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Synergistic Interaction between Xanthan and Tara-Bean Gum*

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ABSTRACT

The non-Newtonian behaviour and dynamic viscoelasticity of a series of aqueous mixtures of xanthan and tara-bean gum were measured with a rheogoniometer.

At a concentration of 0·2% of total gum, gelation did not occur at room temperature, but at a low temperature (0°C). A much stronger interaction was observed with mixtures containing deacetylated, deacylated, or native xanthan than with depyruvated xanthan. The maximum dynamic modulus was obtained when the ratio of xanthan to tara-bean gum was 1:2. The dynamic viscoelastic parameters for mixtures with deacetylated and deacylated xanthan decreased rapidly at temperatures above 25 and 20°C, respectively.

It was concluded that the side chains of the tara-bean gum molecule prevent an intermolecular interaction between xanthan and tara-bean gum. The results obtained support the interaction mechanism between xanthan and locust-bean gum previously proposed.

INTRODUCTION

Xanthan, the extracellular bacterial polysaccharide produced by the plant pathogen *Xanthomonas campestris*, has a pentasaccharide repeating unit formed by a β-(1 → 4)-linked D-glucose backbone (cellulose) substituted through C-3 on alternate glucosyl residues with a charged trisaccharide side chain (Jansson *et al.*, 1975; Melton *et al.*, 1976). The internal D-mannose of the side-chain is substituted at C-6 with an acetyl

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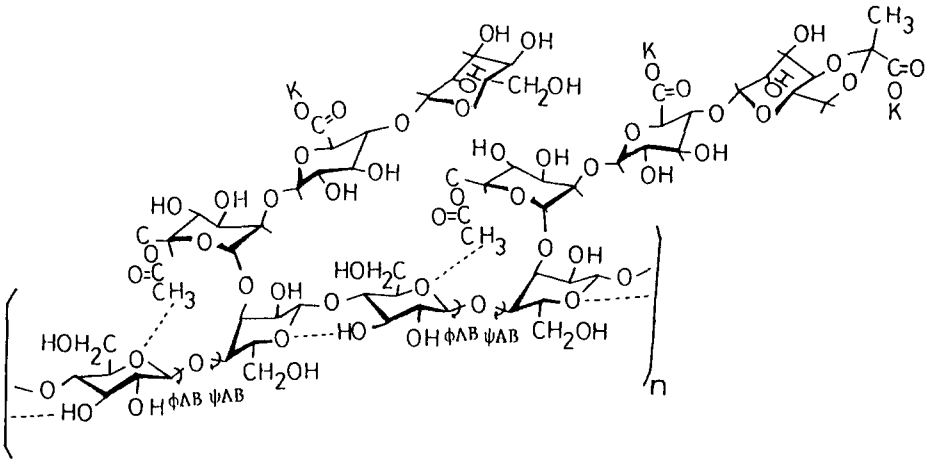
group. About one-half to two-thirds of the terminal D-mannose residue contain a pyruvic acid group. The level of pyruvic acid depends on the culture conditions (Cadmus *et al.*, 1976).

We have proposed a possible mode of intramolecular association involving hydrogen bonding between an alternate hydroxyl group at C-3 and the adjacent hemiacetal oxygen atom of the D-glucosyl residues, and between the methyl group of the acetyl residue and the adjacent hemiacetal oxygen atom of the D-glucosyl residue, as illustrated in Scheme 1

