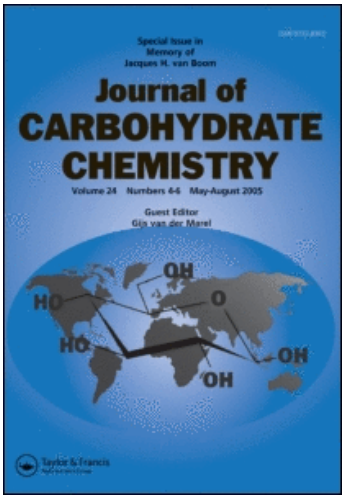


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# THERMAL STABILITY OF SUCCINATE GROUPS IN THE EXOPOLYSACCHARIDE MARGINALAN[1]

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## ABSTRACT

An investigation on the possible effect of temperature on the conformation of the exocellular polysaccharide marginalan, produced by *Pseudomonas marginalis* strain HT041B, in aqueous solutions led to the observation that 30 % of the succinate groups were peeled off from the polymeric backbone when polymeric solutions were heated at 70 °C. This desuccinylation effect was investigated by means of NMR, circular dichroism and viscosity measurements. The experimental findings were compared with those obtained for a sample of chemically desuccinylated marginalan. A possible mechanism for the temperature-induced hydrolysis of succinate groups is discussed.

## INTRODUCTION

Marginalan is an exopolysaccharide produced by *Pseudomonas* marginalis,[2] a microorganism that causes spoilage of stored fruits and vegetables. The polymer is a linear 1→3 linked galactoglucan which contains alternating α-D-galactose and β-D-glucose. The chemical characterisation of marginalan[2,3] demonstrated that two ionic substituents are present on the α-galactose moiety: pyruvyl groups, branching the C-4 and the C-6 position, and succinyl esters at the C-2 position. Both the rheological properties

and the conformational characteristics of marginalan have been investigated and described in a separate paper.[4] The above investigation led to the conclusion that marginalan assumes a disordered conformation in aqueous solution and, in addition to this, a semiflexible behaviour may be assigned to the polymer backbone.

Following the above findings and aiming at a deeper characterisation of the conformational behaviour of marginalan, a study of the effect of the temperature has been carried out. The present paper reports on a peculiar effect of the temperature on the stability of the succinyl esters.

## RESULTS AND DISCUSSION

The temperature dependence of the molar ellipticity measured at 228 nm ($[\theta]_{228}$) of a marginalan solution (0.1 M $NaClO_4$, Cp = 0.92 g/L) is shown in Fig. 1, both in the heating and in the cooling mode. In the heating mode, the molar ellipticity exhibits a linear character as a function of the temperature, further confirming the absence of any conformational transition of the order-to-disorder type. However, the most peculiar aspect of the above findings was the large difference between the $[\theta]$ values measured for the same temperature during the heating process and during the cooling one.

In particular, on increasing the temperature, the magnitude of the molar ellipticity decreased and a further decrease, but to a minor extent, was obtained during cooling. A possible explanation of the chiro-optical behaviour is a progressive loss of chromophoric groups from the polymer backbone due to hydrolysis caused by the experimental conditions. Data showing labile succinyl groups have been already reported in the literature.[5,6] However, no detailed description of the phenomenon has been given.

To better characterise the behaviour observed, the time dependence of the molar ellipticity of marginalan (0.1 M $NaClO_4$, Cp = 0.92 g/L) at a fixed temperature of 70 °C was investigated. As it is shown in Fig. 2, the absolute value of the molar ellipticity progressively decreased with increasing time, and exhibited a faster decrease in the first two hours. It is important to note that, after the first 8 hours at 70 °C, the measurement was stopped and the system was kept overnight at 25 °C. The following day, optical measurements were continued after restoring the 70 °C temperature conditions. As can

