ELSEVIER

Carbohydrate Polymers 42 (2000) 51–57

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Specific modifications of galactomannans

M.R. Sierakowski[a,b], M. Milas[a], J. Desbrières[a], M. Rinaudo[a,*]

[a]*Centre de Recherches sur les Macromolécules Végétales (CNRS) affiliated to the Joseph Fourier University of Grenoble, BP 53, 38041 Grenoble cedex 9, France*

[b]*Laboratory of Biopolymers, Chemistry Department, UFPR-P.O. Box 19081 81531-990, Curitiba, Brazil*

Received 4 March 1999; received in revised form 15 May 1999; accepted 6 August 1999

Abstract

A sample of galactomannan extracted from the seeds of *Leucaema leucocephala* is modified on a specific position using the reactant 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). This method allows oxidation at the C-6 position of galactose and free mannose (not connected to a side group). The kinetics of oxidation is followed under well-defined conditions. Different methods are proposed for the characterization of the modified polysaccharides and NMR spectroscopy is shown to give useful information. New polyelectrolytes are produced with different degrees of oxidation. Successive alkylations on the C-6 sites are investigated. The degree of alkylation obtained remains low but it can be increased easily to reinforce the amphiphilic character.

Keywords: Galactomannan; Specific oxidation; Alkylation; Characterization; Nuclear magnetic resonance; New polyelectrolyte

1. Introduction

Galactomannans are polysaccharides produced by many seeds. We have studied the relation between their chemical structure and their properties (Ganter, Milas & Rinaudo, 1992; Kapoor, Milas, Taravel & Rinaudo, 1994; Kapoor, Milas, Taravel & Rinaudo, 1996; Kapoor, Taravel, Joseleau, Milas, Chanzy & Rinaudo, 1998). These neutral polymers are based on a β (1 → 4) mannose unit (M) polymeric backbone with side groups consisting of a galactose unit (G) which is α (1 → 6) linked. A schematic representation is given in Fig. 1. The galactomannans differ in their content of galactose and its distribution along the chain. The solubility in water increases when the galactose yield increases but it remains relatively low due to the large capability for intermolecular H-bonding. These polysaccharides are considered as semi-rigid polymers with a persistence length in the range of 100 Å (Petkowicz et al., 1999); and because of this they are good thickeners for aqueous systems.

In this work, the objective is to perform specific chemical modifications on galactomannans to extend their range of applications. An initial approach was specific enzymic oxidation on the C-6 of the galactose side units (Frollini, Reed, Milas & Rinaudo, 1995). In this paper, we describe oxidation on the C-6 position using the reagent 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). This has been shown to oxidize different polysaccharides very efficiently (Muzzarelli, Muzzarelli, Cosani & Terbojevich, 1999; de Nooy, Besemer & van Bekkum, 1994; de Nooy, Besemer & van Bekkum, 1995; de Nooy, Besemer, van Bekkum, van Dijk & Smit, 1996; Rinaudo, Roure, Milas & Frollini, 1998). The modified polymer produced is an anionic polyelectrolyte having a greater solubility in aqueous solvent than the original material. In a second step, the modified carboxylated galactomannan prepared is grafted with alkyl chains to develop some amphiphilic character.

2. Experimental

The seeds of *Leucaema leucocephala* were collected in Curitiba-Parana (Brazil). First, the seeds were submitted to extraction of pigments and lipids with 2/1 (V/V) toluene/ethanol in a Soxhlet for 18 h. They were then treated for 15 min with water at 96°C to destroy enzymic activities and extracted exhaustively with water at 25°C. The crude extracts were precipitated with two volumes of EtOH and the precipitated polysaccharide was washed with ethanol and dried. For purification, the crude polysaccharide was redissolved in water to a concentration of 10 g/l and clarified by centrifugation at 14 000 rpm and 35°C for 20 min two times. The supernatant was filtered through Sartorius membranes of 3.0, 1.2 and 0.8 μm pore diameters successively, precipitated with EtOH and dried. The composition was determined by complete hydrolysis with trifluoroacetic

* Corresponding author. Tel.: +33-476037627; fax: +33-476547203.
E-mail address: rinaudo@cermav.cnrs.fr (M. Rinaudo).

