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Tamarind seed polysaccharide: preparation, characterisation and solution properties of carboxylated, sulphated and alkylaminated derivatives

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A range of derivatives of tamarind seed polysaccharide has been prepared, characterised and selected solution properties examined. Following oxidation of terminal sidechain galactose residues with galactose oxidase, subsequent oxidation and reductive amination have been used to prepare a range of carboxylated and alkylaminated derivatives respectively. Sulphated derivatives have been prepared by reaction with a sulphur trioxide–pyridine complex in dimethylformamide. The nature and extent of substitution have been characterised by potentiometric titration, infrared and ^{1}H and ^{13}C NMR spectroscopy.

From the dependence of intrinsic viscosity on ionic strength (Smidsrød & Haug, 1971), carboxylated and sulphated derivatives are found to have characteristically stiffened backbones as found previously for the native polysaccharide (Gidley *et al.*, 1991). Binding of divalent cations to carboxylated derivatives is shown to be relatively weak, although polymer precipitation was noted in the presence of Pb^{2+}.

Alkylaminated polysaccharides show only a modest decrease in surface and interfacial tension compared with the native polysaccharide, although significant foam formation and stabilisation was found for a nonylaminated sample. Following enzymic depolymerisation, this material showed a marked decrease in surface and interfacial tension suggesting that interfacial activity in alkylaminated tamarind polysaccharide is only apparent under disruptive solution conditions. The results of ^{1}H NMR line width and T_1 measurements before and after depolymerisation suggests that this is due to solution viscosity rather than specific interaction effects.

INTRODUCTION

Seeds of the tamarind tree (*Tamarindus indica*) contain a cell wall storage polysaccharide which is composed of a (1 → 4)-β-D-glucan backbone substituted with sidechains of α-D-xylopyranose and β-D-galactopyranosyl (1 → 2)-α-D-xylopyranose linked (1 → 6) to glucose residues (Gerard, 1980; Glicksman, 1986). Tamarind seed polysaccharide therefore belongs to the xyloglucan family of polysaccharides which are widely distributed in plant cell walls (Hayashi, 1989) as well as being a major energy reserve in a range of seeds such as those of the tamarind tree (Kooiman, 1960; Meier & Reid, 1982). The composition of tamarind seed poly-

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saccharide has recently been shown to be 2·8:2·25:1·0, glucose-xylose:galactose by two independent methods (Gidley *et al.*, 1991) indicating a high degree (*c.* 80%) of backbone substitution. Arabinose residues are frequently reported for tamarind seed polysaccharide preparations but these probably arise from contaminating arabinans (Gidley *et al.*, 1991).

Applications have been described both for crushed tamarind endosperms (often called tamarind kernel powder and containing *c.* 65% polysaccharide) in textiles and other industries (Gerard, 1980) as well as for the purified polysaccharide in foods (Glicksman, 1986). A number of chemical modifications of tamarind seed polysaccharide have also been described including acetyl (Rao & Beri, 1955), hydroxyalkyl (Schiavio & Maderno, 1958; Shimohiro *et al.*, 1985), and carboxymethyl (Omya & Tabuchi, 1985; Prabhanjan, 1989; Shimohiro *et al.*, 1985) derivatives.

In this paper we describe the preparation, characterisation and selected properties of three types of derivatives of tamarind seed polysaccharide which will allow comparisons to be drawn with the properties both of similar derivatives of other polysaccharides (Dentini & Crescenzi, 1986) and with underivatised tamarind seed polysaccharide (Gidley *et al.*, 1991). The derivatisation reactions studied are (i) specific (C-6) oxidation of galactosyl residues to uronic acids (ii) non-specific sulphation and (iii) specific (C-6) alkylamination of galactosyl residues.

EXPERIMENTAL METHODS

General

Tamarind seed polysaccharide was obtained from Dainippon Pharmaceutical Co. Ltd, Osaka, Japan ('Glyloid 3S') and was further purified by dissolution in deionised water, centrifugation, extensive dialysis against deionised water, and lyophilisation. The glucose:xylose:galactose ratio in this material was found to be 2·8:2·25:1·0 with a small contamination (*c.* 2%) of arabinan/galactan species (Gidley *et al.*, 1991). Elemental analysis showed no detectable nitrogen content.

NMR spectra were recorded at 80–90°C using a Bruker AM 200 spectrometer operating at 200·13 MHz for ^1H and 50·32 MHz for ^{13}C observation respectively; chemical shifts are referenced to external TMS (0 ppm) in both cases. ^1H T_1 values were determined by the inversion-recovery pulse sequence (180°-τ-90° acquire). Infrared spectra were recorded using a Hitachi 270-30 Infrared Spectrophotometer. Circular dichroism was monitored using a Jasco J-500A dichrograph. Galactose oxidase (EC 1.1.3.9) and catalase (EC 1.11.1.6) were obtained from Sigma. All other reagents were commercial samples used without further purification.

Preparation and characterisation of carboxylate derivatives

The preparation procedure adopted was based on methods developed for other polysaccharides (Rogers & Thompson, 1968; Hall & Yalpani, 1980; Dentini & Crescenzi, 1986) and involved the enzymic oxidation of galactosyl C-6 hydroxymethyl groups to formyl groups, using galactose oxidase/catalase, which were further oxidised *in situ* to carboxylate groups by adding alkaline I_2/I^-. Experimental conditions, described in detail below, were chosen to minimise the enzyme/substrate ratio necessary for achieving reasonable reaction rates in the viscous reaction medium. All quantities quoted below are for 1 mg of polymer-bound galactose.

To a 0·1% w/v solution of purified tamarind seed polysaccharide is added 0·6 ml of 0·1 M phosphate buffer (pH 7), 1200 Units of catalase in 0·1 M phosphate buffer, and 2·2 Units of galactose oxidase in 0·1 M phosphate buffer. The reaction mixture is kept at 27°C for a period long enough to achieve the desired extent of enzymic reaction (see kinetic data in the Results section), and then 10 mg iodine (aqueous 20 mM I_2 containing KI) and 12 mg sodium carbonate are added. The solution is stirred at room temperature for 4 h, neutralised with 0·1 M HCl, heated rapidly to 95°C (to eliminate most of the iodine and to denature the enzymes), cooled to room temperature and filtered through charcoal at room temperature to remove the last traces of iodine and avoid potential polymer degradation. Excess sodium chloride is added to the resulting clear solution which is then dialysed against frequent changes of distilled water, carefully neutralised and lyophilised. Overall yields are 85–90%. Elemental analysis showed no detectable nitrogen, indicating that protein from the enzyme reaction was not present in significant quantities in the product.

The kinetics of the enzymatic reaction were followed by treating aliquots removed after various reaction times with I_2/I^- to complete oxidation, recovering the products as described above, and then recording their circular dichroism (CD) spectra in the 200–250 nm region (water, 25°C). Samples were also characterised by IR and NMR spectroscopy and by potentiometric titration using the experimental procedure described elsewhere (Dentini & Crescenzi, 1986).

Preparation and characterisation of sulphated derivatives

Sulphated tamarind seed polysaccharide derivatives have been prepared by swelling the polysaccharide in dimethylformamide (DMF) and treating with sulphur trioxide–pyridine complex in DMF at 50°C (Focher *et al.*, 1986). The method detailed below was chosen following experimentation to optimise exposure of the polysaccharide chains to the sulphating reagent and to

minimise chain degradation: it was reasoned that this should lead to high molecular weight products with a reasonably regular distribution of sulphate groups along the chains.

Tamarind seed polysaccharide (0·5 g) is dissolved in distilled water (80 ml) and precipitated in a gelatinous state by addition of ethanol (100 ml) under stirring. The precipitate is collected on a G3 glass filter under gentle suction, and washed on the filter with ethanol and then thoroughly with DMF. The product is added to 50 ml DMF and stirred overnight at room temperature. The finely dispersed suspension is cooled to 2°C and 1·7 g of SO_3-pyridine complex (Merck-Schuchard) in 15 ml DMF is added. The mixture is stirred vigorously for 24 h at 50°C, and then dialysed against 5% sodium hydrogen carbonate for 48 h and then against distilled water, exhaustively. After careful neutralisation with dilute sodium hydroxide, the products are recovered by lyophilisation in overall yields of *c.* 85%.

Products were analysed by potentiometric titration as described above and elsewhere (Dentini & Crescenzi, 1986) in order to determine the degree of sulphation, and by IR and NMR spectroscopy.