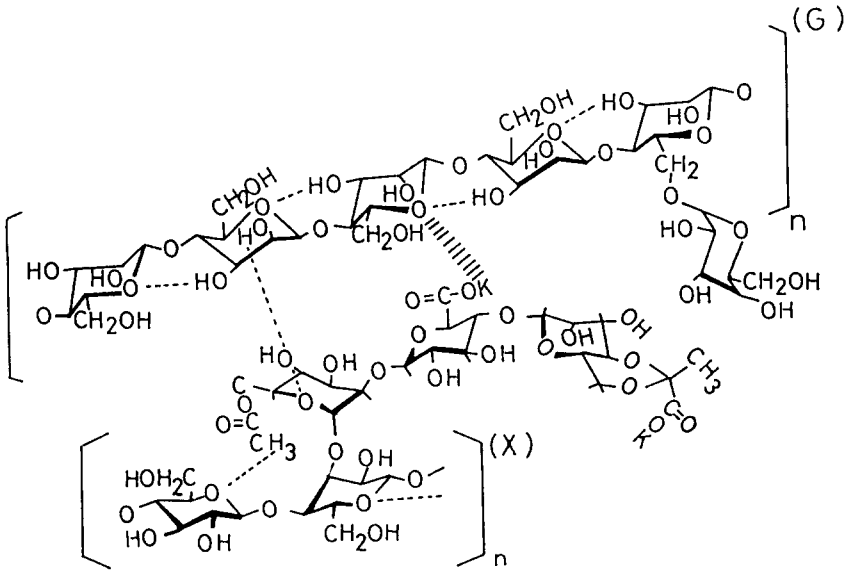


Scheme 1. Possible mechanism for intramolecular associations in xanthan molecules in aqueous solution. The dotted lines show the proposed hydrogen bonds. The model corresponds to a single-stranded helix. The conformational changes in the interaction between alternate neighbouring sugar residues are expressed in terms of the two angles of rotation, ϕ_{AB} and ψ_{AB}.

(Tako & Nakamura, 1989). The intramolecular associations provide an explanation for the rheological characteristics in aqueous solutions. The xanthan molecule may keep an ordered, less extended, single-stranded helix conformation (Moorhouse *et al.*, 1977) owing to the formation of intramolecular associations at room temperature (25°C) in aqueous solution. A sigmoidal curve of viscosity and dynamic viscoelasticity (Jeanes *et al.*, 1961; Tako *et al.*, 1977), with increasing temperature, may be attributed to the breakdown of the two alternate intramolecular associations in the temperature range 25–35 or 55–65°C, and to the formation of an intermolecular association through side chains (Tako *et*

al., 1977; Tako & Nakamura, 1984, 1988, 1989) between different molecules at high temperature (> 45°C).

The synergistic interaction between xanthan and locust-bean gum is well known (Rees, 1972; Dea *et al.*, 1977). Many workers (Dea *et al.*, 1977; Morris *et al.*, 1977; Cairns *et al.*, 1986; Cheetham & Mashimba, 1988) have proposed a mode of interaction between the single-stranded helix of the xanthan molecule and the backbone of the locust-bean gum molecule without taking into consideration the role of the xanthan side chains. We have shown that the characteristics of association between the gums depends on the charged trisaccharide side chains of the xanthan molecule (Tako *et al.*, 1977; Tako & Nakamura, 1984; Tako *et al.*, 1984; Tako & Nakamura, 1985, 1986). Recently, possible binding sites for D-mannose-specific interaction between xanthan and locust-bean gum have been proposed, as illustrated in Scheme 2 (Tako, 1991). Hydrogen bonding may take place between the hemiacetal oxygen atom, which may



Scheme 2. Possible binding sites for a D-mannose-specific interaction between xanthan and galactomannan in aqueous solution: – – –, hydrogen bonding; ||||, electrostatic attraction. The xanthan molecule retains its five-fold, single-stranded helix and its side chains are inserted into the adjacent, unsubstituted segments of the backbone of the galactomannan molecule. This gives a lock-and-key mode of interaction. A molecule of xanthan may combine with two or more molecules of galactomannan, the ratio depending on the preferred conformation in aqueous solution. As the side chains of the native and depyruvated xanthan molecules are rather rigid because of the intramolecular association contributed by the acetyl residues, increased interaction may result from deacetylation. (G) Galactomannan and (X) xanthan.

be involved as a donor, of the inner D-mannose side-chain of xanthan and the hydroxyl group at C-2, which may be an acceptor. The univalent cation (K^+) which associates with the carboxyl oxygen atom of the D-glucuronic acid residue of xanthan may also interact electrostatically with the hemiacetal oxygen atom of the mannan backbone of the locust-bean gum molecule. As the mannan backbone of the locust-bean gum molecule has a rigidity due to an intramolecular hydrogen bonding, O(5)---C(3)′ (Atkins *et al.*, 1988) as illustrated in Scheme 2, the side chains of the xanthan molecule are inserted into the adjacent, unsubstituted segments of the mannan backbone, which is extended into a two-fold, ribbon-like structure.