0144-8617/00/$ - see front matter
PII: S0144-8617(99)00137-X

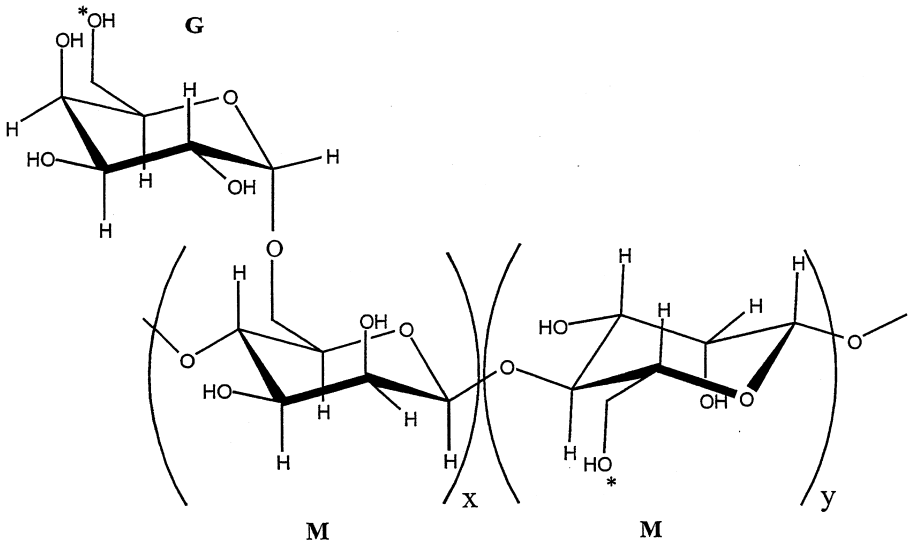

Fig. 1. Schematic representation of the galactomannan molecule. G is the galactose unit, M the mannose unit. x is the number of galactose units and y the number of free mannose units. M/G = $(x + y)/x$; * are the positions available for chemical modification: (a)–CH_2OH native galactomannan; (b)–CH_2OH or –COOH oxidized GM; and (c)–CH_2OH or $-CH_2-NH-(CH_2)_{11}-CH_3$.

acid (TFA) 2 M for 2 h at 100°C; the mannose and galactose formed were separated and quantified with HPLC on a CHO 682 column at 85°C with water as eluent (0.5 ml/min); the M/G ratio is found to be around 1.25. The composition was also obtained by ^{1}H NMR in D_2O at 75°C; from the integrals of the anomeric protons of the two units, the M/G ratio is found to be around 1.3 (Fig. 2a). ^{13}C NMR (Fig. 3) confirms this ratio when the integrals of the C-1 carbons are compared. This means that in the molecular structure shown in Fig. 1 $x = 1$ and $y = 0.3$.

The oxidation was performed as follows. The galactomannan (1 g polysaccharide or $\sim 6.8 \times 10^{-3}$ monomol. expressed in monosaccharide M and G units) was dissolved in 1 l of distilled water. TEMPO (0.020 g or 0.13 mmol) and NaBr (0.1 g or 0.95 mmol) were added and the solution on stirring was cooled in an ice bath (3 ± 1°C). A 15% sodium hypochlorite solution (6 ml or 12.5 mmol) at pH adjusted to 9.3 with 2 M HCl solution and cooled (3 ± 1°C) was mixed with the polymer solution; the pH was maintained at 9.3 by addition of a 0.05 M aqueous NaOH solution for 4 h. After the reaction, sodium borohydride (150 mg or 3.9 mmol) was added and the solution was stirred for 45 min. Then the pH of the mixture was adjusted to 8 by addition of HCl before precipitation by 2 volumes of EtOH in presence of NaCl (up to 10 g/l); the polymer was isolated by centrifugation, washed several times with EtOH, filtered and dried (recovery 92.6%). These conditions concern the higher degree of oxidation obtained (sample 1, Table 1). Different degrees of oxidation were obtained by varying the time of reaction.

The yield of carboxylation is followed by the consumption of NaOH during the reaction. It was controlled after isolation of the polymer by potentiometric titration. It is expressed by the ionic capacity in eq./g of the derivative or the average degree of substitution (DS) of the galactose and free mannose units.