Preparation and characterisation of alkylamine derivatives

Following oxidation of galactosyl hydroxymethyl groups to formyl groups with galactose oxidase/catalase as described above for carboxylate derivatives, a reductive amination reaction (Borch *et al.*, 1971; Hall & Yalpani, 1980) can be used to introduce alkylamine substituents on C-6 of galactose residues (Fig. 1). Ethylamino-, octylamino- and nonylamino- derivatives were prepared by addition of a tenfold molar excess of the appropriate alkylamine to the product of galactose oxidase/catalase reaction as described above. An equimolar amount of sodium cyanoborohydride with respect to the aldehyde groups was then added and the mixture kept at room temperature for 96 h. Reaction was carried out in phosphate buffer at pH 6·5 to limit direct reduction of the aldehyde (Borch *et al.*, 1971). As the formation of the Schiff's base (Fig. 1) is probably the rate determining step in the reductive amination procedure, it may be advantageous to allow the amine to react with the aldehyde for 1–2 h prior to adding the reducing agent. Following reaction, products were dialysed exhaustively against distilled water and lyophilised. Yields were *c.* 80%.

Products were analysed by ^{1}H NMR spectroscopy to determine the extent of reaction as described below. The interfacial properties of native polysaccharide and alkylamine derivatives were assessed both by surface and interfacial tension measurements and from foaming and emulsifying activity. Surface tension and interfacial tension (decane/water) were obtained using a DuNouy ring apparatus (Kruss). Emulsification activity was assessed by mixing aqueous solutions

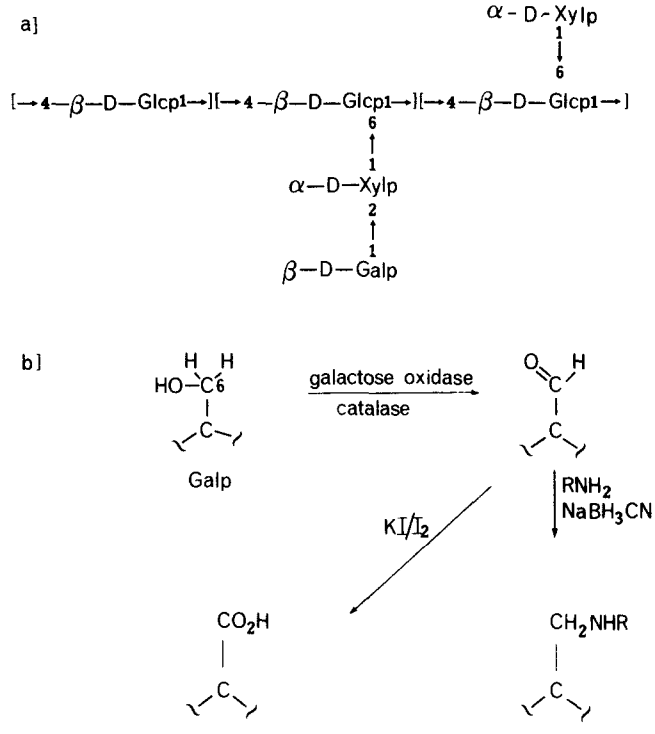

Fig. 1. (a) Structural elements in tamarind seed polysaccharide. (b) Expected mode of action of galactose oxidase on tamarind seed polysaccharide and subsequent oxidation and alkylamination reactions via Schiff's base.

(6 ml) with sunflower oil (4 ml) and homogenising for 1 min at room temperature using an Ultra-Turrax (Janke Kunkel) at full speed. One ml of the resulting mixture was diluted 1:250 and the absorbance at 500 nm used as a measure of emulsification. After standing for 30 min, 1 ml was withdrawn from the bottom of the remaining mixture, diluted 1:250 and the absorbance at 500 nm used to assess the stability of the emulsion. Foaming activity was assessed by vigorously shaking solutions (2 ml) in a 10 ml graduated test tube using a wrist shaker for 5 min. The foam volume was measured following static storage for 10 s, 5 min, 1 h and 20 h after shaking, and the volume of foam expressed as a percentage of liquid volume.

RESULTS AND DISCUSSION

The derivatisation protocols employed were designed to provide site-specific (carboxylate and alkylamine) or non-specific (sulphate) derivatives of tamarind seed polysaccharide. Site selective derivatisation using the reaction of galactose oxidase with polysaccharide galactosyl groups followed by oxidation or reductive amination has previously been demonstrated for the galactomannan family of polysaccharides (Hall & Yalpani, 1980; Dentini & Crescenzi, 1986). The expected reactions for a tamarind seed polysaccharide substrate are shown in Fig. 1. By contrast, non-specific chemical

derivatisation, as exemplified here by sulphation, can potentially lead to substitution at any hydroxyl group in the polysaccharide. In practice, however, it may be expected that steric accessibility could lead to preferred substitution at C-6 hydroxymethyl groups and/or on peripheral sidechain residues or non-glycosylated backbone residues. In the following sections the structures and selected solution properties of each of the three substitution types are discussed.

Carboxylate derivatives 1. Preparation and characterisation

Carboxylated derivatives are formed in two steps (Fig. 1): an initial selective enzymic oxidation with galactose oxidase/catalase followed by I_2/I^- oxidation. The kinetics of the enzymic reaction were studied by submitting aliquots of the reaction mixture, collected after various incubation times, to complete oxidation with I_2/I^-, recovering the products as described above, and then recording their circular dichroism (CD) spectra in the 200–250 nm region (water, 25°). Positive CD bands ($n \rightarrow \pi^*$ transition) are expected in this region for D-galacturonic acid residues (Morris *et al.*, 1975), in contrast to native tamarind seed polysaccharide which shows no CD bands in this region. Kinetic results are shown in Fig. 2 in which the reduced ellipticity at 210 nm (the maximum in the galacturonate CD spectrum see Fig. 3) is plotted against time of enzyme action at 27°C. After about 40 h of reaction, a plateau is reached corresponding (on the basis of potentiometric data reported below) to *c.* 80% oxidation of D-galactose residues in tamarind seed polysaccharide. It is possible that incomplete oxidation may have been due to intramolecular hemiacetal formation between aldehyde groups and flanking hydroxyl groups (Rogers & Thompson, 1968). Following the

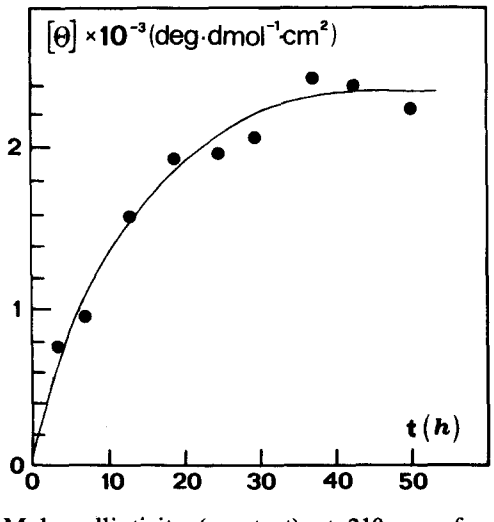

Fig. 2. Molar ellipticity (see text) at 210 nm of partially carboxylated tamarind seed polysaccharide derivatives as a function of enzymic reaction time at 27°C.

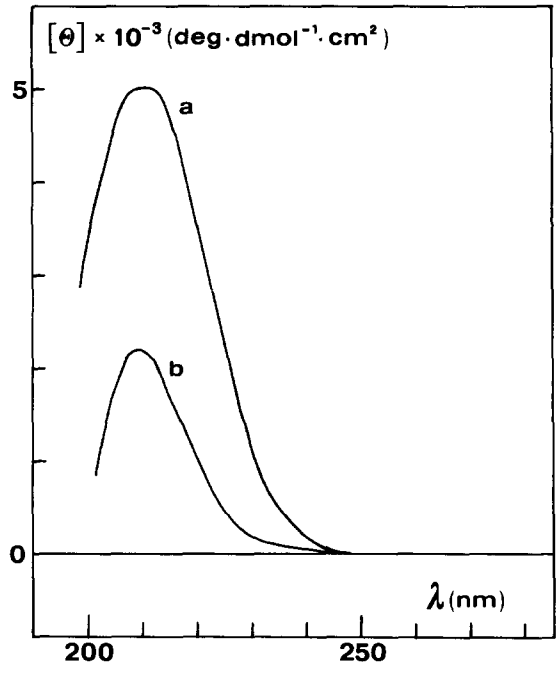

Fig. 3. Circular dichroism spectra of the GS3OX 80 derivative (see text) in water at pH 2 (a) and pH 7 (b).

reaction procedure described above, two (*c.* 1 g) samples of carboxylated tamarind seed polysaccharide have been prepared: these are designated GS3OX 20 and GS3OX 80 respectively.