We describe in this paper the rheological behaviour, with respect to the synergistic interaction, of a mixture of xanthan and tara-bean gum, the structure of which is the same as that of locust-bean gum, except that the ratio of D-mannose to D-galactose was 3·4:1·0.

MATERIALS AND METHODS

Materials

Xanthan, identical with that used in preceding studies (Tako & Nakamura, 1984; Tako *et al.*, 1984; Tako & Nakamura, 1985, 1986, 1988, 1989; Tako, 1990) and tara-bean gum were obtained from Taiyo Kagaku Co., Yokkdichi Ltd. A solution of 0·1% xanthan in distilled water was heated at 90°C for 20 min, and then cooled to room temperature, centrifuged at 46000 *g* for 1 h, and filtered through Celite 545 (which had been treated with boiling 3 M HCl for 30 min and washed with distilled water until the pH was 6·5). In the presence of 0·1% KCl, ethanol (2 vols) was added to the filtrate, and the precipitate was dried *in vacuo*. Purified xanthan was redissolved in water, deionized by passing through a column of Amberlite IR 120 (H^+), and neutralized with 100 mM KOH. Ethanol (2 vols) was added to the filtrate in the presence of 0·1% KCl and the precipitate was dried *in vacuo*.

The pyruvate groups were removed by heating a solution of the purified K salt of xanthan (1 g/litre in 1 mM oxalic acid, 0·1 M KCl, pH 3·0) at 95°C for 2 h (Holzwarth & Ogletree, 1979). After neutralization with 50 mM KOH, the product was isolated in the same manner as described above.

An aqueous 0·2% solution of native or depyruvated xanthan was treated under an atmosphere of nitrogen with 10 mM KOH in the

presence of 0·1% KCl at room temperature for 10 h to accomplish deacetylation (Sloneker & Jeanes, 1962). The solution was neutralized with 50 mM HCl, and then the product was isolated in the same manner as described above.

By a spectrophotometrical estimate as the 2,4-dinitrophenyl derivative, and as the hydroxamic acid, native xanthan ($[\alpha]_{589}^{20}$, $-4°$) had pyruvic and acetic acid contents of 5·7 and 5·4% (w/w), which meant that 67 and 98% of the terminal and inner mannosyl groups contained pyruvic and acetic acid moieties, respectively. The pyruvate groups were removed by heating a solution of native xanthan in 1 mM oxalic acid at 95°C for 2 h. Under these conditions, about 84% of the pyruvate groups were removed, so that $\frac{1}{10}$ of the terminal mannosyl groups contained a pyruvate moiety, but the acetyl groups were unaltered (5·3%); this was depyruvated xanthan ($[\alpha]_{589}^{20}$, $-6°$). The acetyl-free xanthan was prepared by dissolving the native or depyruvated xanthan in distilled water and altering the solvent to 10 mM KOH at room temperature; this was deacetylated ($[\alpha]_{589}^{20}$, $-8°$) or deacylated xanthan ($[\alpha]_{589}^{20}$, $-8°$). Deacetylation of the samples was confirmed by the loss of infrared absorption at 1730 cm^{-1}.

A solution of 0·3% tara-bean gum in hot distilled water (75°C) was filtered through Celite 545, ethanol (2 vols) was added and the precipitate dried *in vacuo*. As the intermolecular interaction between xanthan and galactomannan molecules is closely correlated with the degree of substitution of the mannan chain (Dea *et al.*, 1986), the degree of substitution of tara-bean gum was determined by liquid chromatography and the ratio D-mannose to D-galactose, calculated to be 3·4:1·0. The molecular weight determined from viscosity measurement was 208 000, and $[\alpha]_{589}^{20}$, was $+24°$.