To graft alkyl chain, a primary amine with a C_{12} chain is introduced as the reagent under the following conditions. Firstly, oxidation is performed as previously described. The degree of carboxylation was chosen from the experimental curve established for oxidation and discussed later. We fixed the conditions corresponding to degrees of oxidation of the primary alcohol function (in free mannose and galactose units; see Fig. 1) equal to 0.15 and 0.55 for the two experiments performed. Then, dodecylamine (0.56 mmol) and sodium cyanoborohydride ($NaCNBH_3$, 0.34 g or 5 mmol) were added. The temperature was adjusted to 8 ± 1°C; after 24 h, the pH decreased to 8.7 and 6.8, respectively, for DS = 0.15 and 0.55 (Table 2). $NaBH_4$ (150 mg) was added after alkylation and the solution was stirred for 60 min at 25°C. Then, the pH was adjusted to 8 and the modified polymer was precipitated with two volumes of EtOH in the presence of NaCl (up to 10 g/l), centrifugated, washed with ethanol and dried.

The degree of alkylation τ (average fraction of alkyl chain per galactose or free mannose unit) is determined by ^{1}H NMR using the H-1 signals as internal reference at 30°C to prevent hydrophobic interactions (Table 3).

The viscosity of diluted solutions at 25 ± 0.1°C was determined with a low shear rate viscometer from Contraves (LS 30) allowing extrapolation to zero shear rate.

The potentiometric titrations were performed with a Tacussel Minisis 6000 pH meter equipped with a Tacussel TG100 glass electrode after, the solution of polymer was transformed to the carboxylic acidic form by passage through an ion exchanger Dowex 50X8, H^+ form.

NMR spectra were obtained on a Bruker AC-300 spectrometer. The solution of polymers were prepared