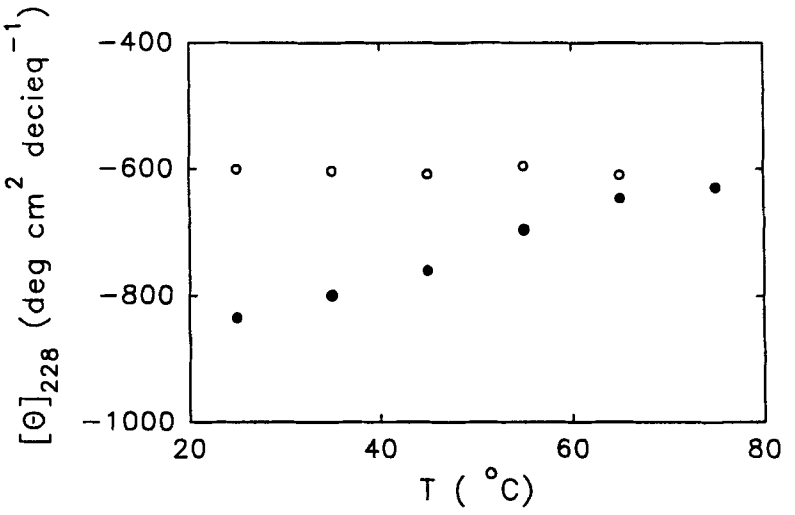



**Figure 1.** Temperature dependence of the molar ellipticity at 228 nm of marginalan in 0.1 M $NaClO_4$, Cp = 0.92 g/L. (•) heating; (o) cooling.



be seen from Fig. 2, the values of the molar ellipticity before and after the overnight interruption were very much the same demonstrating the irreversibility of the process observed and further confirming the "loss-of-chromophore" hypothesis.

In order to verify this hypothesis, $^1H$ NMR spectra of both native and thermally treated (16 h at 70 °C) marginalan samples were collected. The ppm-range relative to the succinate methylenes and the pyruvate methyl groups is shown in Fig.3. As already noted in a previous work describing the full NMR characterisation of marginalan,[3] the native polymer exhibits a complex resonance pattern for the succinate methylenes. Both broad peaks were assigned to the two magnetically different methylene groups and the sharp signal which occurred at the lowest ppm side of the cluster, was due to some free succinate groups which were hydrolyzed during the spectrum collection. In fact, the $^1H$ NMR experiment was carried out at 80 °C due to the high viscosity of the solutions. The integration ratio between the pyruvate and the succinate resonances was found to be 1:0.77 (mole: mole), according to the previous findings.[3]

The $^1H$ NMR spectrum of the thermally treated sample showed a simpler pattern where only one resonance peak was present for the succinate group. The superimposition

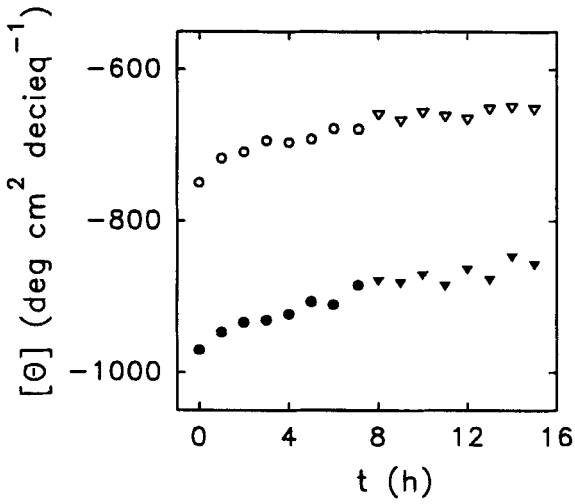



**Figure 2.** Time dependence of the molar ellipticity (70 °C) at 220 nm (full symbols) and 228 nm (empty symbols) of marginalan in 0.1 M $NaClO_4$, Cp = 0.92 g/L. (o,•) before keeping the solution at 25 °C overnight; (▽,▼) after keeping the solution at 25 °C overnight.



of the two resonances of the methylene groups was due to the pH[3] which was not exactly equal to that of the native marginalan solution. In addition to this, no free succinate was detected due to the extensive dialysis carried out after the thermal treatment. The peak integration ratio between the pyruvate and the succinate resonances was found to be 1:0.51 (mole: mole) so that 34 % of the succinate groups were removed by thermal treatment.