Various mixed solutions of xanthan and tara-bean gum having a total concentration of 0·2% were prepared by dissolving tara-bean gum in hot water and adding xanthan (native, deacetylated, depyruvated, and deacylated).

Pyruvic and acetic acid measurements

Pyruvic and acetic acid were measured colorimetrically as the 2,4-dinitrophenylhydrazone (Sloneker & Orentas, 1962), and as the hydroxamic acid and ferric ions (McComb & McCready, 1957), respectively. The deacetylation was also identified by infrared spectroscopy. Spectra were recorded with an infrared spectrophotometer (IR-440, Shimadzu Seisakusho Co. Ltd, Tokyo) for samples dispersed in KBr discs.

Liquid chromatography

A solution of tara-bean gum (50 mg) in 1 M H_2SO_4 (20 ml) was heated at 100°C for 3 h. The hydrolyzate was neutralized with $BaCO_3$, and the solution concentrated and filtered through Celite 545 into a 10-ml volumetric flask. Liquid chromatography was performed with a Hitachi L-6200 chromatograph, equipped with a column of #3013-N. The mobile phase was 0·3 M boric acid (temperature, 60°C; flow rate, 0·5 cm/min).

Specific rotations

Specific rotations were measured at 589 nm on an automatic digital polarimeter DIP-180 (Japan Spectroscopic Co. Ltd, Tokyo) in a 0·5 and 0·1% (w/v) solution of tara-bean gum and xanthan in water, respectively.

Molecular weight

The molecular weight of tara-bean gum was determined from the intrinsic viscosity according to the relationship (Robinson *et al.*, 1982) $[\eta] = 3{\cdot}8 \times 10^{-4} . Mw^{0{\cdot}723}$ determined for guar gum. The intrinsic viscosity $[\eta]$ was determined for each solution by measuring the specific viscosity with an Ostwald-type viscometer at 25°C. The flow time for water was 42 s.

Viscosity and dynamic viscoelasticity measurements

Viscosity at different shear rates (1·19–95·03/sec) and dynamic viscoelasticity at steady angular velocity (3·77 rad/s) were determined with a rheogoniometer consisting of a coaxial cylinder (1·8 cm diameter) with a rotating outer cylinder (2·2 cm diameter), 6·0 cm high (IR-103, Iwamoto Seisakusho, Co. Ltd, Kyoto). The temperature of the sample was controlled by circulating oil from a Thermo-cool (LCH-130F, Toyo Co. Ltd, Tokyo) over the temperature range 0–50°C and raised at a rate of 1°C/min steps. Shear rate (D), shear stress (S), and apparent viscosity (η) were calculated using the Margules equation (Harris, 1977). Dynamic viscosity (η') and elasticity (G') were calculated by a modification of Markovitz's equation (Markovitz, 1952).

RESULTS AND DISCUSSION

To compare the rheological behaviour of a mixed solution of xanthan and tara-bean gum with that of a mixed solution with locust-bean gum (Tako *et al.*, 1984; Tako & Nakamura, 1986; Tako, 1991), and with guar gum (Tako & Nakamura, 1985, 1986; Tako, 1991), the viscosity and dynamic viscoelasticity measurements were performed under the same conditions as those of the preceding studies.

Gelation did not occur for the mixture with xanthan (native, deacetylated, depyruvated, and deacylated) and tara-bean gum at a total concentration of gums of 0·2% at room temperature. The flow curves, at 35°C, of a mixture of native (Fig. 1(A)), deacetylated (Fig. 1(B)), and deacylated (Fig. 1(C)) xanthan with tara-bean gum solutions at varying ratios of the gums, but keeping the total concentration constant at 0·2%, are shown in Fig. 1. The solutions containing deacetylated and native xanthan showed plastic behaviour, in which the values for the shear stress S of the mixtures with deacetylated xanthan were higher than those of the values of the mixture with native xanthan. In the case of the mixture with deacylated xanthan, the solutions were shifted to lower stresses even at high shear rates (D) and showed shear-thinning rather than pseudoplastic behaviour, indicating that there were less intermolecular interactions in these mixtures.