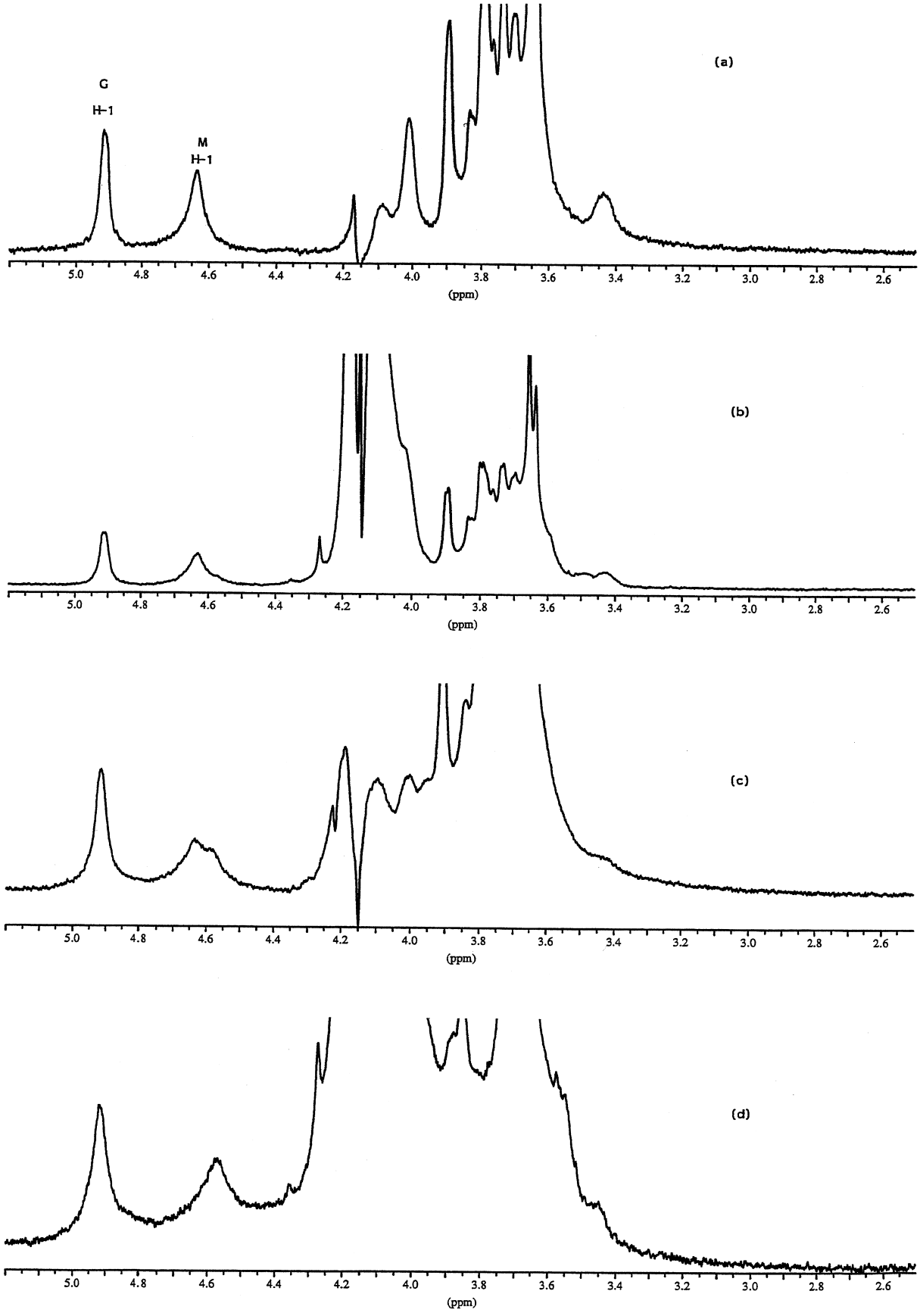

Fig. 2. ^{1}H NMR spectra for (a) native galactomannan; (b) oxidized GM-sample 2; (c) oxidized GM-sample 3; and (d) oxidized GM-sample 1.

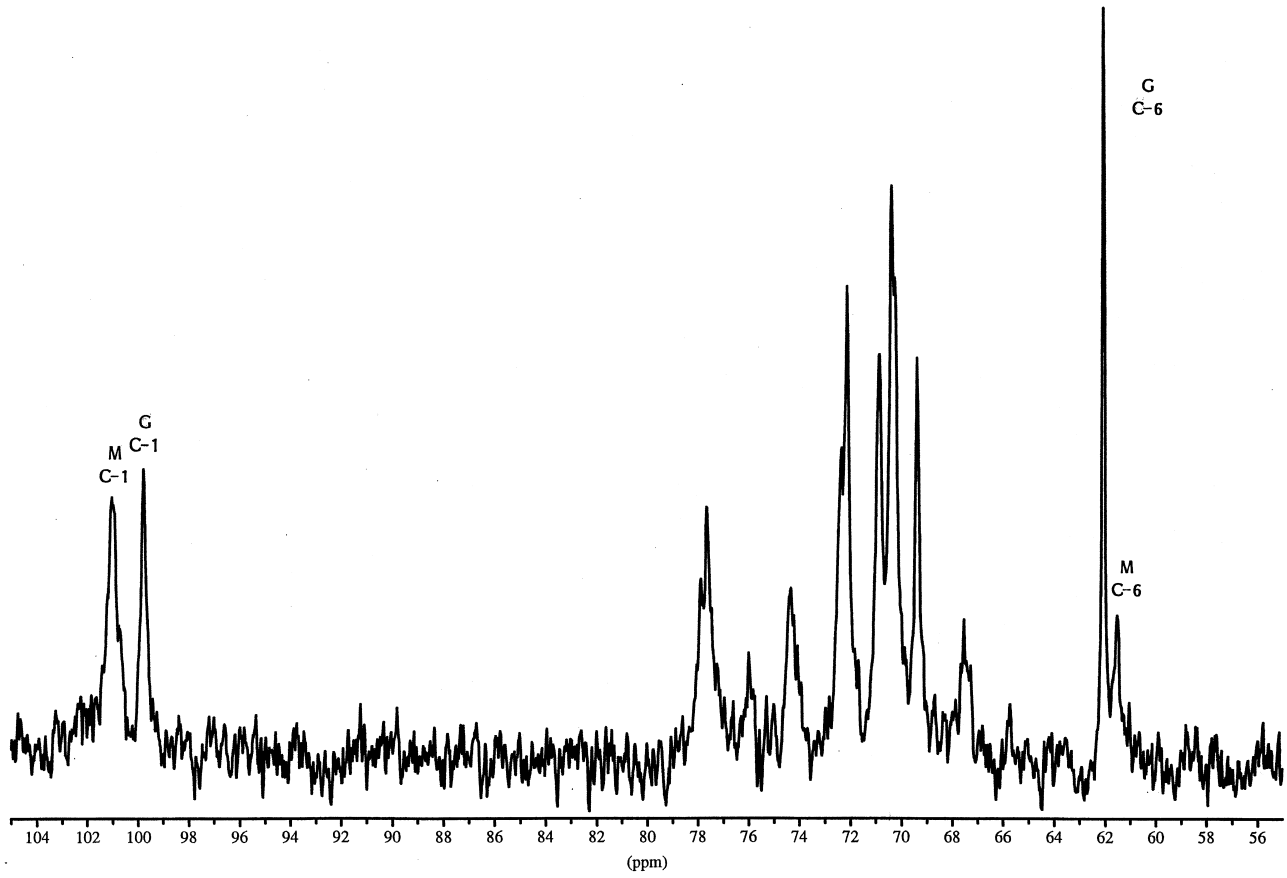

Fig. 3. ^{13}C NMR spectrum for the native galactomannan allowing us to determine the M/G ratio and the fraction of free mannose (from MC-6 signal).