The IR spectrum of a film of GS3OX 80 (Fig. 4) clearly shows the typical carboxylate C=O stretching band at about 1600 cm^{-1} which is absent in the native polysaccharide. Potentiometric titration data, obtained using an experimental procedure described elsewhere for other polycarboxylated polysaccharides (Dentini & Crescenzi, 1986), yielded the following equivalent weight (EW) and percent of oxidation (%O) i.e. carboxylation values:

	GS3OX 20	GS3OX 80
EW	4100	1150
%O	22	82

The %O figures were calculated from the formula:

$$\%O = (911 + 36 \times \%O)/EW$$

where 911 is the weight of an 'average' repeating unit of tamarind seed polysaccharide containing one D-galactose residue (Gidley *et al.*, 1991) and 36 is the weight difference ($COONa - CH_2OH$). The ^{13}C NMR spectrum of GS3OX 80 is shown in Fig. 5(b) and compared with the native polysaccharide (Fig. 5(a)). A number of changes in relative peak intensity and shift are observed in the C-2–5 region (68–85 ppm): more striking differences are observed in the anomeric (C-1) region (100–110 ppm) and the hydroxymethyl region (60–65 ppm). Thus, four C-1 peaks are observed following oxidation with the relative intensity of the galactose C-1 peak (105·5 ppm) greatly reduced upon

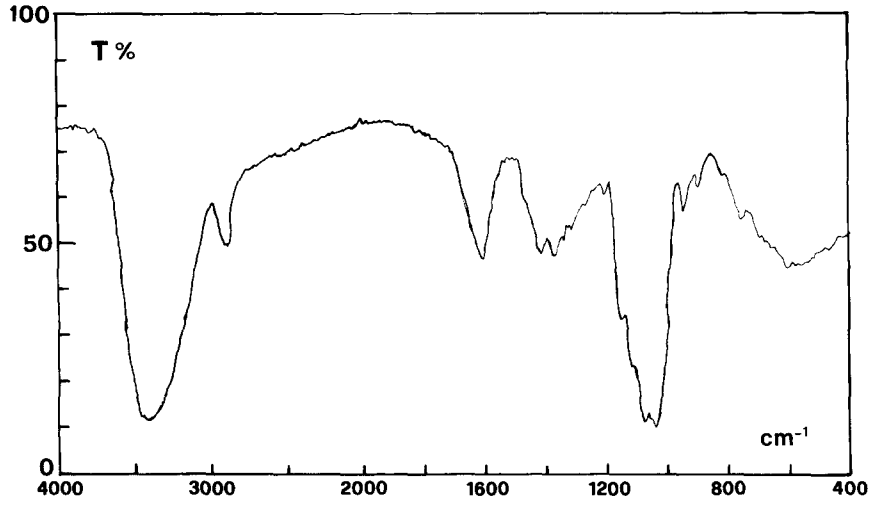

Fig. 4. Infrared film spectrum of the GS3OX 80 derivative.

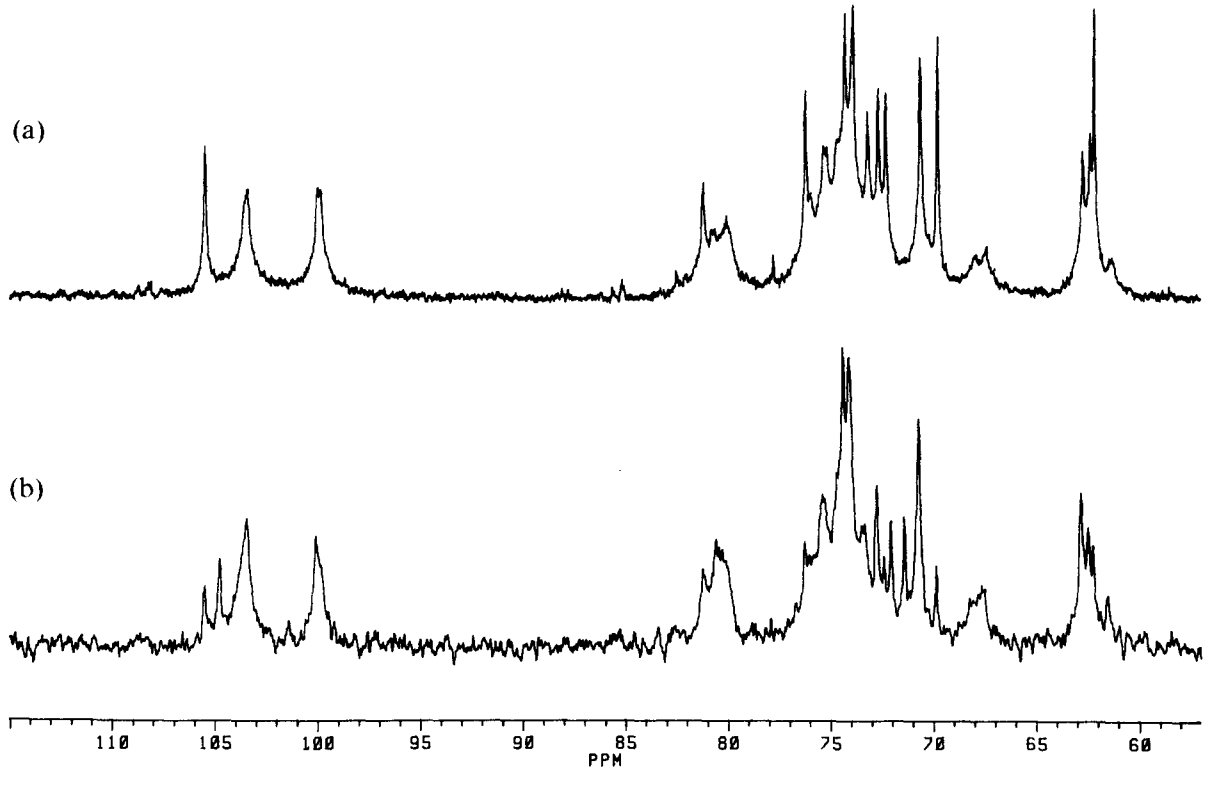

Fig. 5. ^{13}C NMR spectra of solutions (10 mg/ml) of (a) native and (b) GS3OX 80 derivative tamarind polysaccharides.

oxidation compared with the xylose and glucose C-1 peaks (100·0 and 103·4 ppm). The signal at 104·7 which is observed following oxidation is assigned to C-1 of galacturonic acid units: from relative intensities of peaks at 104·7 and 105·5 ppm a percent oxidation value of 70–75% is estimated. This value can only be an estimate as T_1 and n.O.e. (nuclear Overhauser effect) measurements have not been made, but is still in reasonable agreement with the results of potentiometric titration. A major difference is also found in the hydroxymethyl region of the spectrum following oxidation: thus the sharpest signal (62·1 ppm) in the native polysaccharide is much reduced in intensity following oxidation and is assigned to C-6 of galactosyl residues. Under the conditions used for ^{13}C NMR (90° pulse angle, 3·1 s recycle time) the limited signal to noise ratio precluded direct observation of the quaternary carboxyl carbon. Partial depolymerisation may bring the required improvements in signal to noise ratio (Dentini & Crescenzi, 1986). The absence of intermediate aldehyde groups in GS3OX 20 and GS3OX 80 was confirmed by the lack of a diagnostic

aldehyde proton (8–10 ppm) in the ^1H NMR spectrum. The results from CD, IR and potentiometric titration therefore identify the production of carboxylate groups and ^{13}C NMR spectra are completely consistent with the selective oxidation of galactosyl C-6 hydroxymethyl groups as expected (Fig. 1). Information on the molecular weight distribution of carboxylated samples will be published elsewhere. Nevertheless, intrinsic viscosity results (see below) suggest that depolymerisation was not extensive following the derivatisation procedure.

