Carbohydrate Polymers **6** (1986) 493–498

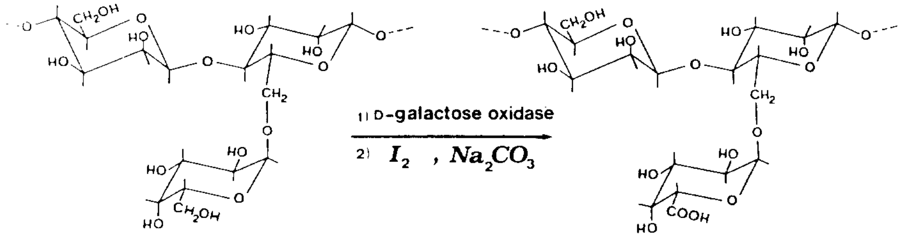
Research Note

On a Polycarboxylate Derived from Guar

Research on new ionic derivatives of industrial polysaccharides with potential interest for practical applications has recently resulted in the preparation of a range of carboxylated polyelectrolytes. For example, starting from scleroglucan ionic derivatives have been prepared by selective oxidation ($NaIO_4/NaClO_2$) of pendant 1,6-β-linked glucopyranose residues (Crescenzi *et al.*, 1983, 1984; Gamini *et al.*, 1984). Similarly, starting from cellulose, amylose and scleroglucan different polyelectrolytes have been obtained by selective oxidation at C(6) in H_3PO_4 (85% w/w)-$NaNO_2$ (Cesaro *et al.*, 1985; Painter *et al.*, 1985).

All these polymeric anionic hydrocolloids exhibit a number of interesting features related to their peculiar solution behaviour and/or gelling ability in aqueous media. One of the derivatives of scleroglucan has been proposed as the main component of a pH-controlled drug delivery system (Alhaique *et al.*, 1985).

We now report the preparation and characterisation of a new carboxylated derivative of guar ('guarox') by enzymic oxidation at C(6) of the galactose side chains, followed by further oxidation with alkaline I_2 (Scheme 1) (Rogers & Thompson, 1968; Hall & Yalpani, 1980).



Scheme 1

Carbohydrate Polymers 0144-8617/86/$03.50 — © Elsevier Applied Science Publishers Ltd, England, 1986. Printed in Great Britain

Guar gum (The Copenhagen Pectin Factory Ltd, Copenhagen) has been purified by precipitation with ethanol from a 0·3% w/v aqueous solution. After washing with ethanol and drying at 40°C under vacuum, the polymer (0·2% w/v in water) was sonicated at *c*. 0°C for 90 min with a 150 W ultrasound source. The solution was filtered through charcoal and then through millipore filters (5 μm and 1·2 μm). One volume of the filtrate was mixed with 0·3 volumes of phosphate buffer (pH 7) containing D-galactose oxidase (EC 1.1.3.g: Sigma product G-3385) (~ 400 Units mg^{-1} of polysaccharide) and peroxidase (EC 1.11.7: Sigma product P-8250) (~ 2×10^5 Units mg^{-1} of polysaccharide). The mixture was left at 20–25°C for 24 h and then added to an I_2 solution (5 g I_2 dm^{-3}) containing KI (~ 1 g dm^{-3}) to reach an I_2/polysaccharide weight ratio of *c*. 3. The pH was raised to 9 by addition of solid Na_2CO_3, and the solution stirred for 4 h at room temperature.

To remove protein, the solution was finally heated to 90°C then cooled to room temperature and filtered through charcoal. The filtrate, made 1 M in NaCl, was extensively dialysed against distilled water and then filtered through 0·45 μm millipore filters. The polymer (guarox:Na^+ salt) was finally recovered by freeze drying.

Analysis of the sample by atomic absorption spectroscopy indicated an equivalent weight of 546 (theoretical weight 522, for 100% oxidation). The ^{13}C-NMR spectrum of guarox is reported in Fig. 1 together with that of the parent polymer (sonicated guar). One salient feature is, as expected, the strong weakening of the C(6) peak of galactose residues (G-6) and the appearance of peaks at around 176 ppm, characteristic of carboxylated groups, in the guarox spectrum.