In order to further confirm the above findings, marginalan was desuccinylated and the optical measurements were repeated. Fig. 4 shows the variation of the molar ellipticity as a function of the temperature for a desuccinylated marginalan. The amount of hysteresis was very low, if any. In addition to this, the time course of the variation of the molar ellipticity at 70 °C (Fig. 5) was measured also for desuccinylated marginalan, using the same experimental procedures as those used for the native polymer. This experiment also led to the conclusion that the splitting of the succinyl esters can account for the temperature-induced decrease of the molar ellipticity of marginalan.

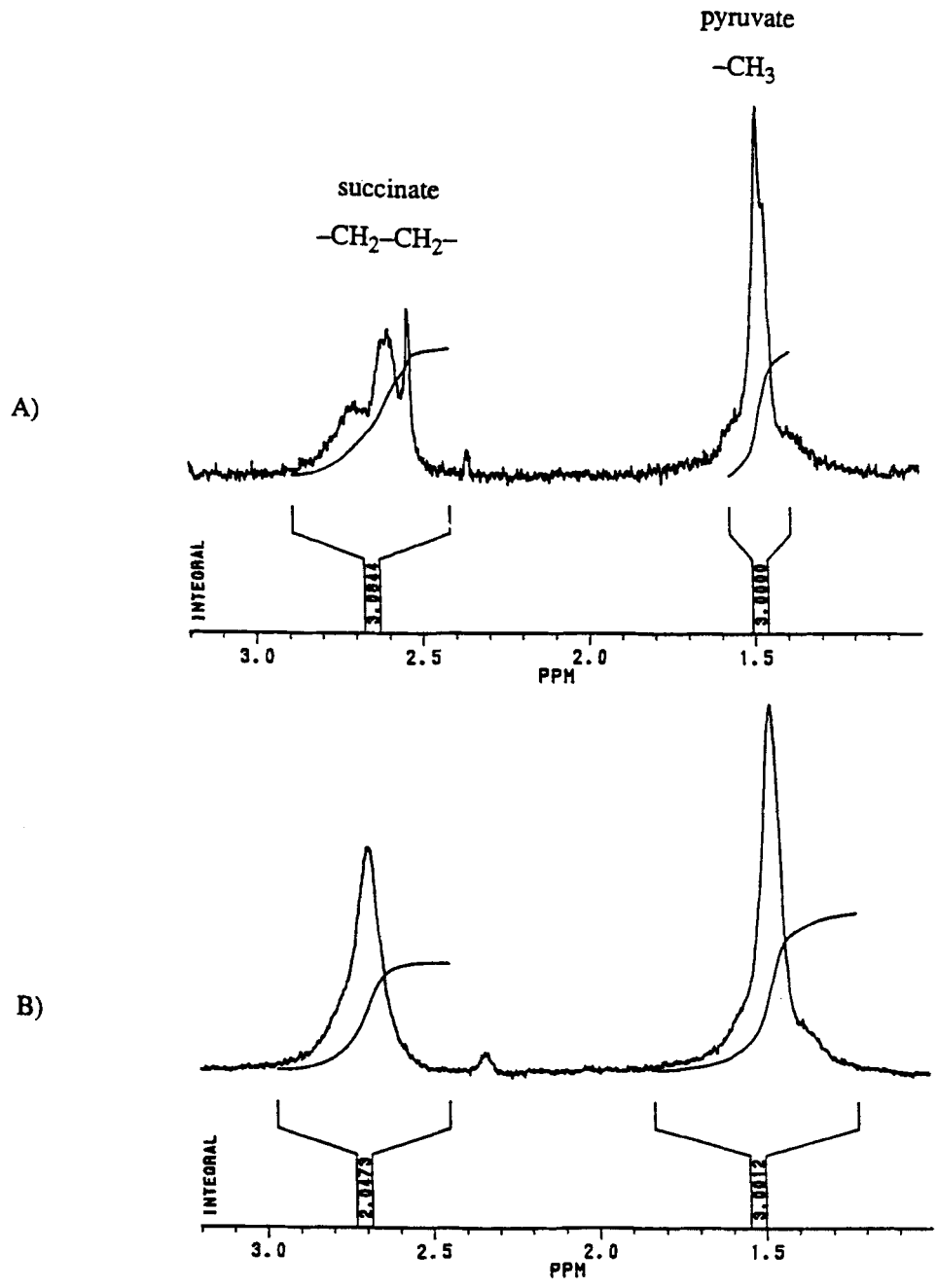