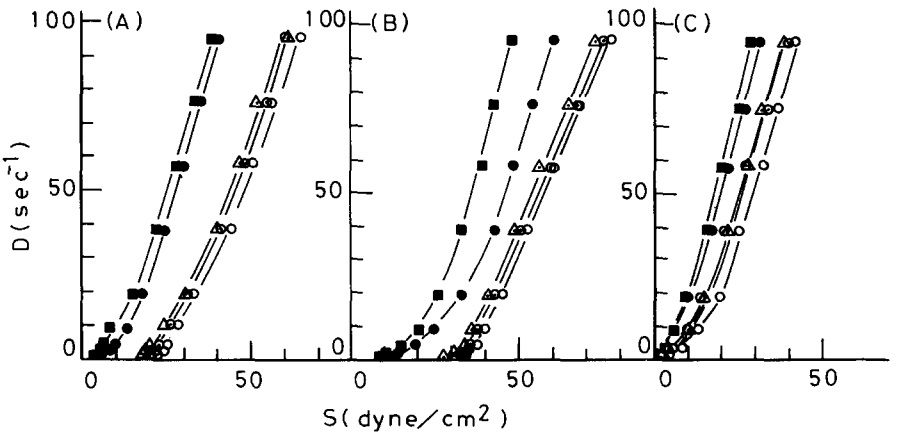


Fig. 1. Flow curves, at 35°C, of xanthan solution mixed with tara-bean gum solution at a total concentration of gum of 0·2%. Ratio of xanthan to tara-bean gum: ■, 1:3; ●, 1:2; ⊙, 1:1; ○, 2:1; △, 3:1. (A) Native xanthan, (B) deacetylated xanthan, and (C) deacylated xanthan.

The effect, at 25°C, of the ratio of xanthan (native, deacetylated, depyruvated, and deacylated) to tara-bean gum in solution on the dynamic modulus of solutions at a total concentration of 0·2% is shown in Fig. 2. A small synergistic increase in dynamic modulus was observed for the mixture containing depyruvated xanthan. However, the synergistic interaction was enhanced in the mixture with native, deacetylated, and deacylated xanthan. The maximum dynamic modulus was achieved when the ratio of xanthan to tara-bean gum was 1:2. In the case of deacetylated xanthan large dynamic moduli were also observed at mixing ratios of 1:4, 1:3, 1:1, 2:1, 3:1, and 4:1. However, the dynamic modulus of the mixture of xanthan (native, deacetylated, depyruvated, and deacylated) and tara-bean gum showed values only about a quarter to a half of that of the mixture with locust-bean gum (Tako *et al.*, 1984; Tako & Nakamura, 1986; Tako, 1991). As reported in previous papers, mixtures of xanthan and galactomannan with low galactose contents, locust-bean gum (20%), formed gels at room temperature with total carbohydrate concentrations as low as 0·2%. However, a galactomannan with a higher galactose content, guar gum (33%), showed only a small degree of interaction with xanthan. This may be due to the greater number of side chains present on the guar gum molecule (Baker & Whistler, 1975; Painter *et al.*, 1979). These side chains might prevent the

