Carbohydrate Research, 147 (1986) 275–294
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

EFFECT OF GALACTOSE-SUBSTITUTION-PATTERNS ON THE INTERACTION PROPERTIES OF GALACTOMANNANS

IAIN C. M. DEA, ALLAN H. CLARK,
Unilever Research, Colworth Laboratory, Sharnbrook, Bedford MK44 1LQ (Great Britain)

AND BARRY V. MCCLEARY
Biological and Chemical Research Institute, NSW Department of Agriculture, Rydalmere 2116, New South Wales (Australia)

(Received June 24th, 1985; accepted for publication, September 13th, 1985)

ABSTRACT

A range of galactomannans varying widely in the contents of D-galactose have been compared for self-association and their interaction properties with agarose and xanthan. Whereas, in general, the most interactive galactomannans are those in which the (1→4)-β-D-mannan chain is least substituted by α-D-galactosyl stubs, evidence is presented which indicates that the distribution of D-galactosyl groups along the backbone (fine structure) can have a significant effect on the interaction properties. For galactomannans containing <30% of D-galactose, those which contain a higher frequency of unsubstituted blocks of intermediate length in the β-D-mannan chain are most interactive. For galactomannans containing >40% of D-galactose, those which contain a higher frequency of exactly alternating regions in the β-D-mannan chain are most interactive. This selectivity, on the basis of galactomannan fine-structure, in mixed polysaccharide interactions *in vitro* could mimic the selectivity of binding of branched plant-cell-wall polysaccharides in biological systems.

INTRODUCTION

Plant-seed galactomannans are polysaccharides that comprise a (1→4)-β-D-mannan main-chain to which are attached single α-D-galactopyranosyl groups at O-6 of some of the D-mannopyranosyl residues. Such galactomannans have attracted attention in relation to their role in polysaccharide interactions. On cooling even concentrated solutions of galactomannans to room temperature, true gels do not form. However, admixture with certain gelling-polysaccharides can increase considerably the gel strength and modify the gel structure. Early investigators[1,2] of the effect of the galactomannan from *Ceratonia siliqua* (~25% D-galactose) on the mechanical properties of kappa-carrageenan and agarose gels reported that even small additions of galactomannan made the gel firmer, less brittle, and more elastic. The galactomannan from *Ceratonia siliqua* has been

0008-6215/86/$ 03.50

fractionated[3] on the basis of water solubility into D-galactose-rich and D-galactose-depleted components. The latter fraction was more effective in strengthening agarose gels. More recently, using a range of native galactomannans[4], with a content of D-galactose varying between 23% and 48%, and a range of D-galactose-depleted galactomannans (D-galactose content, 14–39%), prepared by treatment of *Cyamopsis tetragonolobus* galactomannan with α-D-galactosidase[5,6], it was demonstrated that, in general, the strength of the interaction of galactomannans with kappa-carrageenan and agarose increased with decreasing content of D-galactose.

The interaction of galactomannans with kappa-carrageenan and agarose is not confined to increase of gel strength and modification of mechanical properties. The addition of galactomannans having a low content of D-galactose to non-gelling concentrations of these gelling polysaccharides can result in gel formation[4]. Furthermore, both kappa-carrageenan and agarose can be degraded in a controlled manner by "*kink splitting*" using Smith degradation[7] to give low-molecular-weight "segmented" material. These segmented materials can still undergo the same conformational ordering that occurs on gelation of the parent polysaccharides, but, because of their low molecular weight, they do not form gels at any concentration. However, on admixture with galactomannans having a low content of D-galactose, firm rubbery gels are formed[4]. The interaction of galactomannans with xanthan, the extracellular polysaccharide from *Xanthomonas campestris*, is just as striking. Xanthan alone does not gel at any concentration. However, on admixture with the galactomannan from *Ceratonia siliqua*, firm gels are formed at low concentrations of total polysaccharide[8]. As for the interaction with kappa-carrageenan and agarose, the strength of the xanthan–galactomannan gelling interaction increased with decreasing content of D-galactose in the galactomannan[5,6,8–10].

In addition to the content of D-galactose, it is reasonable to suppose that the distribution of D-galactosyl groups along the D-mannan backbone would affect the interactions of galactomannans with other polysaccharides. Indeed, although the galactomannans from *Leucaena leucocephala* and *Cyamopsis tetragonolobus* have closely similar contents of D-galactose, the degree of interaction of xanthan with the *Leucaena* galactomannan is much greater, and this arises from differences in fine structure between the two galactomannans[10]. There has been considerable debate on the distribution of D-galactosyl groups along the main chains of galactomannans[11]. Regular, block, and random distributions have been considered. Enzymic degradation is a potentially powerful method for determining fine structure and, using this technique, it was concluded[12] that *Ceratonia siliqua* galactomannan had an essentially block-type structure. This conclusion led to proposals that the junction zones in mixed polysaccharide gels containing galactomannans involved binding of the ordered double-helical regions of agarose and kappa-carrageenan and the ordered rigid-rod conformation of xanthan to an extended-ribbon-ordered conformation of unsubstituted blocks of the galactomannan[4,8]. More recently, it has been shown[13], using highly purified enzymes[14] and

rigorous characterisation of the degradation products[15], that *Ceratonia siliqua* galactomannan does not have a block, regular, or statistically random distribution of D-galactosyl stubs along the main chain. Rather, it has a non-regular structure, with a higher proportion of unsubstituted blocks of intermediate length than would be expected for a statistically random distribution. However, comparison of the interaction properties of galactomannans having closely similar contents of D-galactose but different fine structures would help an understanding of the mechanisms of mixed polysaccharide interactions.

In this study, a range of galactomannans having contents of D-galactose varying from 48% to 17% are compared. The temperature dependence of optical rotation is extremely sensitive to polysaccharide interactions in mixed agarose/galactomannan systems, and this parameter can be significantly different for galactomannans having closely similar contents of D-galactose[16]. The degree and strength of the interactions of the galactomannans and agarose were therefore monitored by this technique. The strength of the interaction between the galactomannans and xanthan was measured using non-destructive rheological testing of the mixed gels.