Carboxylate derivatives 2. Selected solution properties

Viscosity and ion-binding measurements have been carried out on both GS3OX 20 and GS3OX 80 in order to assess the influence of two levels of carboxyl derivatisation. A series of viscosity measurements (using Ubbelohde viscometers and an automatic Schotte-Geraete apparatus) in various concentrations of aqueous sodium chloride at 25°C led to the results shown in Fig. 6. It is clear that the specific reduced viscosity and the Huggins constant, K, of the less charged GS3OX 20 sample (1 carboxylate per ~27 sugar residues) are almost insensitive to changes in ionic strength, whereas highly significant effects are observed for the more charged GS3OX 80 sample (1 carboxylate per ~7 sugar residues) as would have been expected. The intrinsic viscosities of these two samples, however, do not differ appreciably in 0·2 M NaCl (370 and 395 ml/g for GS3OX 20 and GS3OX 80 respectively) but are smaller than the intrinsic viscosity of the native polysaccharide (650 ml/g in water, 25°C) indicating that some degradation may have occurred during the oxidation reactions. Alternatively, the observed decrease in intrinsic viscosity may be (partially?) due to an increased stiffness of the chain upon derivatisation causing greater solvent drainage effects (Gidley *et al.*, 1991).

Information on the stiffness of the GS3OX 80 chain can be obtained from the dependence of intrinsic viscosity on ionic strength using the method of Smidsrød & Haug (1971). From the results shown in Fig. 7, a value of the B parameter (Smidsrød & Haug, 1971) of 0·05 is calculated for GS3OX 80. This value is close to those obtained (Smidsrød & Haug, 1971) for carboxylated polysaccharides with characteristically stiffened backbones such as alginate (B = 0·04) and carboxymethyl cellulose (B = 0·065), less than values obtained for carboxylated polysaccharides with more flexible backbones such as carboxymethylamylose (B = 0·20), and very much greater than rigid helices such as DNA (B = 0·0055). The sample GS3OX 80 therefore adopts a relatively stiff (persistent) structure in solution: this finding is confirmed by detailed light scattering observations which will be published elsewhere, and would be predicted based on the relatively high level of chain stiffness in solution found

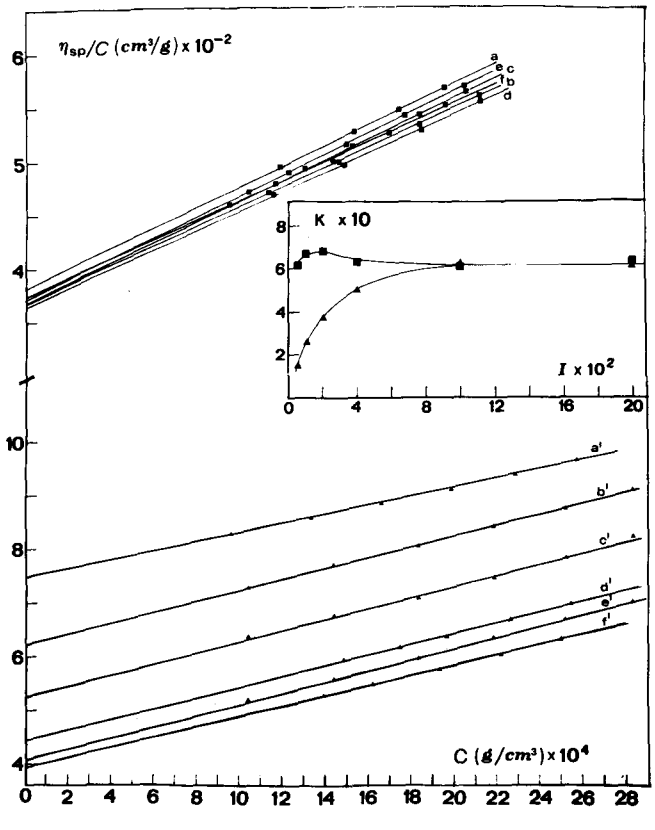

Fig. 6. Specific reduced viscosity of GS3OX 80 (▲) and of GS3OX 20 (■) in aqueous NaCl solutions at 25°C. Concentrations (M × 10^2) of NaCl used were (a) 0·5, (b) 1·0, (c) 2·0, (d) 4·0, (e) 10·0, (f) 20·0.

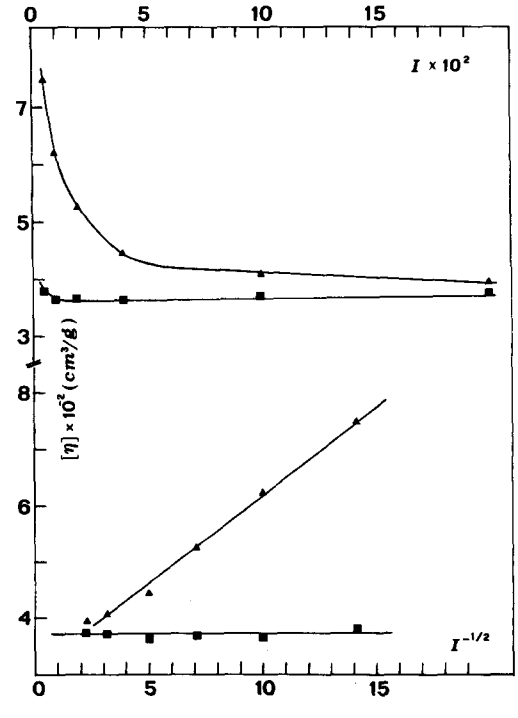

Fig. 7. Ionic strength dependence of the intrinsic viscosity of GS3OX 20 (■) and GS3OX 80 (▲) at 25°C.

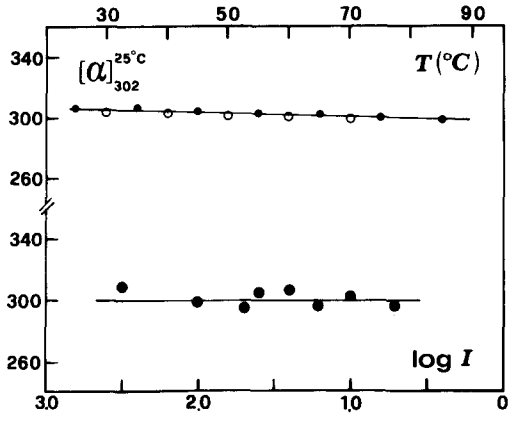

Fig. 8. Ionic strength and temperature dependence of GS3OX 80 optical activity at 302 nm in aqueous solution. Upper trace: heating (●) and cooling (○) in 0·1 M NaCl. Lower trace: constant temperature (25°C) at various NaCl concentrations.

for the native polysaccharide (Gidley *et al.*, 1991).

Optical rotation has been measured at 302 nm, for GS3OX 80 at a range of ionic strengths and a range of temperatures (in 0·1 M NaCl). Results are shown in Fig. 8, from which it can be seen that there is no evidence for any conformational transition in the regimes studied.

By analogy with other carboxylated polysaccharides such as alginate and pectin, it is of interest to study the binding of divalent cations to oxidised tamarind seed polysaccharide. The interactions between GS3OX 80 and Ca^{2+}, Cu^{2+}, Zn^{2+} and Pb^{2+} in dilute solution have been studied by calorimetry and potentiometric titration. In all cases binding capacities and strengths are modest, which is perhaps not surprising considering the low charge density (1 carboxylate per ~7 residues). As an example, Fig. 9 shows a titration of $Ca(ClO_4)_2$

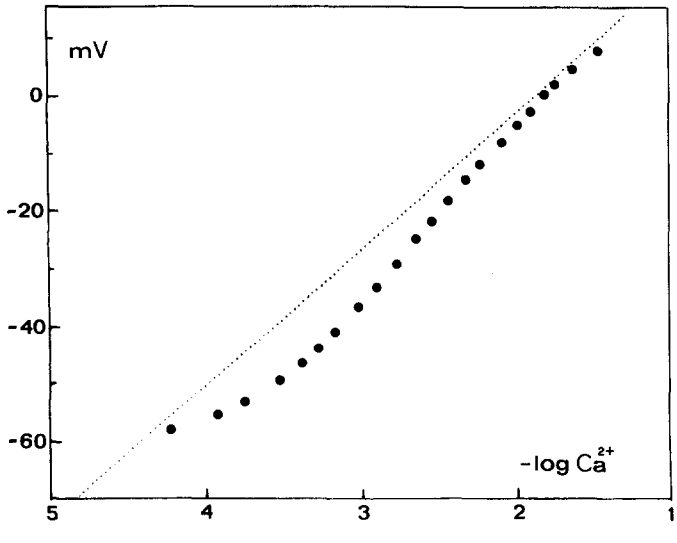

Fig. 9. Emf readings taken with a sodium-electrode (vs calomel) upon addition of aqueous $Ca(ClO_4)_2$ to a solution of GS3OX 80 (1·3% w/v) at 25°C. The dotted line is the calibration plot (additions of $Ca(ClO_4)_2$ to distilled water).

against 1·3% w/v GS3OX 80. From this data an apparent activity coefficient of *c.* 0·45 (Ca^{2+} stoichiometric concentration = 1 mM) is calculated: this is in qualitative agreement with expectations based on a GS3OX 80 'linear charge density' (projected on the backbone) of *c.* 0·6 unit charges/nm.