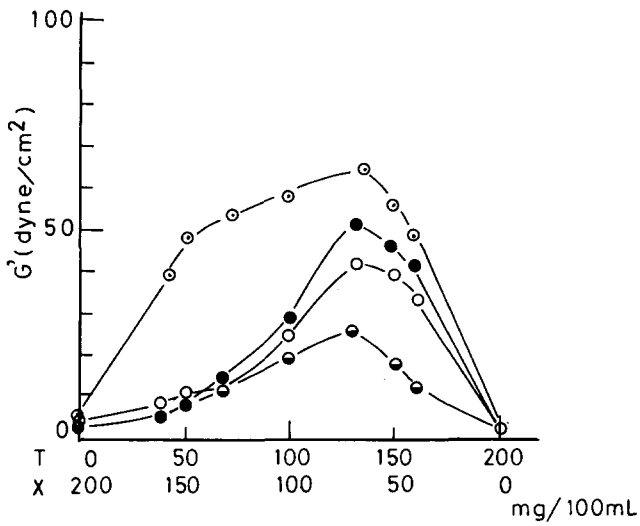


Fig. 2. Dynamic modulus, at a frequency of 3·77 rad/s and 25°C, of a 0·2% xanthan–tara-bean gum solution as a function of the ratio of components: ○ native; ⊙ deacetylated; ◒ depyruvated; ● deacylated xanthan. (T) Tara-bean gum and (X) xanthan.

insertion of the charged trisaccharide side-chains of the xanthan molecule into the backbone of the guar-gum molecule. However, a synergistic interaction occurred with deacetylated and deacylated xanthan even at room temperature (25°C), indicating that the xanthan molecule had become flexible and could associate with the guar-gum molecules more easily, because they were free from the intramolecular hydrogen bonding to which the acetyl residues contributed (Tako & Nakamura, 1984, 1989). In spite of the low galactose content of tara-bean gum (23%), only a small synergistic interaction was observed. This may be caused by the difference in the distribution of the galactose side chains along the galactomannan molecule. Though the side chains of locust-bean gum may be distributed in uniform blocks along the backbone of the mannan molecule (Baker & Whistler, 1975; Painter *et al.*, 1979), the fine structure of tara-bean gum is not yet known (Dea *et al.*, 1977; McCleary *et al.*, 1985). The experimental results suggest that it may have random, or regular units of substitution of galactose residue. The intramolecular hydrogen bonding between O(5)---C(3)′ of mannose residues on the mannan backbone may break due to the kinetic energy and Brownian motion of the galactose side-chains and solvent at room temperature (Zugenmaier, 1974). This suggests that the small synergistic interaction may be attributed not only to the distribution of the galactose side chains but also to breakdown of the intramolecular hydrogen bonding of the mannan backbone at room temperature. This is because unsubstituted mannose regions of the galactomannan result in extensive intramolecular hydrogen bond formation. The interaction may be enhanced by deacetylation of xanthan, as in a mixture with locust-bean gum (Tako *et al.*, 1984; Tako & Nakamura, 1986; Tako, 1991). Though the maximum dynamic modulus was achieved when the ratio of xanthan to tara-bean gum was 1:2 as with mixtures with locust-bean gum, a mixture with deacetylated xanthan had large values over a wide range of the ratios (4:1 ~ 1:4), suggesting that the pyruvic acid residues may also take part in the interaction with the backbone of the tara-bean gum molecule. Furthermore, for ratios of xanthan to tara-bean gum in the range 4:1 ~ 2:1, and in the range 1:3 ~ 1:4, self-association of the xanthan and tara-bean gum molecules also appear to take place, respectively, to which the pyruvate and mannose residues may contribute (Tako & Nakamura, 1984; Dea *et al.*, 1986).

The effect of pH, at 25°C, on the dynamic modulus of a mixed solution of tara-bean gum with native, deacetylated, depyruvated, and deacylated xanthan in a combination ratio of 1:2 at 0·2% total gums is shown in Fig. 3. The dynamic modulus of the mixed solution was nearly independent of pH between 5 and 9. This is in agreement with observa-