EXPERIMENTAL

Materials. — Seeds of *Ceratonia siliqua, Sophora japonica, Caesalpinia vesicaria, Gleditsia triacanthos, Caesalpinia pulcherima, Cyamopsis tetragonolobus, Leucaena leucocephala, Medicago sativa,* and *Trigonella foenum-graecum* were obtained as previously described[13], and the galactomannans were purified as described[15]. The agarose used was a production batch (No. 202) obtained from Seravac Labs, was essentially free of substituents, and was that used in earlier investigations[6]. The extracellular polysaccharide from *Xanthomonas campestris* was a commercial sample of xanthan obtained from Kelco Co. and was also used in earlier investigations[6,8]. *A. niger* β-D-mannanase was purified[17] by affinity chromatography on mannan–AH-Sepharose.

Hydrolysis of galactomannan by β-D-mannanase. — To a 0.4% solution of galactomannan in 20 mM acetate buffer (pH 4.5) was added β-D-mannanase (400 nkat/g of galactomannan), and the solution was incubated at 40° for 20 h. Reaction was terminated by heating at 100° for 10 min, and the solution was centrifuged (20,000*g*, 30 min), then concentrated under reduced pressure (below 40°), and adjusted to 4% carbohydrate. Aliquots (2–6 mL) were fractionated on Bio-Gel P-2 (5.0-mL fractions). Aliquots were removed for the determination of total carbohydrate by the anthrone procedure[19]. Oligosaccharides were characterised as previously described[15].

Computer studies. — Computer programmes described previously[13] were used to simulate the synthesis of galactomannan by chain extension. The simulated galactomannan had a distribution of D-galactosyl groups biased towards a particular pattern in response to nearest-neighbour/second-nearest-neighbour interaction. Four probabilities are involved, namely, P_{00}, P_{01}, P_{10}, and P_{11} (where 0 represents

an unsubstituted D-mannosyl residue and 1 a substituted residue). These probabilities were optimised by minimisation of the sums of the squared differences between a range of supplied experimental data and the corresponding computed values. The supplied experimental data comprised the content of D-galactose of the galactomannan, the degree of hydrolysis of the galactomannan by *A. niger* β-D-mannanase, and the amounts and structures of the oligosaccharides of d.p. 2–7 released. The computed values for the amounts and structures of the oligosaccharides were obtained by subjecting the simulated galactomannan to "attack" by computer-simulated *A. niger* β-D-mannanase using the known action pattern and sub-site binding requirements of the enzyme.

D-Galactose/D-mannose ratios of galactomannans. — These ratios were determined by g.l.c. of the alditol acetates[20] and also determined enzymically[15].

Freeze–thaw treatment of galactomannans. — A measurement of the ability of galactomannans to self-associate was obtained by quantifying the amount of galactomannan precipitated after freeze–thaw treatment. Galactomannan solutions (0.1%) were quiescently frozen to −20°. After 24 h, the samples were melted at room temperature and the precipitated material was removed by using a bench centrifuge. The proportion of galactomannan precipitated was determined by comparing the contents of polysaccharide of the supernatant and the original solution, using the phenol–sulfuric acid method[21].

Optical rotation measurements. — These were carried out at 436 nm on agarose–galactomannan mixtures, using a Perkin–Elmer 241 polarimeter and 10-cm cells. The experimental procedures and calculations employed have been described[4,6].

Rheological characterisation of the interaction of galactomannan and xanthan. — Samples were prepared to give a concentration of 1% of galactomannan and 0.5% of xanthan. The constituents were dispersed (Atomix) in water (15 mL), and the dispersions were autoclaved for 5 min at 120°, mixed (Atomix), and then centrifuged (3000 r.p.m., 2 min) to remove bubbles. The gels formed were remelted in a hot-water bath and stirred to ensure homogeneity. Measurements were made on a Rheometrics Mechanical Spectrometer. Samples were poured onto the plate hot, and the cone was lowered before the gel set. Measurements of the storage modulus (G′) were made at 25°. The temperature was then raised and G′ was measured with increasing temperature up to 70° so that the melting temperature of the gel could be estimated.

Intrinsic viscosity. — Measurements were made using an Ubbelohde suspended-level viscometer[6].

RESULTS

Self association of galactomannans. — Evidence that the extent of substitution by D-galactosyl groups can affect the self-association properties of galactomannans was obtained from the solubility properties of the galactomannans from

Ceratonia siliqua (25% D-galactose) and *Cyamopsis tetragonolobus* (40% D-galactose). The former has to be heated to 90° for complete dissolution, whereas the latter is completely soluble at room temperature. Further evidence for differences in self association was obtained from freeze–thaw treatments. Thus, for *Cyamopsis tetragonolobus* galactomannan solutions of up to 2% concentration, there was no evidence of permanent chain association since solutions with unchanged viscosity properties were recovered. In contrast, the *Ceratonia siliqua* galactomannan formed a weak but cohesive gel network at a concentration as low as 0.5%. On lowering the concentration to 0.25%, the self association of *Ceratonia siliqua* galactomannan on freeze–thaw treatment resulted in the formation of gel islands, and, at 0.1%, a gelatinous precipitate was formed.

This behaviour associated with freeze–thaw treatment permits a quantitative comparison of galactomannan self-association. The percentage of galactomannan precipitated on freeze–thaw treatment of 0.1% solutions for a range of ten different galactomannans was therefore examined. The results (Table I) confirm the general relationship between increase in self-association and decrease in the content of D-galactose. The two most self-associating galactomannans were the hot-water-soluble fraction from *Ceratonia siliqua* and the galactomannan from *Sophora japonica*. The former showed a small but significantly higher degree of freeze–thaw-induced precipitation. Each galactomannan contained 18% of D-galactose, and the patterns of the amounts of oligosaccharides produced on hydrolysis by *A. niger* β-D-mannanase[15], together with the degrees of hydrolysis, are shown in Fig. 1 and Table II. Using the computer programme employed in earlier studies of the elucidation of the fine structure of *Ceratonia siliqua* galactomannan[13], it was found that the distribution of D-galactosyl stubs along the mannan backbone of *Sophora japonica* galactomannan was essentially statistically random. The values for the P_{00}, P_{01}, P_{10}, and P_{11} probabilities for the *Sophora japonica* and hot-water-soluble *Ceratonia siliqua* galactomannans were 0.22, 0.22, 0.22, and 0.22, and 0.19, 0.14, 0.34, and