Table 1
Conditions for galactomannan oxidation

Samples	Time (min)	Equivalents of OH^- consumed per gram of GM in reaction ($\times 10^3$)	Equivalents of OH^- consumed per gram of carboxylated GM by titration ($\times 10^3$)	DS[a]
1	240	3.43	2.0	0.66
2	25	1.03	0.8	0.25
3	55	1.64	1.2	0.38

[a] Calculated from consumed OH^- determined by titration. DS is expressed as the average fraction of modified free mannose and galactose units on the basis of M/G = 1.3.

Table 2
Conditions for alkylation of oxidized galactomannans (C_p = 1 g/l)

Samples	DS[a]	TEMPO (mg/l)	pH oxidation	Amine (mmol/l)	pH at end of amination
4	0.15	40	9.3	0.565	8.7
5	0.55	20	9.3	0.565	6.8

[a] Related to equivalents of OH^- consumed by reaction (meq./g) (assuming M/G = 1.3). This value is overestimated.

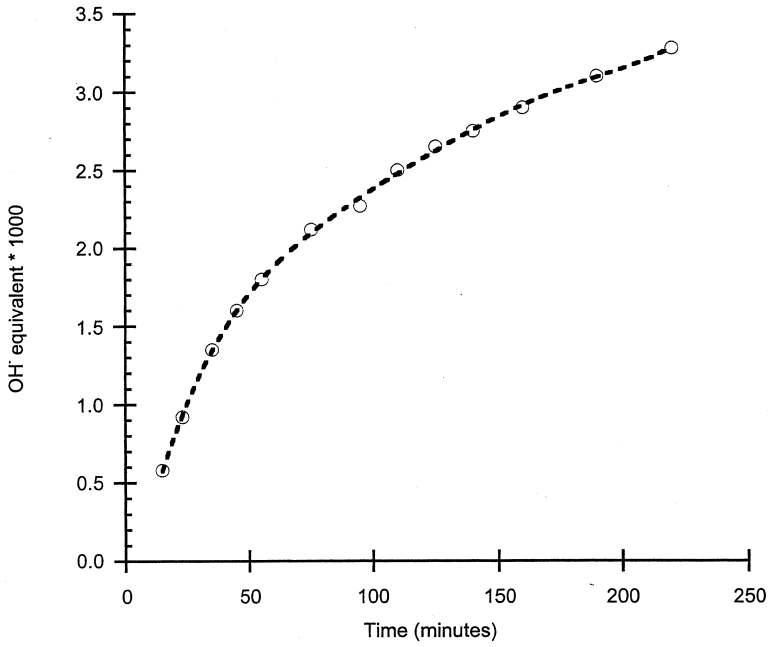

Fig. 4. Kinetics of oxidation of the galactomannan by TEMPO. Amount of OH^- consumption as a function of time (expressed in minutes).