**Figure 3.** Succinate and pyruvate $^1H$ NMR spectra of: A) native marginalan and B) marginalan after the thermal treatment (16 h, 70 °C). Spectra were recorded at 80 °C; the integration figures are reported normalised for the protons of the pyruvate methyl group.

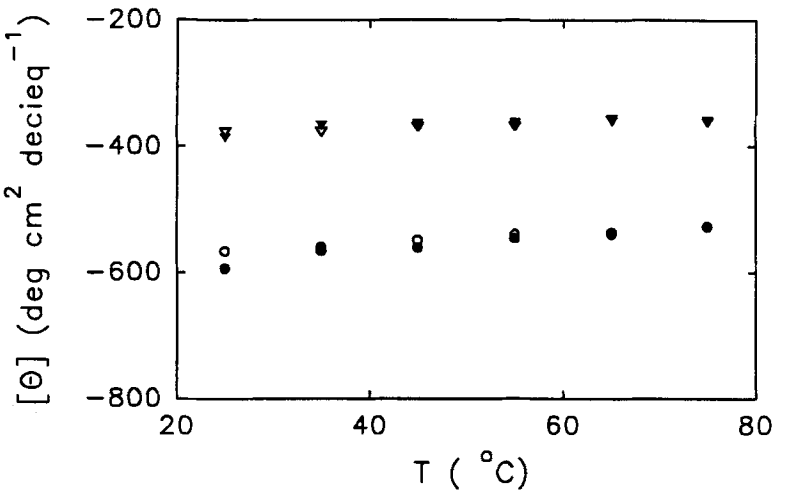



**Figure 4.** Temperature dependence of the molar ellipticity at 228 nm [(•) heating; (○) cooling] and at 236 nm [(▾) heating; (▽) cooling] of desuccinylated marginalan in 0.1 M $NaClO_4$, Cp = 0.92 g/L.

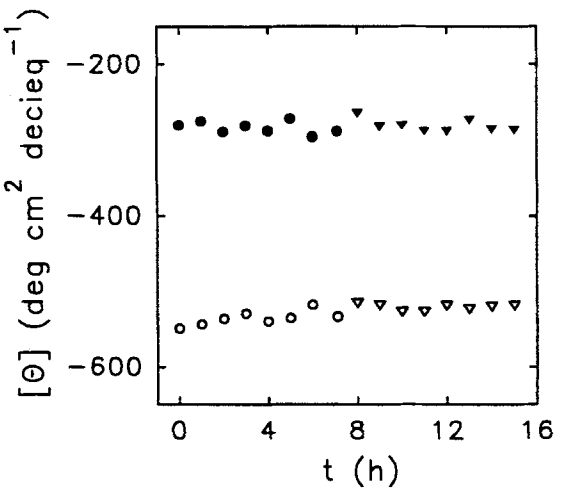



**Figure 5.** Time dependence of the molar ellipticity (70 °C) at 220 nm (full symbols) and 228 nm (empty symbols) of desuccinylated marginalan in 0.1 M $NaClO_4$, Cp = 0.92 g/L. (•,○) before keeping the solution at 25 °C overnight; (▾,▽) after keeping the solution at 25 °C overnight.