In the case of Pb^{2+}, although spectroscopic (UV absorption of chelated Pb^{2+} at *c.* 220 nm) and calorimetric evidence points to a weak binding by GS3OX 80 chains, polymer precipitation is observed down to a Pb^{2+}/polycarboxylate equivalent ratio of *c.* 0·1. This is in contrast to the other divalent cations studied for which no phase separation was observed even at charge ratios close to unity. It therefore appears that GS3OX 80 chain aggregation is promoted selectively (amongst the cation group studied) by Pb^{2+}, a process which is deserving of more detailed investigation.

Sulphated derivatives

Derivatisation reactions were carried out in suspensions of tamarind seed polysaccharide in dimethylformamide using a sulphur trioxide-pyridine complex (Focher *et al.*, 1986). The kinetic course of the reaction was followed by removing aliquots at regular time intervals and determining the degree of sulphation by potentiometric titration (as described for the carboxylate derivatives) following dialysis. Equivalent weight (EW) data were used to derive the degree of substitution (DS) per sugar residue for each sample from the equation:

$$DS = (150 + 102\,DS)/EW$$

where 150 is the average residue weight for the glucose, xylose and galactose composition of the polysaccharide (Gidley *et al.*, 1991) and 102 is the weight difference ($OSO_3Na - OH$).

For a mole/residue ratio (*r*) of SO_3-pyridine/polysaccharide of 3·1 at 50°C, the results shown in Fig. 10 were obtained after various reaction times. Following reaction at 50°C for 24 h using different *r* values, the degrees of substitution shown in Fig. 11 were obtained. It was noted during the course of certain reactions (particularly at high *r* values) that the reaction mixtures became more homogeneous as judged by optical transparency, whereas in other reactions a certain degree of inhomogeneity persisted. The results obtained under the more homogeneous conditions are represented by open circles in Figs 10 and 11 and show consistently higher levels of substitution than those obtained in more heterogeneous mixtures (filled circles in Figs 10 and 11). As might be expected, therefore, more homogeneous reaction conditions lead to a more efficient rate of substitution.

The presence of sulphate groups in polysaccharide products was confirmed by infrared spectroscopy: the spectrum of a film of a derivative with DS = 0·44 is shown in Fig. 12. A strong band at *c.* 1230 cm^{-1} and a

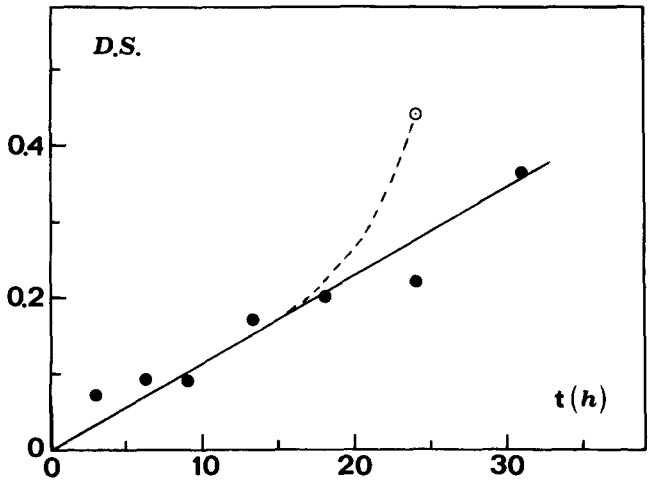

Fig. 10. Dependence of the substitution degree, DS, of tamarind polysaccharide sulphated derivatives as a function of reaction time for $r = 3{\cdot}1$ (see text) at 50°C.

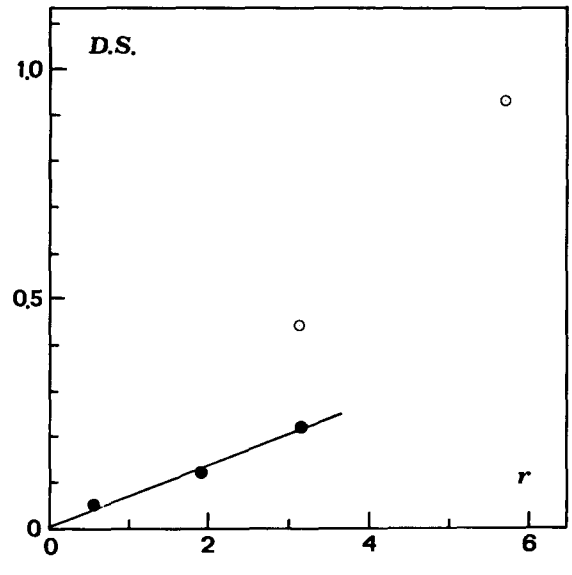

Fig. 11. Dependence of DS (see Fig. 10) on r (see text) after 24 h reaction.

weaker band at *c.* 820 cm^{-1} are observed in the derivative but not the native material and are assigned to S=O and C—O—S stretching vibrations respectively.

Information on the site(s) of sulphate substitution are available in principle from ^{13}C NMR spectroscopy as *O*-sulphate substitution has been shown (Honda *et al.*, 1973; Archbald *et al.*, 1981) to result in a 6–10 ppm downfield shift of attached carbons and a 1–2·5 ppm upfield displacement for adjacent carbons. The ^{13}C NMR spectrum of a sulphated derivative of DS = 0·42 is shown in Fig. 13 together with the native material. Derivatisation is seen to result in a major new resonance at 69·7 ppm and increased intensity in the range 77–87 ppm including a resolved resonance at 84·2 ppm. In contrast to spectra of carboxylated polysaccharide (Fig. 5) no obvious splitting of anomeric

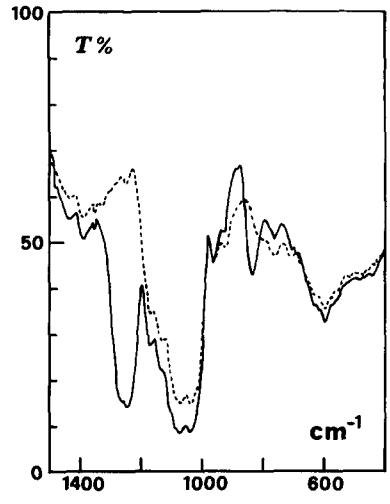

Fig. 12. Infrared film spectrum of sulphated tamarind polysaccharide with DS = 0·44 (dotted line is the spectrum of native polysaccharide).

resonances (100–108 ppm) is observed. Based on the known ^{13}C NMR shifts induced by sulphation (Honda *et al.*, 1973; Archbald *et al.*, 1981), the new signal at 69·7 ppm can be assigned to C-6 sites substituted with sulphate i.e. a downfield shift of 5–6 ppm from the non-substituted resonance. Similarly the increased signal intensity in the 77–87 ppm is assigned to sulphated carbon sites whose corresponding non-substituted resonance lies in the 70–78 ppm range. Signals at 81–83 ppm in the native polysaccharide are probably due to glycosidically linked carbon sites: the apparent absence of signals 5–10 ppm downfield from these positions is therefore expected as these sites cannot be substituted directly. ^{13}C NMR results are therefore consistent with a range of substitution sites, although C-6 positions (on galactose and unsubstituted backbone glucose residues) appear to be particularly highly substituted presumably due to steric accessibility.