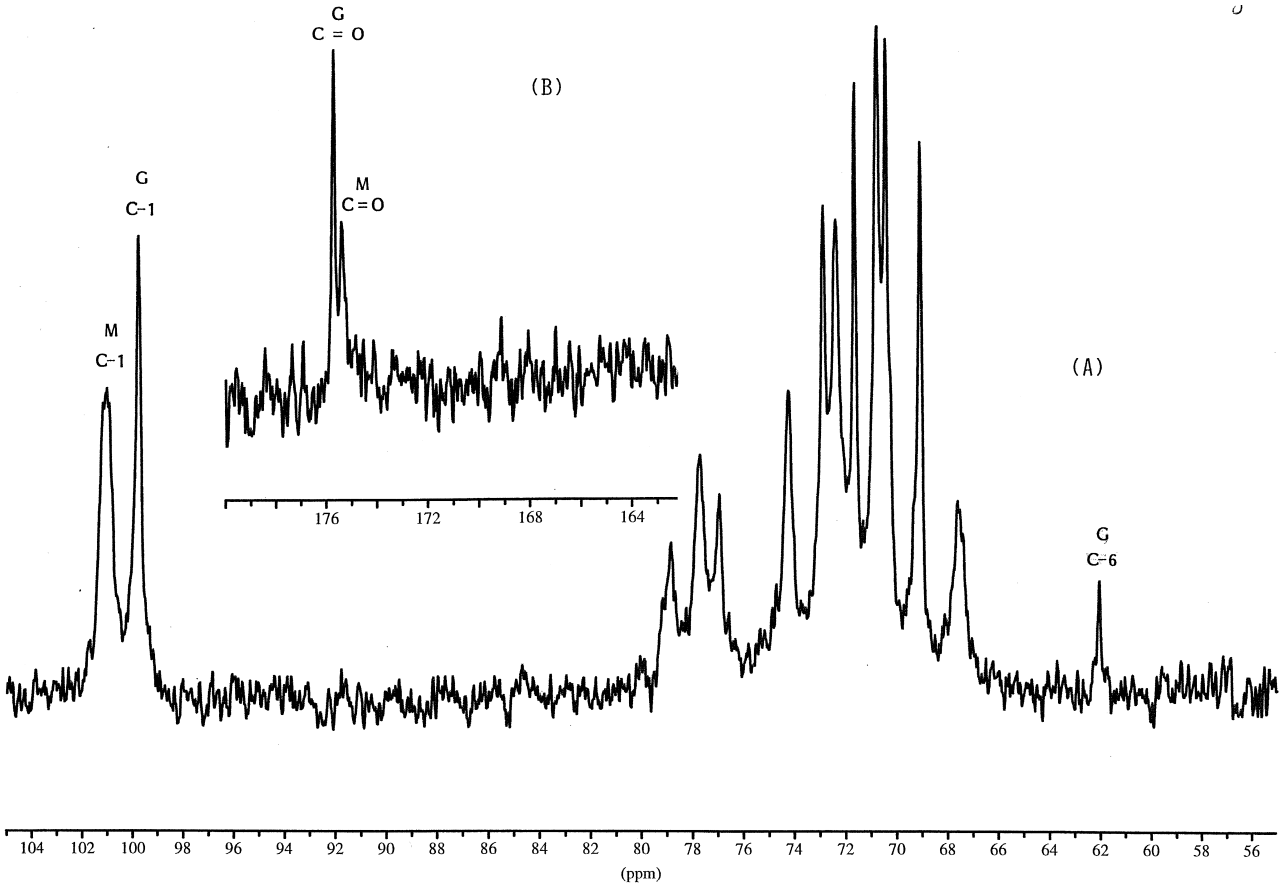

Fig. 5. ^{13}C NMR spectrum of oxidized galactomannan (sample 1, DS = 0.66) allowing us to determine the residual unreacted C-6 positions and showing the two C=O formed: (a) partial attribution of C signals showing residual free C-6 of galactose units; and (b) partial spectrum of the C=O indicating the carboxylation. Polymer concentration 15 g/l in D_2O at 75°C.

Table 3
Characterization of alkylated oxidized galactomannans

Samples	τ[a] from NMR	[η] (ml/g)	
		0.01 M NaCl	0.1 M NaCl
4	< 0.01	–	1260
5	0.06	3100	840

[a] τ = average degree of alkylation determined per modified free mannose and galactose units on the basis of M/G = 1.3.

Table 4
Characterization of oxidized galactomannans (native [η] = 1600 ml/g in H_2O and 0.1 M NaCl)

Samples	[η] (ml/g) (NaCl 0.1 M)	M_W ($\times 10^{-6}$)
1	1 060	1.33
2	800	1.12
3	820	0.8

at 10 and 15 g/l in D_2O respectively for ^1H and ^{13}C experiments.

3. Results and discussion

3.1. Carboxylation of galactomannan

The reaction was performed under the conditions given in Section 2 and Table 1. During the reaction, the quantity of NaOH consumed (to maintain the pH) allows the yield of carboxylation (expressed in millie-quivalent of carboxylic groups per gram of modified polymer) to be determined as a function of the time of reaction. The total degree of carboxylation is also determined by titration of the acidic form of the oxidized sample. The evolution obtained for sample 1 is given in Fig. 4. The characteristics of the different samples are given in Tables 1 and 4.