TABLE I

SELF-ASSOCIATION PROPERTIES OF A RANGE OF GALACTOMANNANS AFTER FREEZE–THAW TREATMENT

Galactomannan sample	*Galactose content (%)*	*Intrinsic viscosity (dL/g)*	*Amount precipitated on freeze–thaw treatment (%)*
Medicago sativa	48	13.0	0
Trigonella foenum-graecum	48	—	0
Leucaena leucocephala	40	11.0	0
Cyamoposis tetragonolobus	40	14.3	0
Caesalpinia vesicaria	29	13.3	7
Gleditsia triacanthos	27	13.8	9
Ceratonia siliqua	25	9.9	37
Caesalpinia pulcherima	24	11.1	6
Ceratonia siliqua (hot-water-soluble fraction)	19	11.7	88
Sophora japonica	17	15.7	82

TABLE II

OLIGOSACCHARIDES RELEASED ON HYDROLYSIS OF THE GALACTOMANNANS FROM *Sophora japonica* AND THE HOT-WATER-SOLUBLE FRACTION OF *Ceratonia siliqua* BY *A. niger* β-D-MANNANASE

Measured parameter	*Experimental data*	*Theoretical data*	
		Random model	*Nearest-neighbour/second-nearest-neighbour model*
Sophora japonica			
Man_2 + Man_3 (wt.%)	40	40	40
Gal^1Man_2	24	20	21
Gal^1Man_3	12	14	16
$Gal^{3,4}Man_5$	7	4	4
>Heptasaccharide	17	22	19
D-Galactose content (%)	17	18	18
Degree of hydrolysis (%)	30	31	31
Goodness of fit index (S)[a]		31	36
Best fully random model	P_{00} 0.22, P_{01} 0.22, P_{10} 0.22, P_{11} 0.22		
Best non-random model	P_{00} 0.24, P_{01} 0.19, P_{10} 0.19, P_{11} 0.14		
The structure does not deviate significantly from fully random.			
Hot-water-soluble fraction of Ceratonia siliqua			
Man_2 + Man_3 (wt.%)	45	42	44
Gal^1Man_2	16	20	14
Gal^1Man_3	7	14	10
$Gal^{3,4}Man_5$	11	4	8
>Heptasaccharide	21	20	24
D-Galactose content (%)	19	17	16
Degree of hydrolysis (%)	26	31	30
Goodness of fit index (S)[a]		152	48
Best fully random model	P_{00} 0.21, P_{01} 0 21, P_{10} 0.21, P_{11} 0.21		
Best non-random model	P_{00} 0.19, P_{01} 0.14, P_{10} 0 34, P_{11} 0.06		
The non-random model is significantly preferred.			

[a] $S = \Sigma(\text{Obs.} - \text{Calc.})^2$ for all independent observations.

0.06, respectively. The significantly non-regular, non-statistically random structure of the *Ceratonia siliqua* galactomannan was biased towards substituted couplet and against triplet structures.

The differences between the fine structures of these two galactomannans are illustrated by plotting the calculated weight distributions for the lengths of the unsubstituted D-mannan sequences as a function of increasing length (Fig. 2). From these data, the weight-average length of unsubstituted D-mannose sequences was calculated as 7.9 for *Sophora japonica* galactomannan and 9.4 for the hot-water-soluble *Ceratonia siliqua* galactomannan. In addition, the weight fraction of unsubstituted D-mannose sequences longer than 10 was 0.25 and 0.35 for *Sophora japonica* and the *Ceratonia siliqua* galactomannans, respectively. This greater

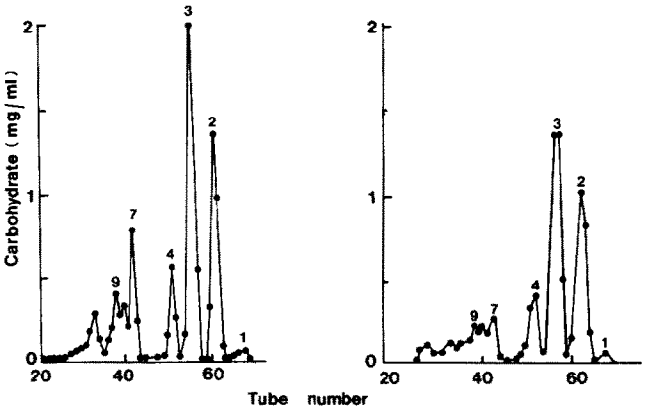

Fig. 1. Elution (5-mL fractions), from a column (2.5 × 90 cm) of Bio-Gel P2 (<400 mesh) with distilled water at 60°, of the oligosaccharides produced on hydrolysis of the hot-water-soluble fraction of *Ceratonia siliqua* galactomannan (left) and *Sophora japonica* galactomannan (right) by *A. niger* β-D-mannanase.

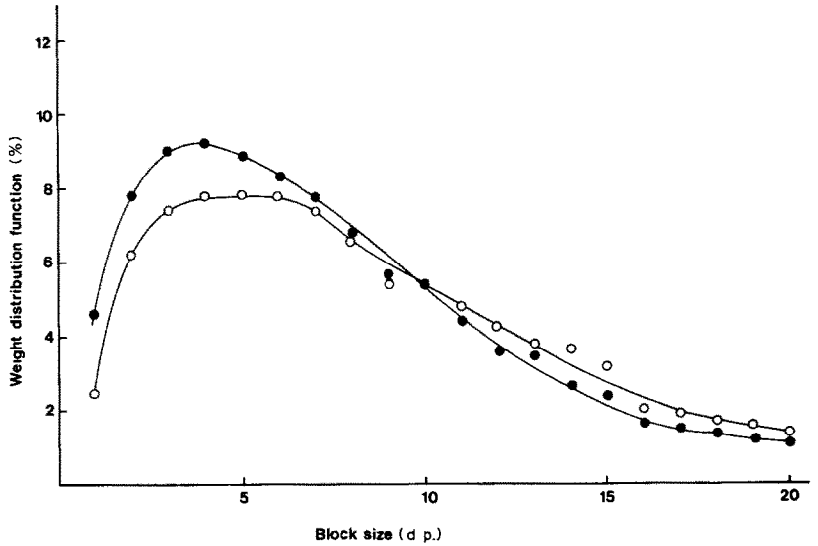

Fig. 2. The theoretical distribution functions of unsubstituted D-mannan block-size (*i.e.*, the weight of unsubstituted blocks of a particular d.p. as a percentage of the weight of all unsubstituted blocks) for the best models for *Sophora japonica* galactomannan (●, P_{00} 0.22, P_{01} 0.22, P_{10} 0.22, P_{11} 0.22) and the hot-water-soluble fraction of *Ceratonia siliqua* galactomannan (○, P_{00} 0.19, P_{01} 0.14, P_{10} 0.34, P_{11} 0.06).

average length of unsubstituted D-mannose sequences and the higher frequency of unsubstituted D-mannose sequences of intermediate length for the *Certonia siliqua* galactomannan were accompanied by a greater freeze–thaw-induced precipiation compared with *Sophora japonica* galactomannan (88% and 82%).