The carboxylate groups are the chromophores responsible for the circular dichroism band exhibited by guarox in water with a peak at 210 nm (Fig. 2). In fact, the circular dichroism spectrum of β-D-galacturonoside (Na^+ salt) (Morris *et al*., 1975) results in quantitative agreement with that given in Fig. 2.

A summary of additional data is given in Fig. 3. The viscosity plot shown in Fig. 3(a) indicates, by comparison with similar results for other ionic polysaccharides (Smidsrod & Haug, 1971), that guarox chains are rather expanded in dilute aqueous solution with only a moderate contraction in average hydrodynamic size upon increasing the ionic strength from 0·02 to 1·0 mol dm^{-3} (NaCl, 25°C).

Calorimetric data collected in Fig. 3(b) show that in the case of guarox both the enthalpy of protonation and the enthalpy of inter-

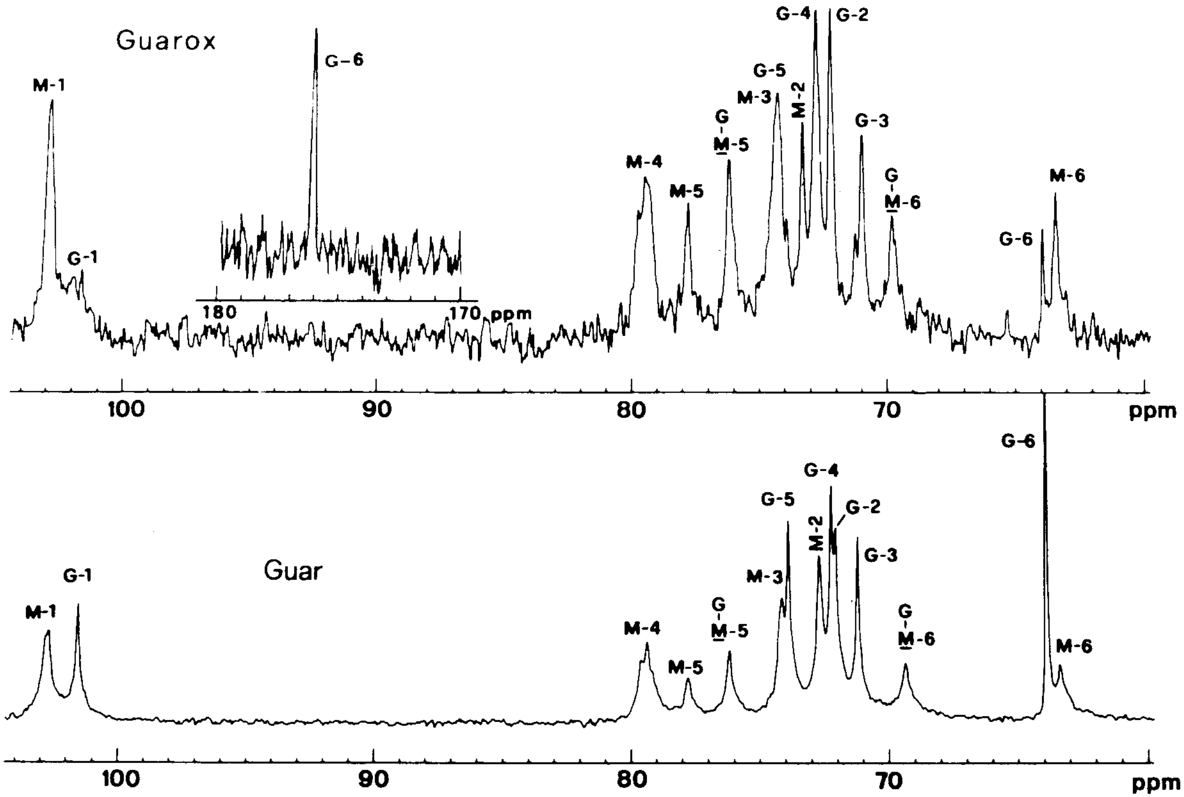


Fig. 1. ^{13}C-NMR spectrum of guarox (upper) and of sonicated guar (lower) in *c.* 5% w/v D_2O solution at 90°C. The experiments were performed in the F-T mode at 50 MHz with a Brucker WP-200 spectrometer. Chemical shifts were measured with respect to external 1,4-dioxane and are referred to a tetramethylsilane (TMS) scale.