The ^{13}C NMR spectrum obtained for sample 1 having the larger degree of substitution is given in Fig. 5. From comparison with the spectrum obtained for the native galactomannan (Fig. 3), it is clear that the signal of the C-6 carbon of free mannose units disappears (which means a complete substitution), but that few free galactose units are left; quantitative esti-mation from the integral of the corresponding signal compared with that of the C-1 of the total galactose units gives a fraction of 0.15 unmodified units. From this estimation, it is apparent that the DS equals 0.88

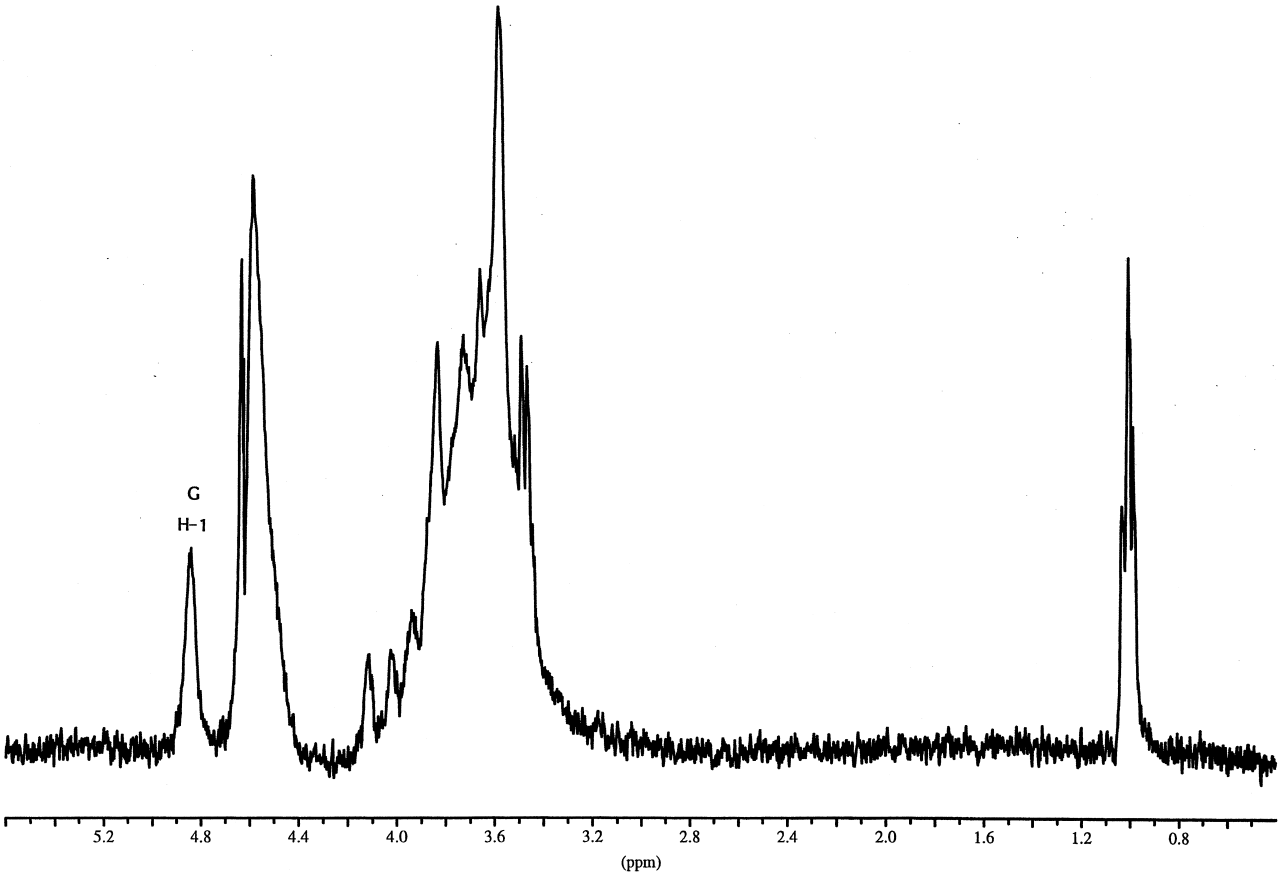

Fig. 6. ^1H NMR spectrum of alkylated oxidized galactomannan (sample 5, $\tau = 0.06$) allowing us to quantify the content of alkylated chains. Polymer concentration 10 g/l in D_2O at 30°C.

fraction of free C-6 which is in relatively good agreement with the previous characteristics (Table 1). In addition, from the analysis of the signals corresponding to the C=O carbons (Fig. 5), it is also clear that oxidation occurs on the two different units (free mannose and galactose) with a larger reactivity of the mannose units. Fig. 2 shows the evolution of the ^{1}H NMR partial spectrum (for the hydrogens H-1) for the derivatives with different degrees of oxidation. The H-1 of the mannose units is perturbed by the oxidation. The signal is displaced by 0.06 ppm from 4.63 ppm on the native sample (Fig. 2a) to 4.57 ppm for the larger degree of oxidation (Fig. 2d).