The most striking result is a comparison of the data in Table I for the galactomannans from *Ceratonia siliqua* (25% D-galactose) and *Caesalpinia pulcherima*

(24% D-galactose). Although *Caesalpinia pulcherima* galactomannan has a content of D-galactose similar to that of the *Ceratonia siliqua* galactomannan, significantly less was precipitated on freeze–thaw treatment. This significant difference in self-association was not a result of large differences in molecular weight since the galactomannans had closely similar intrinsic viscosities. Examination of the array of oligosaccharides produced by *A. niger* β-D-mannanase hydrolysis, together with the degree of hydrolysis, indicated that the two galactomannans differed significantly in the distribution of D-galactosyl groups along the main chain (see Fig. 3 and Table III). Using the above computer programme, it was found that the nearest-neighbour/second-nearest-neighbour models required to match the experimental data for released oligosaccharides were very different for the two galactomannans, as evidenced by the values of the P_{00}, P_{01}, P_{10}, and P_{11} probabilities (Table III).

From these probabilities, it can be seen that *Caesalpinia pulcherima* galactomannan had essentially a statistically random distribution of D-galactose along the mannan backbone. As reported previously[13], the *Ceratonia siliqua* galactomannan had a non-regular, non-statistically random distribution of D-galactose, with a higher proportion of substituted couplets and of unsubstituted blocks of intermediate length than for a statistically random distribution. The data reported here accord with the earlier findings. The difference between the fine structures of these two galactomannans is illustrated by plotting the calculated weight distributions for the lengths of unsubstituted D-mannose sequences as a function of increasing length (Fig. 4). From these data, the weight-average length of unsubstituted D-mannose sequences was calculated as 6.4 for *Ceratonia siliqua* galactomannan and 5.6 for *Caesalpinia pulcherima* galactomannan. In addition, the weight fractions of unsubstituted D-mannose sequences longer than 10 were 0.14 and 0.10 for *Ceratonia*

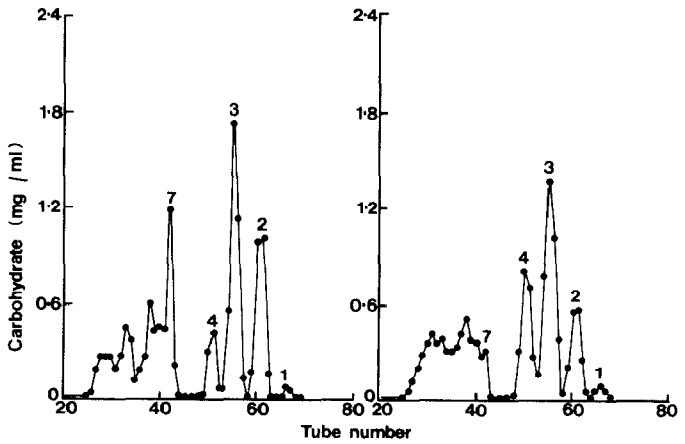

Fig. 3. Elution (5-mL fractions), from a column (2.5 × 90 cm) of Bio-Gel P2 (<400 mesh) with distilled water at 60°, of the oligosaccharides produced on hydrolysis of *Ceratonia siliqua* galactomannan (left) and *Caesalpinia pulcherima* galactomannan (right) by *A. niger* β-D-mannanase.

TABLE III

OLIGOSACCHARIDES RELEASED ON HYDROLYSIS OF THE GALACTOMANNANS FROM *Caesalpinia pulcherima* AND *Ceratonia siliqua* BY *A. niger* β-D-MANNANASE

Measured parameter	*Experimental data*	*Theoretical data*	
		Random model	*Nearest-neighbour/second-nearest-neighbour model*
Caesalpinia pulcherima			
Man_2 + Man_3 (wt.%)	26	26	25
Gal^1Man_2	16	18	16
Gal^1Man_3	16	13	12
$Gal^{3,4}Man_5$	8	5	7
>Heptasaccharide	34	38	40
D-Galactose content (%)	24	23	23
Degree of hydrolysis (%)	22	25	24
Goodness of fit index (S)[a]		32	23
Best fully random model	P_{00} 0.30, P_{01} 0.30, P_{10} 0.30, P_{11} 0.30		
Best non-random model	P_{00} 0.32, P_{01} 0.22, P_{10} 0.37, P_{11} 0.17		
The structure does not deviate significantly from fully random.			
Ceratonia siliqua			
Man_2 + Man_3 (wt.%)	33	29	29
Gal^1Man_2	14	19	11
Gal^1Man_3	7	13	9
$Gal^{3,4}Man_5$	15	5	11
>Heptasaccharide	31	34	40
D-Galactose content (%)	25	22	21
Degree of hydrolysis (%)	22	26	24
Goodness of fit index (S)[a]		202	65
Best fully random model	P_{00} 0.28, P_{01} 0.28, P_{10} 0.28, P_{11} 0.28		
Best non-random model	P_{00} 0.28, P_{01} 0.07, P_{10} 0.50, P_{11} 0.03		
The non-random model is significantly preferred.			

[a]See Table II.

siliqua and *Caesalpinia pulcherima* galactomannans, respectively. This higher proportion of unsubstituted blocks of intermediate length is consistent with the finding that the galactomannan from *Ceratonia siliqua* self-associates to a much greater extent than that from *Caesalpinia pulcherima*.