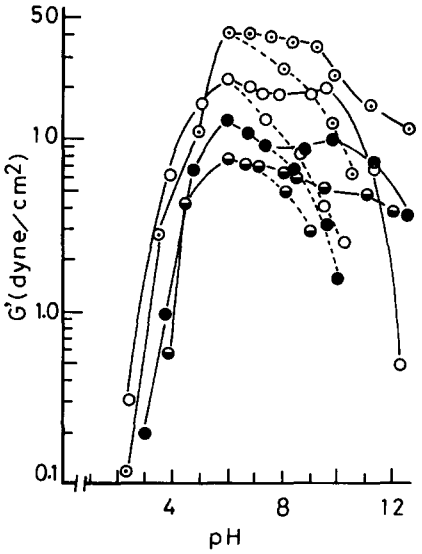


Fig. 3. Dynamic modulus of xanthan–tara-bean gum solution as a function of pH at 3·77 rad/s and 25°C: ○ native; ⊙ deacetylated; ◒ depyruvated; ● deacylated xanthan. Xanthan/tara-bean gum, 1:2 (0·2% total gum). The full lines refer to addition of HCl and KOH (100 mM), and the broken lines to $Ca(OH)_2$.

tions made on mixtures of xanthan and locust-bean gum (Tako, 1991). However, a lower dynamic modulus was observed than with locust-bean gum. After the pH values reached 5·2, 6·2, 4·8, and 6·2 for mixtures with native, deacetylated, depyruvated, and deacylated xanthan, respectively, the dynamic modulus decreased rapidly. At alkaline pHs, a small decrease in the dynamic modulus was observed on addition of KOH, but it decreased rapidly on addition of $Ca(OH)_2$. The rapid decrease in the dynamic modulus on addition of $Ca(OH)_2$ may be due to the formation of self-association within xanthan molecules via Ca^{2+} with ionic bonding on the carboxyl groups of the D-glucuronic acid residues of the intermediate side chains of the xanthan molecule.

The effect of temperature on the dynamic viscoelasticity of a mixed solution of deacylated xanthan and tara-bean gum is shown in Fig. 4. The dynamic modulus had a large value at low temperatures (0°C), and was insensitive to an increase of the temperature from 0 to 20°C, but it decreased rapidly with further temperature increases. The dynamic viscosity of the mixture with deacylated xanthan also showed a similar transition point at the same temperature. However, in mixed solutions of deacetylated xanthan and tara-bean gum the transition increased to 25°C (not shown in the figure). The sharp transition temperatures observed