The loss of the charged succinyl substituents significantly lowers the negative charge-density of the polymer so that a decrease of the viscosity of the system was expected. In addition to this, the succinate released slightly increased the ionic strength of the solution giving rise to a further decrease of the viscosity.

The reduced specific viscosity measurements as a function of the temperature for native marginalan are shown in Fig. 6, where two sets of data are reported at ionic strength equal to 0.1 mol/L and 0.005 mol/L. The data represented in Fig. 6 demonstrated two points. First, following a typical polyelectrolyte behaviour, the higher the ionic strength the lower the specific viscosity of the system. Second, upon heating and cooling the reduced specific viscosity decreased and the decrease was greater at the lower ionic strength used. The trend of the viscosity, as a function of both temperature and ionic strength, further confirmed the hypothesis of the temperature-induced splitting of succinyl esters. It has to be said, however, that some decrease of the molecular weight of the polymer cannot be excluded, especially at the highest temperature used.



It is reasonable that the desuccinylation reaction proceeds by a simple hydrolysis mechanisms (Scheme) and, based upon some preliminary molecular modelling studies,[7] possibly involving formation of a cyclic transition state with the carboxyl group in the protonated form.

In fact, potentiometric titrations carried out on the native polysaccharide showed that at the pH value measured during the thermal treatment (pH = 5.8) more than 60 % of the succinyl acidic groups were in the undissociated form.

## EXPERIMENTAL

The marginalan sample was kindly supplied by Dr. S.F. Osman, U.S. Department of Agriculture, Philadelphia, Pennsylvania, U.S.A..

Marginalan, utilised for the CD measurements, was purified by enzymatic treatment with DNase (from bovine pancreas, Sigma) and protease (pronase E type XXV, Sigma). DNase was added to a polysaccharide solution ($C_p$ = 1 g/L) in 50 mM Tris - HCl buffer (pH = 7.5) to a final concentration of 33 μg/mL and incubated for 7 h at 37 °C. Protease was then added to a final concentration of 67 μg/mL and the solution kept at 37

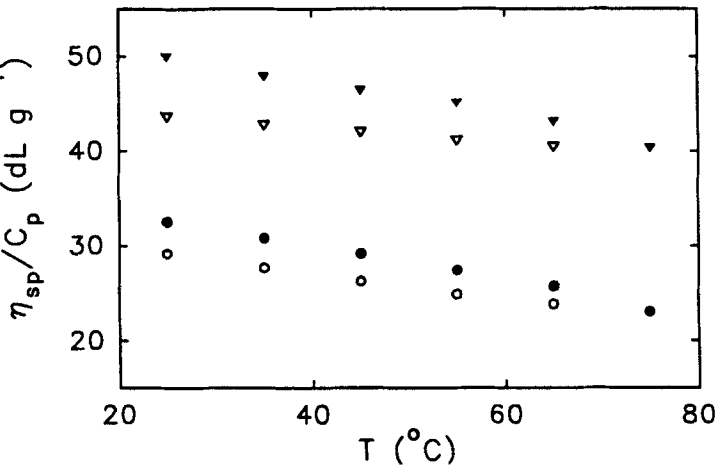
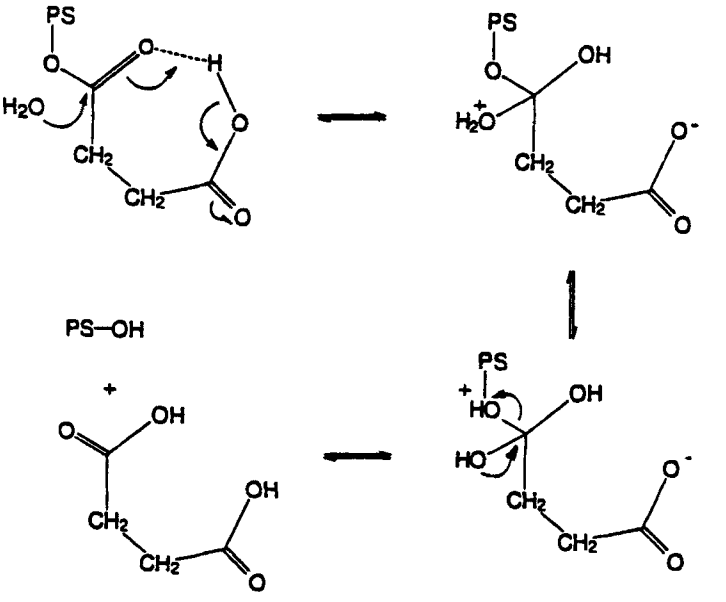