Comparison of the galactomannans from Ceratonia siliqua *and* Caesalpinia pulcherima *in their interaction with xanthan and agarose.* — Since these two galactomannans have already been shown to differ significantly in the distribution of D-galactosyl groups along the D-mannan backbone and in their ability to self-associate, it is reasonable to compare their interactive properties with other polysaccharides. Each galactomannan formed a firm, rubbery gel on admixture with xanthan. However, comparison of the storage modulus (G′) of mixed galacto-

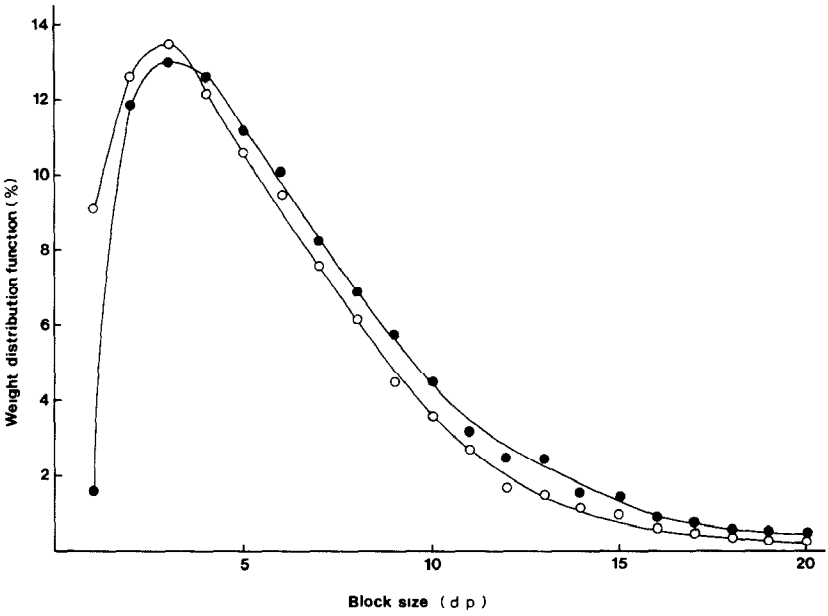

Fig 4. The theoretical distribution functions of unsubstituted D-mannan block-size (*i.e.*, the weight of unsubstituted blocks of a particular d.p. as a percentage of the weight of all unsubstituted blocks) for the best models for *Ceratonia siliqua* galactomannan (●, P_{00} 0.28, P_{01} 0.07, P_{10} 0.50, P_{11} 0.03) and *Caesalpinia pulcherima* galactomannan (○, P_{00} 0.30, P_{01} 0.30, P_{10} 0.30, P_{11} 0.30).

TABLE IV

INTERACTION PROPERTIES OF A RANGE OF GALACTOMANNANS WITH XANTHAN AND AGAROSE

Galactomannan sample	*Galactose content (%)*	*Storage modulus*[a] *($d.cm^{-2} \times 10^{-3}$)*	*Gel m.p.*[a] *(degrees)*	*Optical rotation change*[b] *(degrees)*
Caesalpinia pulcherima	24	2.5	32–33	0.025
Ceratonia siliqua	25	3.4	38–40	0.037
Gleditsia triacanthos	27	1 4	30–31	0.023
Caesalpinia vesicaria	29	0.7	30–31	0.016
Leucaena leucocephala	40	[c]	[c]	0.019
Cyamopsis tetragonolobus	40	[c]	[c]	0.008

[a]Obtained from examination of mixed gels of xanthan (0.5%) and galactomannan (1.0%). [b]Positive contribution to optical rotation change from galactomannan at 436 nm. Obtained from the temperature dependence of optical rotation traces for mixtures of agarose (0.05%) and galactomannan (0.3%). See Figs. 5, 6, and 9. [c]Not determined.

mannan (1%)–xanthan (0.5%) gels indicated that the gel formed with *Ceratonia siliqua* galactomannan was significantly firmer (Table IV). Furthermore, the melting temperature of these gels can be determined by measuring the temperature dependence of G′, and the gel formed with *Ceratonia siliqua* galactomannan was more temperature-stable (Table IV).

Examination of mixed agarose–galactomannan gelling systems also indicated the *Ceratonia siliqua* galactomannan to be the more interactive. The temperature dependences of optical rotation for the two agarose (0.05%)–galactomannan (0.3%) mixed systems are shown in Fig. 5. The lower degree of interaction of the *Caesalpinia pulcherima* galactomannan was demonstrated by the smaller positive contribution to the change in optical rotation on cooling the mixed system[6] for this galactomannan (Fig. 5, Table IV). In addition, the positive rise in optical rotation on cooling the *Ceratonia siliqua* galactomannan–agarose mixture showed significantly wider hysteresis, indicating that the stability of the interaction in this system was greater. This observation is consistent with the higher melting-temperatures for the mixed *Ceratonia siliqua* galactomannan–xanthan gels.

As discussed above, the distributions of D-galactosyl groups along the main chains of these two galactomannans are significantly different. *Caesalpinia pulcherima* galactomannan has a distribution approaching statistically random, whereas *Ceratonia siliqua* galactomannan has a non-regular, non-statistically random distribution with a higher proportion of unsubstituted blocks of intermediate length than the *Caesalpinia pulcherima* galactomannan. This is consistent with the greater extent and stability of the interaction of *Ceratonia siliqua* galactomannan with agarose and xanthan.

Comparison of the galactomannans from Gleditsia triacanthos *and* Caesalpinia vesicaria *in their interaction with xanthan and agarose.* — On admixture with xanthan over a range of concentrations, the galactomannan from *Gleditsia triacanthos* gave mechanically strong gels, whereas the gels made with the galactomannan from *Caesalpinia vesicaria* were considerably weaker. Comparison of the storage modulus of mixed galactomannan (1%)–xanthan (0.5%) gels confirmed that the gel formed with *Caesalpinia vesicaria* galactomannan was significantly weaker (Table IV).

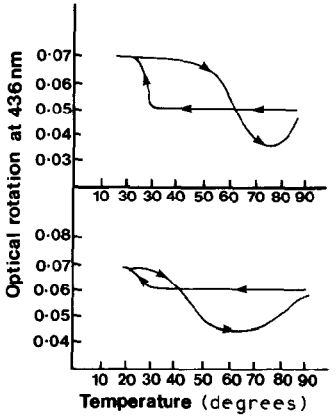

Fig. 5. Comparison of the variations in optical rotation with temperature for (top) a mixture of agarose (0.05%) and *Ceratonia siliqua* galactomannan (0.3%), and (bottom) a mixture of agarose (0.05%) and *Caesalpinia pulcherima* galactomannan (0.3%).