The dependence of sulphated polysaccharide (DS = 0·44) viscosity on ionic strength (NaCl) at 25°C has been studied as for GS3OX 80 (Fig. 6). The intrinsic viscosity of the sample in 0·1 M NaCl was found to be ~500 ml/g, only slightly lower than that of the native material (650 ml/g), thereby suggesting that only slight chain degradation occurred during the derivatisation reaction. As shown in Fig. 14, sulphated polysaccharide shows marked ionic strength effects on both specific reduced viscosity and Huggins constant, K. Elaboration of intrinsic viscosity data for the same sample at different ionic strengths according to the method of Smidsrød & Haug (1971) yields a value of $B = 0{\cdot}04$ (Fig. 15). This is close to the B value estimated for the polycarboxylate GS3OX 80 and suggests a high rigidity. The similarity in B values for a low level of carboxyl substitution (DS = 0·14, $B = 0{\cdot}05$) and a higher level of sulphate substitution (DS = 0·44, $B = 0{\cdot}04$) is consistent with the relatively rigid

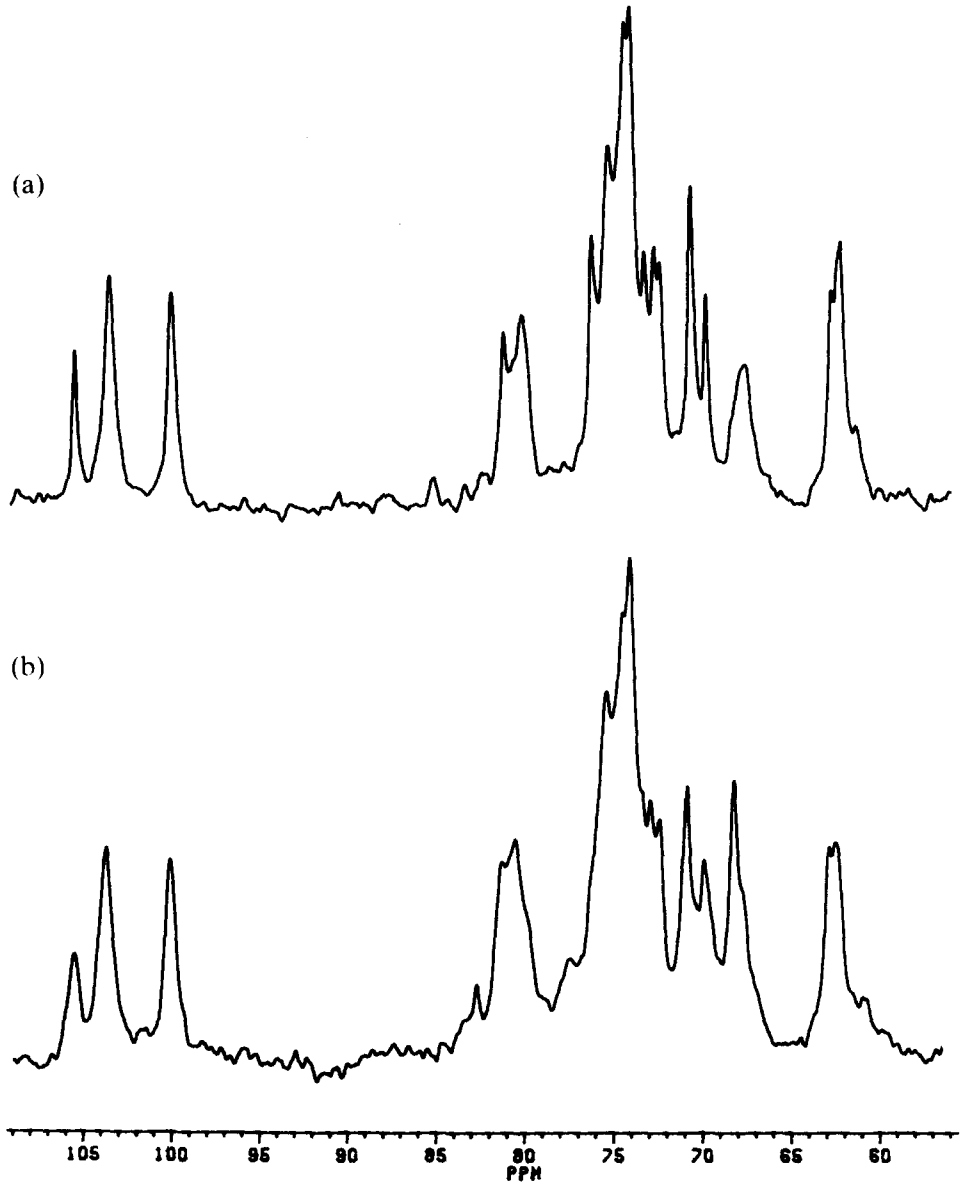

Fig. 13. ^{13}C NMR spectra of (a) native tamarind polysaccharide and (b) sulphated derivative of DS = 0·42.

($C^{\infty} \sim 100$) solution conformation characterised for the native polysaccharide (Gidley *et al.*, 1991).

Alkylamine derivatives

Alkylamine substituents were introduced into tamarind seed polysaccharide by a two step process (Fig. 1) involving formation of the C-6 aldehyde of galactosyl residues with galactose oxidase, and subsequent reaction with an appropriate alkylamine in the presence of sodium cyanoborohydride. Although cyanoborohydride preferentially reduces Schiff's bases in the presence of aldehydes (Borch *et al.*, 1971) under the experimental conditions chosen, a relatively inefficient rate of formation of Schiff's base (particularly for the relatively insoluble octylamine and nonylamine) is expected and hence some reduction of the aldehyde back to galactose was thought likely to occur. In practice, substitution levels up to 23 and 40% of galactose residues were achieved as assayed by ^{1}H NMR (see below) for alkylamine derivatives based on nonylamine and ethylamine respectively.

The ^{1}H NMR spectrum of a nonylamine derivative of tamarind seed polysaccharide is shown in Fig. 16. Polysaccharide resonances, particularly in the diagnostic anomeric region (4·4–5·4 ppm) are closely similar to those of the native polysaccharide. Additional resonances are observed at ~3·0, ~1·7, 1·3–1·4 and ~0·9 ppm: from their chemical shifts and relative intensities these may be assigned as shown below:

$$\text{Polysaccharide—NH—}\underset{3\cdot0}{CH_2}\text{—}\underset{1\cdot7}{CH_2}\text{—}[\underset{1\cdot35}{CH_2}]_6\text{—}\underset{0\cdot9\ \text{ppm}}{CH_3}$$

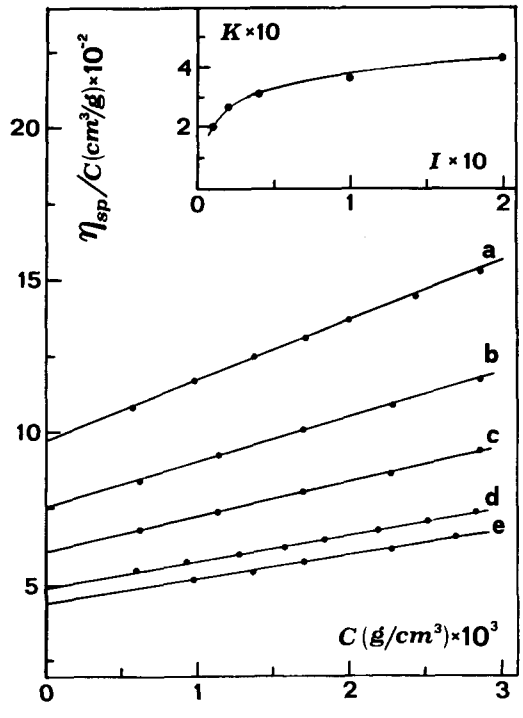

Fig. 14. Dependence of the reduced specific viscosity of sulphated tamarind polysaccharide (DS = 0·44) on ionic strength (NaCl) at 25°C. NaCl concentrations (M × 10^2) used were (a) 1·0, (b) 2·0, (c) 4·0, (d) 10·0 and (e) 20·0. The insert shows the variation of Huggins constant, K, with ionic strength.

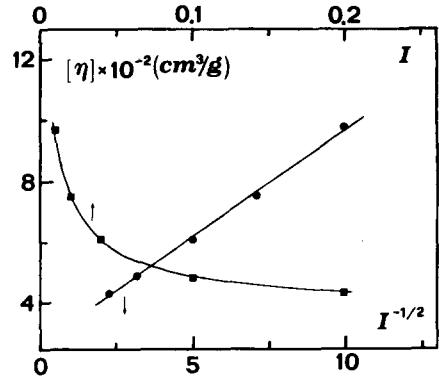

Fig. 15. Dependence of sulphated tamarind polysaccharide (DS = 0·44) intrinsic viscosity on ionic strength (NaCl) at 25°C. ■: int. visc. *vs* I; ●: int. visc. *vs* $I^{-1/2}$.