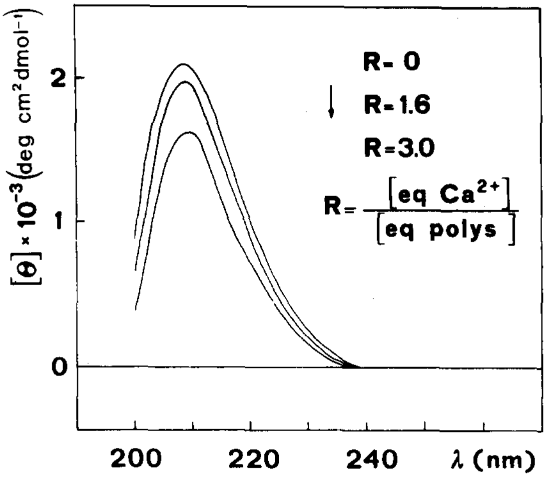


Fig. 2. Circular dichroism spectrum of guarox in water and in the presence of added $Ca(ClO_4)_2$ at 25°C. *R* is the stoichiometric ratio between added Ca^{2+} ions and guarox (fixed) concentrations, both in charge equivalent dm^{-3}. Polymer concentration equals $1{\cdot}8 \times 10^{-3}$ equiv dm^{-3}. A Jasco J-500 A dichrograph was used.

action with added Ca^{2+} ions exhibit regular trends, typical of disordered, random coil polyelectrolytes (Crescenzi, 1985). In addition, Ca^{2+} binding by guarox leads only to a modest change in ellipticity at around 210 nm (Fig. 2), to no change in optical activity at 302 nm (Fig. 3(c)) and to no development in turbidity, even in the presence of excess Ca^{2+} ions.

In conclusion, it appears that aqueous solution properties of guarox are those typical of a rigid chain polycarboxylate with no symptoms of a conformational dependence on parameters such as pH, ionic strength and divalent counterions concentration. This behaviour, rather unusual among artificial and natural carboxylated polysaccharides, may be qualitatively explained as follows.

Guar chains molecularly dispersed in an aqueous environment are quite expanded and stiff mainly because the intrinsically small 'conformational space' available to the β(1,4)-D-mannan backbone is further restricted by the galactose side groups linked β(1,6) to every second mannose residue (on average) along the main chain. Oxidation of C(6) of the galactose side chains, with introduction of $-COO^-$ groups, would only moderately perturb this conformational situation. Evidently, the electrostatic interactions among fixed charges along