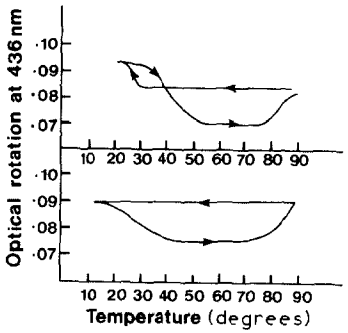

Fig. 6. Comparison of the variations in optical rotation with temperature for (top) a mixture of agarose (0.05%) and *Gleditsia triacanthos* galactomannan (0.3%), and (bottom) a mixture of agarose (0.05%) and *Caesalpinia vesicaria* galactomannan (0.3%).

The temperature dependences of optical rotation for the two agarose (0.05%)–galactomannan (0.3%) mixed systems are shown in Fig. 6. The low ability of *Caesalpinia vesicaria* galactomannan to interact with agarose is clearly seen and quantified by the very small positive contribution to the change in rotation on cooling the mixed system for this galactomannan (Table IV), and the virtual absence of a hysteresis loop (Fig. 6).

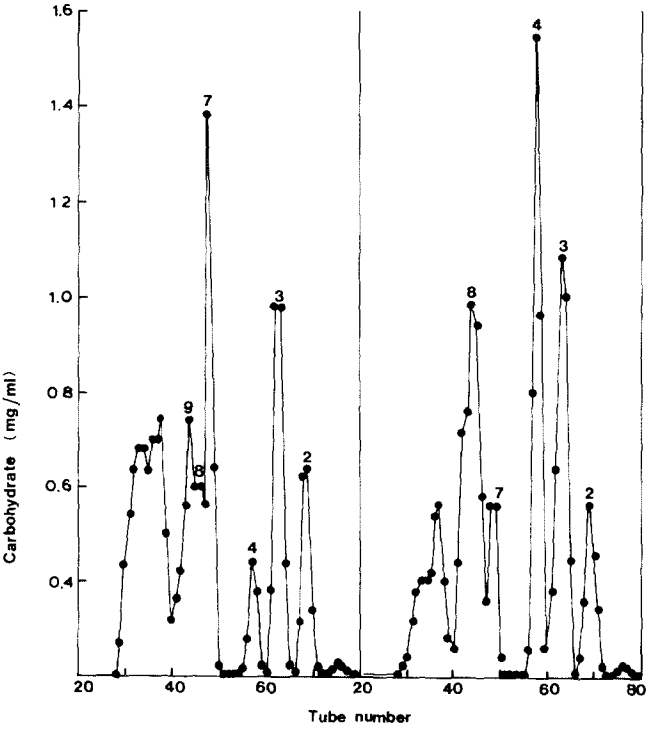

Fig. 7. Elution (5-mL fractions), from a column (2.5 × 90 cm) of Bio-Gel P2 (<400 mesh) with distilled water at 60°, of the oligosaccharides produced on hydrolysis of *Gleditsia triacanthos* galactomannan (left) and *Caesalpinia vesicaria* galactomannan (right) by *A. niger* β-D-mannanase

TABLE V

OLIGOSACCHARIDES RELEASED ON HYDROLYSIS OF THE GALACTOMANNANS FROM *Gleditsia triacanthos* AND *Caesalpinia vesicaria* BY *A. niger* β-D-MANNANASE

Measured parameter	*Experimental data*	*Theoretical data*	
		Random model	*Nearest-neighbour/second-nearest-neighbour model*
Gleditsia triacanthos			
$Man_2 + Man_3$ (wt.%)	17	15	20
Gal^1Man_2	9	15	10
Gal^1Man_3	5	10	6
$Gal^{3,4}Man_5$	16	5	11
>Heptasaccharide	53	55	53
D-Galactose content (%)	27	28	24
Degree of hydrolysis (%)	18	19	20
Goodness of fit index (S)[a]		188	49
Best fully random model		P_{00} 0.39, P_{01} 0.39, P_{10} 0.39, P_{11} 0.39	
Best non-random model		P_{00} 0.32, P_{01} 0.27, P_{10} 0.57, P_{11} 0.00	
The non-random model is significantly preferred.			
Caesalpinia vesicaria			
$Man_2 + Man_3$ (wt.%)	12	19	13
Gal^1Man_2	14	17	16
Gal^1Man_3	23	11	23
$Gal^{3,4}Man_5$	7	5	5
>Heptasaccharide	44	48	43
D-Galactose content (%)	29	26	26
Degree of hydrolysis (%)	19	22	22
Goodness of fit index (S)[a]		224	27
Best fully random model		P_{00} 0.35, P_{01} 0.35, P_{10} 0.35, P_{11} 0.35	
Best non-random model		P_{00} 0.59, P_{01} 0.14, P_{10} 0.29, P_{11} 0.09	
The non-random model is significantly preferred.			

[a]See Table II.

It is difficult to account for the very low interactive properties of *Caesalpinia vesicaria* galactomannan with agarose and xanthan on the basis of its slightly higher content of D-galactose (29% compared with 27% for *Gleditsia triacanthos* galactomannan). In addition, its poorer interaction properties cannot be explained by differences in molecular weight since it has an intrinsic viscosity comparable to that of *Gleditsia triacanthos* galactomannan. Therefore, it appears that differences in fine structure play a role in determining the extent and stability of the interactive properties for this pair of galactomannans. Examination of the array of oligosaccharides produced by *A. niger* β-D-mannanase hydrolysis, together with the degrees of hydrolysis, indicated that these two galactomannans differed significantly in the distribution of D-galactosyl groups along the main chain (see Fig. 7 and

Table V). Using the computer programme employed in the elucidation of the fine structure of *Ceratonia siliqua* galactomannan[13], it was found that the nearest-neighbour/second-nearest-neighbour models required to match the experimental data for released oligosaccharides were very different for the two galactomannans, as can be seen from the values for the P_{00}, P_{01}, P_{10}, and P_{11} probabilities of 0.32, 0.27, 0.57, and 0.00 for *Gleditsia triacanthos* galactomannan, and 0.59, 0.14, 0.29, and 0.09 for *Caesalpinia vesicaria* galactomannan.