For ethylamine derivatives (Fig. 16), chemical shifts of ~3·0 and 1·27 are found for the methylene and methyl groups respectively. It is interesting to note that alkyl resonances show a marked broadening for sites close to the polysaccharide both in the nonylamine and to a lesser extent ethylamine derivatives (Fig. 16). All of a range of alkylamine substituted tamarind polysaccharides showed this effect although it was more marked for octyl and nonyl than ethyl derivatives. This observation suggests restricted motion for those sites with broadened resonances. T_1 data (Fig. 17) were

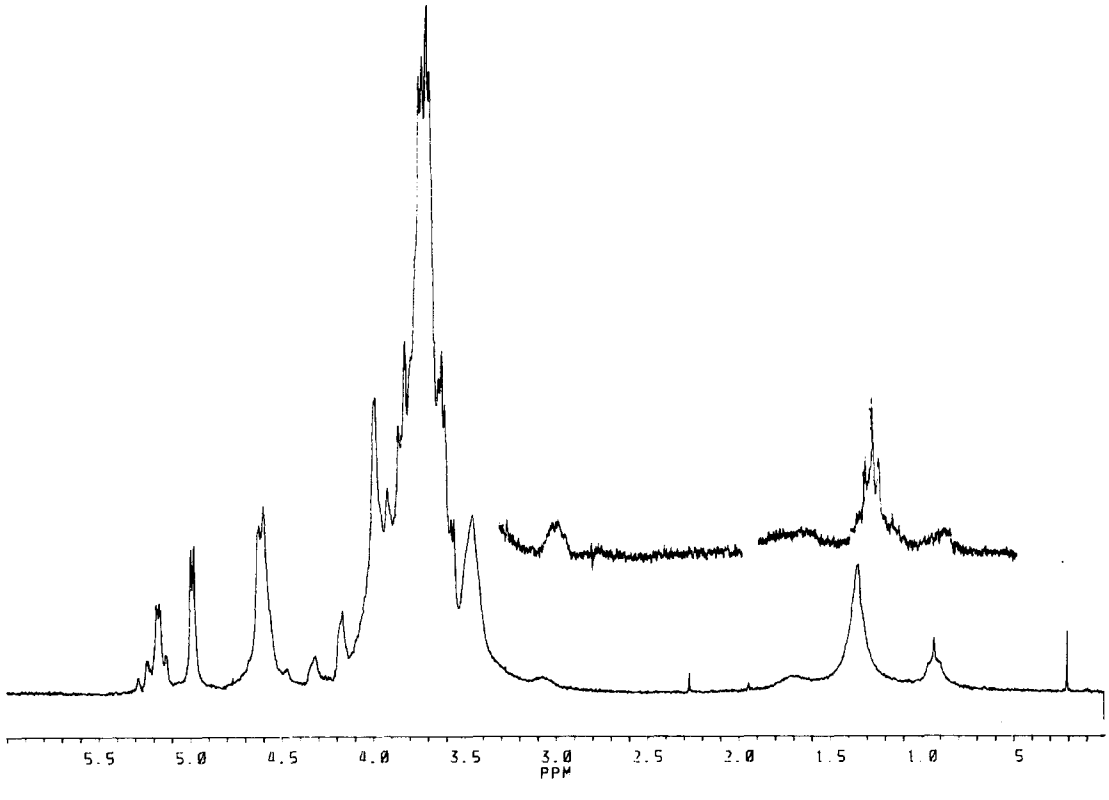

Fig. 16. ^{1}H NMR spectra of nonylaminated tamarind seed polysaccharide (DS = 0·04). Upper trace shows partial (alkyl region) spectrum of an ethylaminated derivative of DS = 0·03.

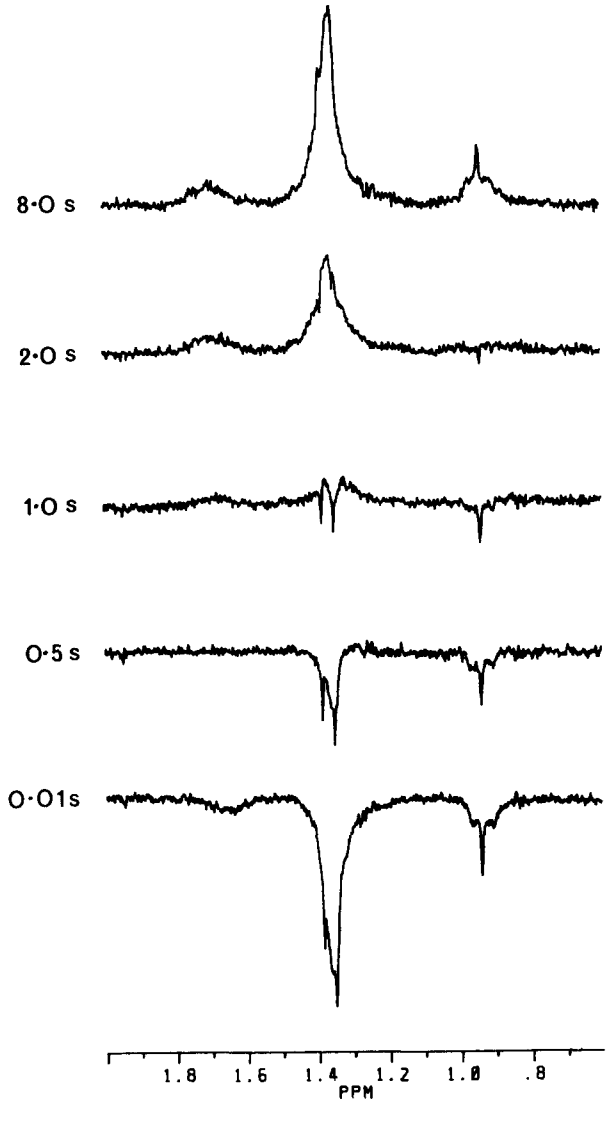

Fig. 17. Partially relaxed ^{1}H NMR spectra of alkyl resonances for nonylaminated tamarind polysaccharide of DS = 0·04. Spectra were obtained for various τ values following the pulse sequence 180°-τ-90° acquire. Individual resonances appear as negative peaks for $\tau = 0$ and progress towards positive peaks with increasing τ values determined by the time constant T_1. Those resonances which progress most slowly to positive intensity therefore have the longest T_1 values and are usually associated with sites of relatively greater mobility.

consistent with this interpretation showing an increase in alkyl T_1 values with remoteness from the polysaccharide chain. Partially relaxed spectra (Fig. 17) also identified a sharp component in the major 1·35 ppm signal with a longer relaxation time than the rest of the signal. This component was assigned to the CH_2 group adjacent to the terminal CH_3 group. T_1 values for a nonylamine tamarind derivative (Fig. 17) are shown below:

Polysaccharide—NH—CH_2—CH_2—$[CH_2]_5$—CH_2—CH_3

0·5 s 0·7 s 1·2 s 1·6 s 2·5 s T_1

Polysaccharide T_1 values of 0·5–0·7 s for anomeric protons were found, an identical range to that found for the native polysaccharide (Gidley *et al.*, 1991). Linewidths of polysaccharide resonances were also essentially identical before and after derivatisation. ^{1}H NMR results therefore suggest that regions of alkyl chains close to the point of polysaccharide substitution experience considerable motional constraints; however no obvious effects of substitution on the relaxation behaviour (i.e. T_1 and linewidth) of the polysaccharide were detected.

As T_1 values have been determined, ^{1}H NMR can be used to assess the degree of substitution provided a delay between observation pulses sufficient to allow full relaxation is employed. From comparison of alkyl and anomeric proton signals, two nonylamine derivatives were characterised as having 6 and 23% substitution at galactose residues. Ethylamine derivatives of 10, 19 and 41% substitution and an octylamine derivative of 7% substitution were also characterised. In order to convert these values to degrees of substitution (DS) with respect to carbohydrate residues, percentage values need to be divided by *c.* 6. The foaming capacity of alkylamine derivatives (see below) prevented detailed viscosity measurements (particularly using capillary viscometers) from being made.

In order to explore surface active properties we have measured surface tension and interfacial tension (decane/water) for a range of native and substituted polysaccharides using a DuNouy ring apparatus. Results are shown in Table 1: comparisons between native and derivatised tamarind polysaccharide show no significant difference in surface activity but a trend (barely significant) for decreasing interfacial tension with increasing hydrophobic character (i.e. ethyl > octyl > nonyl) is observed. The surface tension values may be compared with those (Dea & Madden, 1986) for other polysaccharides with known surface activity such as methyl cellulose of DS 1·6 (48 dyne cm^{-1}), gum tragacanth (43 dyne cm^{-1}) and sugar beet pectin (53 dyne cm^{-1}). These values are all significantly lower than those observed (Table 1) for the same concentrations of alkylated derivatives of tamarind seed polysaccharide.