The weight average molecular weights obtained by GPC and the intrinsic viscosities in 0.1 M NaCl, used to screen the electrostatic interactions, demonstrate a partial degradation when these results are compared with the native polymer ($[\eta] = 1600$ ml/g in water or in NaCl 0.1 M). The data given in Table 4 show that this degradation remains relatively moderate.

3.2. Alkylation of carboxylated galactomannan

The reaction was performed in two successive steps from the native galactomannan without isolation of the polymer between the two steps; the formation of aldehydic groups is not only needed to get carboxylation by oxidation and then to increase the solubility of galactomannan but also to add the hydrophobic character by reductive amination in the presence of an amine having a long alkyl chain (C_{12}):

$$R\text{–}CH_2OH \rightarrow R\text{–}CHO$$

$$(1 - x)R\text{–}CHO \rightarrow (1 - x)R\text{–}CO_2H$$

$$xR\text{–}CHO + xR'\text{–}NH_2 \rightarrow xR\text{–}CH_2\text{–}NH\text{–}R'$$

Different reactions of alkylation were performed and quantified by ^{1}H NMR (Fig. 6). The data concerning the experimental conditions are given in Table 2. From this, it is clear that in the case of sample 4, no significant alkylation occurred; this suggested a competition between carboxylation and reductive amination in relation at the low degree of oxidation chosen. The characteristics of the modified polysaccharides are given in Table 3. It is also shown that the intrinsic viscosities in 0.1 M NaCl are in the same range as for the previous derivatives (Table 4). For lower ionic strength, the intrinsic viscosity is much larger and also larger than that of the native polymer. This is an indication of a larger expansion of the chain due to residual electrostatic repulsions.

4. Conclusion

The conversion of *L. leucocephala* galactomannan in a polyelectrolyte was performed and characterization of derivatives were presented using NMR spectroscopy and viscosity. The C-6 carboxylation is demonstrated to be more effective on mannopyranose units than on the galactose units. To obtain an amphiphilic polymer, we observed that the aldehydic groups, as an intermediate product of the oxidation reaction, constitutes a good basis for alkylation.

Acknowledgements

The authors thank CNRS and CNPq which support this cooperative project.

References

Frollini, E., Reed, W. F., Milas, M., & Rinaudo, M. (1995). *Carbohydrate Polymers, 27*, 129–135.

Ganter, J., Milas, M., & Rinaudo, M. (1992). *Carbohydrate Polymers, 17*, 171–175.

Kapoor, V. P., Milas, M., Taravel, F. R., & Rinaudo, M. (1994). *Carbohydrate Polymers, 25*, 79–84.

Kapoor, V. P., Milas, M., Taravel, F. R., & Rinaudo, M. (1996). *Food Hydrocolloids, 10*, 167–172.

Kapoor, V. P., Taravel, F. R., Joseleau, J. P., Milas, M., Chanzy, H., & Rinaudo, M. (1998). *Carbohydrate Research, 306*, 231–241.

Muzzarelli, R. A. A., Muzzarelli, C., Cosani, A. & Terbojevich, M. (1999). *Carbohydrate Polymers*, (accepted).

de Nooy, A. E. J, Besemer, A. C., & van Bekkum, H. (1994). *Recl. Trav. Chim. Pays-Bas, 113*, 165–166.

de Nooy, A. E. J., Besemer, A. C., & van Bekkum, H. (1995). *Carbohydrate Research, 269*, 89–98.

de Nooy, A. E. J., Besemer, A. C., van Bekkum, H., van Dijk, J. A. P. P., & Smit, J. A. M. (1996). *Macromolecules, 29*, 6541–6547.

Petkowicz, C. L. O., Milas, M., Mazeau, K., Bresolin, T., Reicher, F., Ganter, J., & Rinaudo, M. (1999). *Food Hydrocolloids, 13*, 263–266.

Rinaudo, M., Roure, I., Milas, M., & Frollini, E. (1998). *Polymer, 39*, 5441–5445.