For both galactomannans, the best models were significantly non-regular and non-statistically random. However, the non-random nature of the structures are different. For the *Gleditsia triacanthos* galactomannan, there is a bias towards -0110- structures and against -101- and -111- structures (where 1 represents a D-mannose residue substituted by D-galactose, and 0 represents an unsubstituted D-mannose residue). In contrast, for the *Caesalpinia vesicaria* galactomannan, there

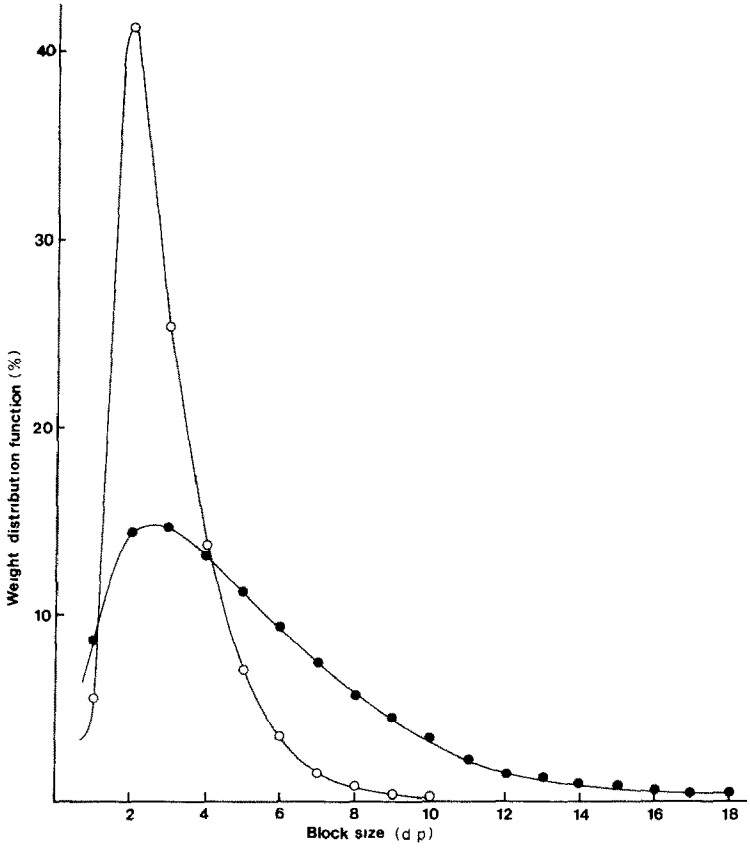

Fig. 8 The theoretical distribution functions of unsubstituted D-mannan block-size (*i.e.*, the weight of unsubstituted blocks of a particular d.p. as a percentage of the weight of all unsubstituted blocks) for the best models for *Gleditsia triacanthos* galactomannan (●, P_{00} 0.32, P_{01} 0.27, P_{10} 0.57, P_{11} 0.00) and *Caesalpinia vesicaria* galactomannan (○, P_{00} 0.59, P_{01} 0.14, P_{10} 0.29, P_{11} 0.09)

is a bias towards -00100- structures, a bias against -101- and -0110- structures, and a strong bias against -111- structures. These differences are particularly well illustrated by plotting the calculated weight distribution for the lengths of unsubstituted D-mannose sequences as a function of increasing chain length for the galactomannans (Fig. 8). From these data, the weight-average length of unsubstituted sequences was calculated as 5.3 for *Gleditsia triacanthos* galactomannan, but only 3.0 for *Caesalpinia vesicaria* galactomannan. In addition, the weight fractions of unsubstituted D-mannose sequences longer than 5 and 10 were 0.38 and 0.08 for *Gleditsia triacanthos* galactomannan, compared with values of 0.06 and 0.00 for *Caesalpinia vesicaria* galactomannan. Therefore, it is clear that the galactomannan from *Caesalpinia vesicaria* has a significantly lower frequency of unsubstituted D-mannose sequences of intermediate length compared with that of *Gleditsia triacanthos* galactomannan, and this major difference in fine structure is consistent with the different extents of interaction with xanthan and agarose.

Comparison of the galactomannans from Cyamopsis tetragonolobus *and* Leucaena leucocephala *in their interaction with xanthan and agarose.* — The interactive properties of the galactomannans from *Cyamopsis tetragonolobus* and *Leucaena leucocephala* have been compared visually, over a wide concentration range, with xanthan. The *Leucaena* galactomannan exhibited a significantly stronger interaction with xanthan since, even at quite low concentrations (0.1% galactomannan, 0.1% xanthan), very weak but cohesive gels are formed. In agreement with previous findings[8,22], *Cyamopsis tetragonolobus* galactomannan did not form gels with xanthan at any concentration. This demonstration of greater interactive properties for *Leucaena leucocephala* galactomannan agrees with an earlier report in which the strength of interaction of xanthan and galactomannan was measured using a Brabender Amylograph, a Brookfield Synchro-Lectric Viscometer, and a salt precipitation technique[10].

The temperature dependence of optical rotation for the two agarose (0.05%)–galactomannan (0.3%) mixed systems is shown in Fig. 9. A greater interactive ability of the *Leucaena* galactomannan was indicated by the presence of a net

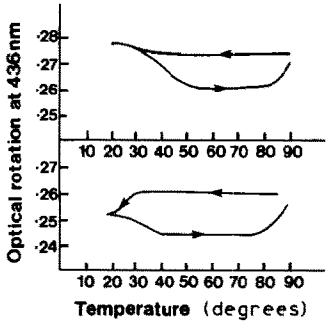

Fig. 9. Comparison of the variations in optical rotation with temperature for (top) a mixture of agarose (0.05%) and *Leucaena leucocephala* galactomannan (0.3%), and (bottom) a mixture of agarose (0.05%) and *Cyamopsis tetragonolobus* galactomannan (0.3%).

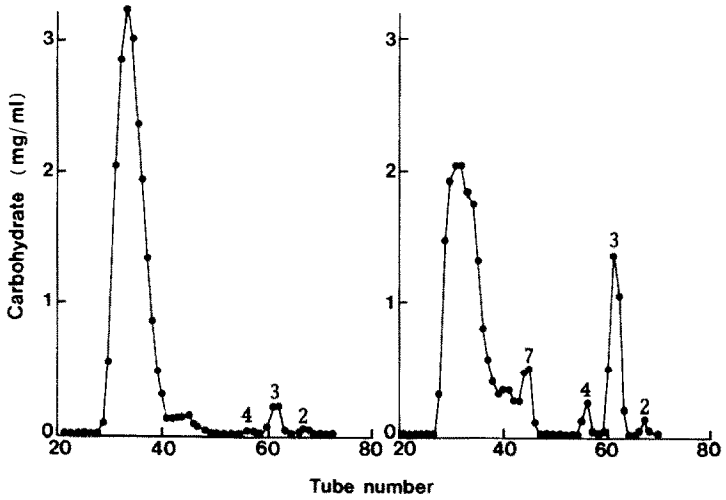

Fig. 10. Elution (5-mL fractions), from a column (2.5 × 90 cm) of Bio-Gel P2 (<400 mesh) with distilled water at 60°, of the oligosaccharides produced on hydrolysis of *Cyamopsis tetragonolobus* galactomannan (left) and *Leucaena leucocephala* galactomannan (right) by *A. niger* β-D-mannanase.

positive change in optical rotation on cooling the agarose–galactomannan mixture, compared with only a reduced negative change in optical rotation for the agarose–*Cyamopsis tetragonolobus* galactomannan mixture. The greater interactive properties of the *Leucaena* galactomannan are emphasised by the values of the positive contributions to optical rotation change listed in Table IV.