Although surface and interfacial tension results suggest only modest surface activity for alkylated derivatives, the values obtained under the limited perturbing conditions of the experiment may not relate directly to surface activity following disruptive treatments as would typically be employed in the production of emulsions and foams. We have therefore attempted to characterise emulsifying and foaming properties using very simple laboratory tests.

Emulsifying activity was assessed for 0·5% w/v solutions in a 60/40 water/sunflower oil mixture following homogenisation for 1 min at room temperature. From immediate absorbance measurements,

Table 1. Surface and interfacial tensions of tamarind seed polysaccharide and alkylated derivatives (0·1% solutions) measured using a DuNouy ring apparatus

Sample	% substitution at galactose	Surface tension[a] (air/water) dyne cm^{-1}	Interfacial tension[a] (decane/water) dyne cm^{-1}
H_2O	—	71·5	43·1
0·1 M NaCl	—	69·2	42·5
Tamarind/H_2O	—	61·3	34·9
Ethyl tamarind/H_2O	19	65·1	34·2
Octyl tamarind/H_2O	7	55·6	32·7
Octyl tamarind/0·1 M NaCl	7	59·3	30·6
Nonyl tamarind/H_2O	23	59·3	29·6

[a] ± 2 dyne cm^{-1}.

initial emulsification was characterised. After 30 min the absorbance measurements were repeated to provide a measure of emulsion stability. For the positive control, gum arabic, at three different concentrations (0·1, 0·5 and 1·0%) both initial emulsification and emulsion stability consistently increased with concentration as would be expected. However, for all tamarind polysaccharide derivatives as well as carboxymethyl cellulose (a presumed negative control) variable values for initial emulsification were obtained although all solutions showed very good apparent emulsion stability. This behaviour was ascribed predominantly to viscosity effects (gum arabic has a negligible viscosity in the concentration range utilised). It was concluded that such a simple test could not discriminate between viscosity and surface activity contributions to apparent emulsification.

Measurement of foaming properties was, however, more informative. Following vigorous shaking of aqueous solutions for 5 min, the volume of foam produced was measured; this measurement was repeated after 5 min, 1 h and 20 h to provide an indication of foam stability. Gum arabic and a commercial whipping protein (D100) were used as positive controls, and carboxymethyl cellulose and underivatised tamarind seed polysaccharide were used as negative controls. Results are shown in Table 2: positive and negative controls are found to act in the expected manner. The foaming activity of alkylaminated tamarind derivatives increases with alkyl chain length and degree of substitution as might be expected. A particularly noteworthy feature is the foam stability exhibited by nonylaminated tamarind of DS 0·04 (Table 2) which is superior to that of gum arabic and comparable to a commercial whipping protein. This behaviour may be contrasted to the very weak surface and interfacial activity exhibited by all alkylaminated tamarind derivatives under less per-

Table 2. Foaming activity of alkylamine tamarind derivatives and controls

Sample	Concentration (%)	Foam volumes[a]			
		Initial	+5 min	+1 h	+20 h
Underivatised tamarind	0·5	0	0	0	0
Ethylaminated tamarind DS[b] 0·02	0·5	0	0	0	0
Ethylaminated tamarind DS[b] 0·07	0·5	2·5	0	0	0
Octylaminated tamarind DS[b] 0·015	0·5	40	40	30	5
Nonylaminated tamarind DS[b] 0·01	0·5	20	20	20	5
Nonylaminated tamarind DS[b] 0·04	0·5	250	250	200	200
D100 whipping protein	1·0	300	300	250	200
Gum arabic	1·0	80	60	30	10
Carboxymethyl cellulose	1·0	0	0	0	0

[a] Following vigorous shaking for 5 min and after standing for indicated times, expressed as a percentage of liquid volume.
[b] Degree of substitution expressed against all carbohydrate residues. DS values expressed relative to galactose are approximately 6 times higher.

turbing conditions (Table 1), and suggests that surface activity in these materials is only apparent following mechanical disruption of solutions. There are at least two possible explanations for this behaviour. One is that the high viscosity of solutions may retard or prevent access of lipophilic groups to the interface in the absence of disruptive perturbation. A second possibility is that alkylaminated tamarind derivatives have more specific inter-chain interactions which limit the availability of lipophilic groups at the interface. A third possible explanation could be that protein contaminants are responsible for surface active properties. However, elemental analysis has shown undetectably low nitrogen contents in both native and enzyme-derivatised tamarind polysaccharides used in this study.

In order to investigate further the properties of alkylaminated tamarind polysaccharide, a sample of nonylaminated polymer (DS 0·04) was treated exhaustively with a xyloglucan-specific endo-(1 → 4)-β-D-glucanase from germinating nasturtium seeds (Edwards *et al.*, 1986). The limit digest product was found to be similar to that produced from underivatised tamarind polysaccharide both with respect to the pattern of oligosaccharides separated by thin layer chromatography (Fanutti *et al.*, 1991) and ^{1}H NMR spectra. From the latter technique, the average degree of polymerisation (DP_n) was estimated to be 30 ± 5, a value which is in the range (30–50) typically found for underivatised tamarind. It therefore appears that the action of the enzyme on the polysaccharide is not hindered significantly by the presence of nonyl substituents on galactose sidechains.

It was noted during the course of endo-(1 → 4)-β-D-glucanase treatment of nonylaminated tamarind that the characteristic foam (Table 2) collapsed partially. As NMR analysis shows that the ratio of nonyl groups to polysaccharide is unchanged following enzyme digestion, this suggests that some of the foaming characteristics of the nonylaminated tamarind can be traced to the presence of a polymeric backbone. We have therefore compared the surface, interfacial, and foaming properties of nonylaminated tamarind before and after depolymerisation with nasturtium endo-(1 → 4)-β-D-glucanase. The results are summarised in Table 3: surface and interfacial tensions are shown to be significantly lower following depolymerisation, although foam stability is also decreased. It was noted that foams of the depolymerised material were more open (i.e. cell size was larger) and fragile than for the corresponding non-depolymerised sample. This difference in foam properties is probably due to the rheological properties of the interfacial films within the foam. Thus, in protein-based foams (Dickinson, 1986), stability and small air cells are correlated with rheologically strong cohesive films. For nonylamine substituted tamarind polysaccharide, our results suggest that the stiff polymer backbone (Gidley *et al.*, 1991) is a major determinant of foam stability and air cell size.

Table 3. Comparison of interfacial behaviour of nonylaminated tamarind of DS 0·04 before and after enzymic depolymerisation

	Nonylaminated tamarind	Depolymerised nonylaminated tamarind
Surface tension at 0·1% (dyne cm^{-1})	59·3	45·9
Interfacial tension (decane/water) at 0·1% (dyne cm^{-1})	29·6	15·0
Foam volumes [a] (0·5% solutions)		
Initial	250	250
+5 min	250	200
+1 h	200	160
+20 h	200	110

[a] As defined in Table 2.

The decrease in surface and interfacial tension exhibited following depolymerisation (Table 3) suggests that access of the hydrophobic groups to the interface is increased following depolymerisation of the nonylaminated polysaccharide, as the overall hydrophilic/lipophilic balance is not changed by depolymerisation. This is consistent with the apparent discrepancy between surface activity and foaming properties of alkylaminated polysaccharides (Tables 1 and 2), and we have speculated above that this may be due to viscosity effects or specific inter-chain associations involving alkyl chains. In order to probe the solution environment of carbohydrate and alkyl regions in depolymerised nonylaminated polysaccharides we have investigated ^{1}H NMR linewidths and T_1 values, and compared them to results for the non-depolymerised material. Apart from features associated with carbohydrate end-groups produced by enzyme action (Fanutti *et al.*, 1991) the NMR spectrum of depolymerised nonylaminated polysaccharide is virtually identical to that of the polymer (Fig. 16) i.e. both linewidths and chemical shifts are essentially unchanged. Furthermore, both partially relaxed spectra (Fig. 17) and calculated T_1 values for alkyl resonances are essentially identical before and after enzyme depolymerisation, whereas carbohydrate anomeric proton T_1 values are increased by up to 30% following depolymerisation. These results show that the local (i.e. motional) environment of pendant alkyl chains is similar for polymeric or depolymerised backbones: the difference in surface activity observed following depolymerisation is therefore more likely to be due to decreased solution viscosity rather than the loss of specific group interactions.

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