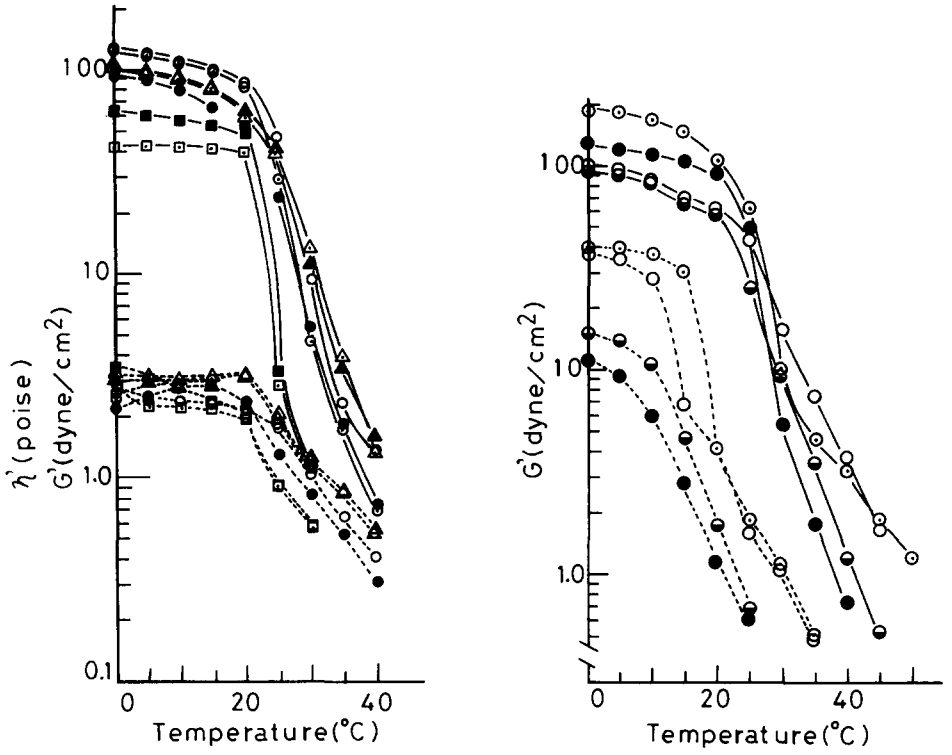


Fig. 4. Effect of temperature on dynamic viscoelasticity of deacylated xanthan–tara-bean gum solution at 0·2% total gum and a frequency of 3·77 rad/s. The broken lines refer to dynamic viscosity and full lines to dynamic modulus. Ratios of xanthan to tara-bean gum; ⊡ 4:1; ■ 3:1; ● 2:1; ⊙ 1:1; ○ 1:2; ▲ 1:3; ◬ 1:4.

Fig. 5. Effect of temperature on the dynamic modulus of xanthan–tara-bean gum (1:2) solution at 0·2% total gum with addition of urea at 3·77 rad/s; ○ native; ⊙ deacetylated; ⊖ depyruvated; ● deacylated xanthan. The full lines refer to the polysaccharide alone and the broken lines to systems containing 4 M urea.

imply that the intermolecular interaction dissociates above these temperatures.

Figure 5 shows the effect of temperature on the dynamic modulus of a mixed solution of tara-bean gum with native, deacetylated, depyruvated, and deacylated xanthan at a ratio of 1:2, and a total concentration of 0·2%. The dynamic modulus showed a very large value and gelation occurred at low temperatures (0°C). The transition temperature of the dynamic modulus was observed at 20 and 25°C for the mixture with deacylated and with deacetylated xanthan, respectively. The dissociation that occurs above these temperatures may be due to the kinetic energy and Brownian motion of carbohydrates and solvent. The dynamic

modulus, however, of mixed solutions had very low values in the presence of urea (4·0 M) even at low temperature (0°C). This was in agreement with observations for mixtures of xanthan and locust-bean gum (Tako, 1991).

CONCLUSIONS

The results presented here support the binding sites between xanthan and locust-bean gum molecules previously proposed (Scheme 2). In spite of the low galactose content of tara-bean gum, only a small synergistic interaction was observed for mixtures with xanthan. This may be caused by the difference in the distribution of the galactose side chains along the tara-bean gum molecules. Tara-bean gum molecules may have random, or regular units of substitution of galactose side chains. These side chains might prevent the insertion of the charged trisaccharide side-chains of the xanthan molecule into the backbone of the tara-bean gum molecules. The pyruvate residues may also take part in the interaction with the backbone of tara-bean gum molecules. Furthermore, a self-association of xanthan, and of tara-bean gum molecules appears to take place when the ratio of xanthan to tara-bean gum was 4:1 ~ 2:1, and 1:3 ~ 1:4, respectively. This suggests that the mannose residues may take part in self-association within tara-bean gum molecules and within xanthan molecules, as well as in synergistic interactions between xanthan and tara-bean gum.

Gelation occurred in mixtures of xanthan and tara-bean gum at low temperatures. The interaction that takes place at low temperature may be due to the extensive formation of intramolecular hydrogen bonding in the mannan backbone of the tara-bean gum molecules; this will enhance analogous hydrogen bonding between the hemiacetal oxygen atom of the inner mannose side chains of xanthan and hydroxyl groups at C-2 of the mannan backbone of the tara-bean gum molecules.

The present and previous studies (Tako, 1991) indicate that the content of galactose and the distribution of galactose residues in galactomannan can have a significant effect on the interaction properties with xanthan molecules. Thus, the interaction between the extracellular bacterial polysaccharide, xanthan, and the galactomannan components of plant cell wall may play a role in the host–pathogen relationship, since *Xanthomonas* species is one of the plant-pathogen bacteria (Morris *et al.*, 1977). Furthermore, the mode of the interaction (Scheme 2) may provide D-mannose-specific binding sites in several cell-recognition processes.

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