodium bromide and 93 mL of 5.25% (w/v) sodium hypochlorite solution. The pH was adjusted to 10.8 with 0.5 M sodium hydroxide and maintained with a pH-controller. The majority of the reactions were conducted at 0° ±1 °C, with the exception of cellulose and chitin, which also were conducted at 25° ±1 °C. The time of reaction varied for each polysaccharide (see Table 1). When 1 mmol of hydroxide/mmol of primary alcohol had been added, the reaction was quenched by the addition of 5 mL of ethanol/200 mL of solution, followed by neutralization with 4 M HCl.

Work-up of the Oxidized Polysaccharides: The oxidized polysaccharide was precipitated by the addition of 2 volumes of ethanol, followed by centrifugation and trituration of the centrifuged polysaccharide with acetone (5-6 times) and by a final time with ethanol. The treated polysaccharide was then dried at 50°C in a vacuum oven.

Analysis of the Reaction: The amount of acid produced (*i.e.*, oxidation of the primary alcohol group to carboxylate group) was obtained by measuring the amount of sodium hydroxide that was required to maintain the pH at 10.8. The uronic acid content of the final oxidized polysaccharide was obtained using the Dische carbazole method[17] after acid hydrolysis of the oxidized polysaccharide.

Acid Hydrolysis of the Oxidized Polysaccharide: A solution of the oxidized polysaccharide (10 mg/mL) was prepared; 10 μL of concentrated HCl was added to each of several 100 μL samples; the samples were sealed in ampules and placed into a boiling water bath for 60 min. The hydrolysis occurred in 1.2 M HCl at 99 °C for 60 min in a sealed ampule. TLC analysis showed that the hydrolysis of the polysaccharides was complete. After cooling, the amount of uronic acid was determined by the Dische carbazole method.[17]

^{13}C-NMR Analysis ^{13}C NMR spectra were obtained for the oxidized polysaccharides dissolved (25 mg/mL) in D_2O, using an AC-200 NMR instrument with deuterated DMSO as an internal standard in a capillary tube.

Determination of the Water Solubility: A suspension of polysaccharide (600 mg/mL) was prepared; 2 mL was put into a screw-cap tube and autoclaved at 121°C for 15 min. The tube was cooled to 20 °C and centrifuged at 6,000 rpm for 5 min; 1.0 mL of the clear supernatant was removed and 3 mL of ethanol was added; the precipitated polysaccharide was centrifuged at 10,000 rpm for 5 min; the supernatant was removed and the centrifuged polysaccharide was triturated with acetone 5-6 times and a final time with ethanol; it was dried at 50 °C in a vacuum oven. The weight of the dried polysaccharide was taken as the solubility in 1.0 mL of water.

Determination of the Specific Optical Rotation: The optical rotations were measured at 20 °C on 10 mg/mL samples of polysaccharides in a 1 dm cell, using a Rudolph polarimeter with a sodium lamp. Samples were determined ten times, five from the left and five from the right. The mean and the mean deviation were computed.

Determination of the Viscosity: The viscosity of the polysaccharides was measured on 2 mg/mL samples in water at 25 °C, using an Ostwald viscometer. Samples were determined in triplicate and the mean and the mean deviation computed.

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NOTE

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REFERENCES

1. T. Miyazawa, T. Endo, S. Shiihashi, and M. Okawara, *J. Org. Chem.*, **50**, 332 (1985).
2. P. L. Anelli, C. Biffi, F. Montanari, and S. Quici, *J. Org. Chem.*, **52**, 2559 (1987).
3. A. E. J. De Nooy, A. C. Besemer, and H. Van Bekkum, *Recl. Trav. Chim. Pays-Bas*, **113**, 165 (1994).
4. A. E. J. De Nooy, A. C. Besemer, and H. Van Bekkum, *Carbohydr. Res.*, **269**, 89 (1995).
5. H. Bender, J. Lehmann, and K. Wallenfels, *Biochim. Biophys. Acta*, **36**, 309 (1959).
6. K. Wallenfels, G. Keilich, G. Brechtler, and D. Freudenberger, *Biochem. Z.*, **341**, 433 (1965).
7. F. R. Seymour, R. D. Knapp, E. C. M. Chen, and S. H. Bishop, *Carbohydr. Res.*, **74**, 41 (1979).
8. F. R. Seymour, E. C. M. Chen, and S. H. Bishop, *Carbohydr. Res.*, **68**, 113 (1979).
9. G. H. Therkelsen, *Industrial Gums: Polysaccharides and Their Derivatives*, 3rd ed. (R. L. Whistler and J. N. BeMiller, Eds.) pp 145--180, Academic Press, Inc., p 145 (1993).
10. K. Clare, *Industrial Gums: Polysaccharides and Their Derivatives*, 3rd ed. (R. L. Whistler and J. N. BeMiller, Eds.), Academic Press, Inc., p 105 (1993).
11. K. S. Kang and D. J. Pettit, *Industrial Gums: Polysaccharides and Their Derivatives*, 3rd ed. (R. L. Whistler and J. N. BeMiller, Eds.), Academic Press, Inc. p 341 (1993).
12. S. Lansky, M. Kooi, and T. J. Schoch, *J. Am. Chem. Soc.*, **71**, 4066 (1949).
13. J. N. BeMiller, *Methods Carbohydr. Chem.*, **5**, 103 (1965).
14. G. L. Côté and J. F. Robyt, *Carbohydr. Res.*, **101**, 57 (1982).
15. C. A. Wilham, B. H. Alexander, and A. Jeanes, *Arch. Biochem. Biophys.*, **59**, 61 (1955).
16. G. L. Côté and J. F. Robyt, *Carbohydr. Res.*, **119**, 141 (1983).
17. Z. Dische, *Methods Carbohydr. Chem.* **1**, 498 (1962).