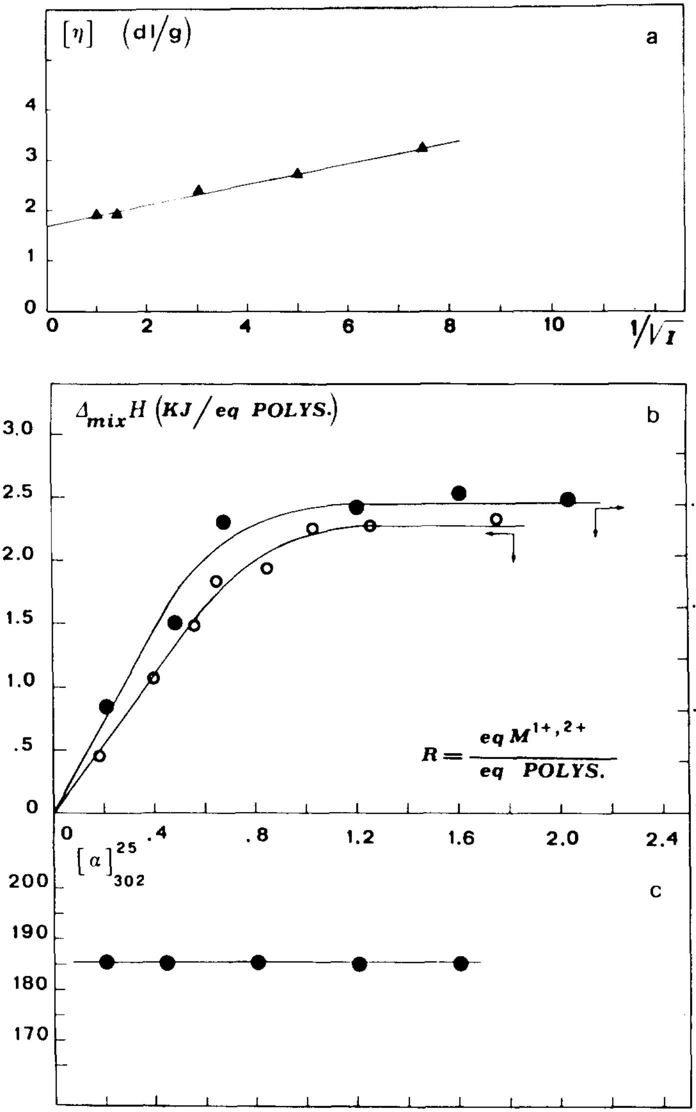


Fig. 3. (a) Dependence of guarox intrinsic viscosity (25°C) on ionic strength, *I* (NaCl). (b) Isothermal calorimetry data (25°C): ○, heat of mixing of guarox (Na^+ form) with HCl dilute aqueous solutions; ●, heat of mixing of guarox (Na^+ form) with $Ca(ClO_4)_2$ dilute aqueous solutions. The enthalpy values (kJ equiv. polysaccharide^{-1}) are corrected for dilution effects. A LKB batch-calorimeter has been used. (c) Optical activity ($\lambda = 302$ nm, 25°C) of guarox in the presence of $Ca(ClO_4)_2$. In (b) and (c) the polymer concentration is 1×10^{-3} equiv. dm^3.

guarox chains do not promote any further expansion of the already quite extended mannan backbone, and do not result in any distinct, conformationally favourable state for the polyanions.

It will be of interest to investigate whether similar behaviour is exhibited also by derivatives of more densely substituted galactomannans (i.e. fenugreek gum) prepared by an oxidation procedure like that reported in this paper for guar gum.

ACKNOWLEDGEMENTS

The authors wish to thank Professor A. L. Segre (Area della Ricerca di Roma, CNR, Italy) for the NMR measurements.

This work has been carried out with the financial support of the Progetto Finalizzato Chimica Fine–CNR, Rome, Italy.

REFERENCES

Alhaique, F., Riccieri, F. M., Santucci, E., Crescenzi, V. & Gamini, A. (1985). *J. Pharm. Pharmacol.* **37**, 310.

Cesaro, A., Delben, F., Painter, T. J. & Paoletti, S. (1985). In *New developments in industrial polysaccharides*, eds V. Crescenzi, I. C. M. Dea, & S. S. Stivala, Gordon & Breach, New York, p. 307.

Crescenzi, V. (1985). *Therm. Acta* **96**, 299.

Crescenzi, V., Gamini, A., Paradossi, G. & Torri, G. (1983). *Carbohydr. Polymers* **3**, 273.

Crescenzi, V., Dentini, M., Meoli, C., Casu, B., Naggi, A. & Torri, G. (1984). *Int. J. Biol. Macromol.* **6**, 142.

Gamini, A., Crescenzi, V. & Abruzzese, R. (1984). *Carbohydr. Polymers* **4**, 461.

Hall, L. D. & Yalpani, M. (1980). *Carbohydr. Res.* **81**, C-10.

Morris, E. R., Rees, D. A., Sanderson, G. R. & Thom, D. (1975). *J. Chem. Soc. (Perkin II)* **13**, 1418.

Painter, T. J., Cesaro, A., Delben, F. & Paoletti, S. (1985). *Carbohydr. Res.* **140**, 61.

Rogers, J. K. & Thompson, N. S. (1968). *Carbohydr. Res.* **7**, 66.

Smidsrod, O. & Haug, A. (1971). *Biopolymers* **10**, 1213.

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(Received: 7 April 1986)