**Figure 6.** Temperature dependence of the reduced specific viscosity of marginalan in 0.1 M NaCl (Cp = 0.28 g/L, • and ○) and in 0.005 M NaCl (Cp = 0.14 g/L, ▼ and ▽). Full symbols refer to measurements carried out in the heating mode; empty symbols refer to measurements carried out in the cooling mode.






Proposed mechanism for the desuccinylation reaction.

**Scheme**

°C with stirring overnight. After dialysis against Milli-Q water, the solution was filtered (1.2 μm - Millipore filter) and the purified sample was recovered by freeze-drying.

Desuccinylated marginalan was prepared by adding 0.1 M NaOH to an aqueous solution of the purified sample ($C_p$ = 2 g/L) to give a final pH of 12.5. The succinate ester hydrolysis was allowed to proceed for 2.5 h with stirring at room temperature. After neutralisation by addition of HCl and dialysis against Milli-Q water, the desuccinylated polymer was recovered by freeze-drying.

Solutions were prepared by dissolving known weights of polysaccharide in the proper buffer solutions by stirring for at least 3 h at room temperature. Concentrations were corrected for the water content of the lyophilised samples (Karl-Fischer titration).

Capillary viscometry measurements were carried out using a Schott-Geräte automatic viscometer equipped with an Ubbelohde capillary (0.46 mm) immersed in a Lauda thermostat bath and with an AVS 440 measuring system.

The flow times $t_0$ and t were measured on the solvent (filtered through a 0.45 μm Millipore filter) and on the polymer solution (filtered through a 1.2 μm Millipore filter), respectively.

Reduced specific viscosity, $\eta_{sp}/C_p$, defined as the value of $(t/t_0-1)/C_p$, was calculated in order to determine the temperature dependence of viscosity.

Circular dichroism measurements were carried out with a Jasco J-600 spectropolarimeter. The molar ellipticity, [θ], was calculated on the basis of the CD spectra corrected with the baseline obtained from the measurement on the solvent.

$^1H$ NMR spectra were recorded in $D_2O$ (Cp = 10 g/L) at 80 °C by means of a Bruker AC 200 spectrometer equipped with a 5 mm multinuclear probe.



## ACKNOWLEDGEMENTS

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## REFERENCES

1. Presented at the *XVIIIth International Carbohydrate Symposium*, Milan, Italy, July 21-26, 1996.

2. S.F. Osman and W.F. Fett, *J. Bacteriol.*, **171**, 1760 (1989).
3. M. Matulová, L. Navarini, S.F. Osman and W.F. Fett, *Carbohydr. Res.*, **283**, 195 (1996).
4. A. Abatangelo, R. Gilli, L. Navarini, R. Rizzo, S.F. Osman and W.F. Fett, *J. Carbohydr. Chem.*, see immediately preceding paper.
5. G. Gravanis, M. Milas and M. Rinaudo, *Int. J. Biol. Macromol.*, **12**, 195 (1990).
6. M. Matulová, R. Toffanin, L. Navarini, R. Gilli, S. Paoletti and A. Cesàro, *Carbohydr. Res.*, **265**, 167 (1994).
7. Molecular modelling was carried out by using NMR GRAPH Molecular Simulation Software, Burlington, Massachusetts, 1991.