The significantly superior interactive properties of *Leucaena leucocephala* galactomannan are not caused by differences in molecular weight since *Cyamopsis tetragonolobus* galactomannan has a substantially higher intrinsic viscosity (Table I). However, the action of *A. niger* β-D-mannanase on these two galactomannans was quite different. The degree of hydrolysis was almost twice as great for the *Leucaena* galactomannan (10% compared with 5%). Furthermore, examination of the array of oligosaccharides produced by the action of β-D-mannanase indicated large differences between the galactomannans (Fig. 10). The hydrolysate of the *Leucaena* galactomannan contained high proportions of trisaccharide (almost exclusively 6^1-α-D-galactosyl-β-D-mannobiose). The high molar proportion of this oligosaccharide (~16% of the hydrolysate) indicated that there must have been frequent regions of the main chain in this galactomannan in which every second D-mannosyl residue was substituted by D-galactose, and which, therefore, in the ordered two-fold ribbon conformation, would have unsubstituted sides[8,10,23,24].

The enzyme hydrolysate of *Cyamopsis tetragonolobus* galactomannan contained a much smaller amount of this trisaccharide, and consequently this galactomannan had many fewer regions in the main chain with every second D-mannosyl residue substituted with D-galactose. Since these two galactomannans are heavily substituted with D-galactose, they will have no substantial regions of main chain unsubstituted by D-galactose (confirmed by the virtual absence of mannobiose and

mannotriose in the β-D-mannanase hydrolysate). The basis of their ability to interact with agarose and xanthan therefore arises from binding of regions of the main chain, with alternating D-galactose substitution, *via* their unsubstituted sides in the junction zone. The differences in fine structure between the two galactomannans, outlined above, therefore account for their very different interactions with xanthan and agarose.

DISCUSSION

The results presented here are consistent with previous reports[3–5,8–10] that, in general, the most effective galactomannans in the co-gelling interactions with agarose and xanthan are those in which the mannan main-chain carries fewest D-galactosyl stubs. However, the present results also indicate that the distribution of D-galactosyl groups along the main chain can have a significant effect on the interactive properties of galactomannans.

Prior to this study, there had only been one report[10] concerning the fine structure of galactomannans having a major effect on the interaction properties. In this earlier study, two heavily substituted (40% galactose) galactomannans were compared. The rheological interaction of xanthan and *Leucaena leucocephala* galactomannan was much stronger than that with *Cyamopsis tetragonolobus* galactomannan. This difference has now been confirmed. *Leucaena leucocephala* galactomannan–xanthan mixtures form cohesive, although weak, gels down to concentrations of 0.1% galactomannan plus 0.1% xanthan, whereas, at all the concentrations studied, *Cyamopsis tetragonolobus* galactomannan only increased the viscosity properties of xanthan. Also, the superior interaction properties of the *Leucaena* galactomannan extend to the co-gelling interaction with agarose. Enzymic hydrolysis of the two galactomannans indicated major differences in the distribution of D-galactosyl groups along the main chain. Thus, *Leucaena leucocephala* galactomannan had a significant proportion of the chain in which alternative mannosyl residues are substituted by D-galactose, so that, in the ordered two-fold ribbon conformation, one side of these segments of the main chain is unsubstituted. *Cyamopsis tetragonolobus* galactomannan had a much more non-regular distribution of D-galactosyl stubs along the main chain. These findings are consistent with previous proposals[8,10,24] that such heavily substituted galactomannans interact with other polysaccharides by binding of the unsubstituted sides of exactly alternating regions of the molecules in the mixed polysaccharide junction zones.

This study also indicates that the effect of distribution of D-galactosyl groups along the D-mannan backbone on the interaction properties of galactomannans is important at much lower contents of D-galactose. Thus, a pair of galactomannans with intermediate contents (27% and 29%) of D-galactose and a pair with low contents (24% and 25%) were studied, and the differences in interaction properties were shown to correlate with differences in fine structure. These differences involve

the relative frequency of unsubstituted blocks of intermediate length along the mannan backbone, and the results are consistent with the proposal[4] that galactomannans having intermediate-to-low contents of D-galactose interact with other polysaccharides by ordered conformation of unsubstituted blocks binding in mixed polysaccharide junction zones.

Perhaps the most striking illustration of the influence of the fine structure of galactomannans on interaction properties is the observation that differences in fine structure can result in galactomannans with a high content of D-galactose having interaction properties as good as or superior to those of galactomannans with a significantly lower content of D-galactose. Thus, the galactomannan from *Leucaena leucocephala* (40% galactose) has interaction properties similar to those of that from *Caesalpinia vesicaria* (29% galactose).

Although this study has been restricted to the effect of the fine structure of a galactomannan on self-association and the interactions with agarose and xanthan, it is reasonable to assume that fine structure will influence the interaction of galactomannans and kappa-carrageenan in the same way. In addition, it has been reported recently that galactomannans can influence the gelatinisation of starch[25]. This effect probably arises from an interaction of the double-helical-ordered conformation of the starch components[26] and an ordered-ribbon conformation of the galactomannans. This proposal is consistent with our observation[27] that the presence of small levels of galactomannan can significantly accelerate the retrogradation of amylose. Therefore, we would expect that differences in the patterns of D-galactosyl stubs would also influence the interactions of starch components and galactomannans.

The influence of the fine structure of galactomannans on interaction properties and the extension of this concept to related polysaccharides such as substituted (1→4)-β-D-glucans (*e.g.*, xyloglucan) and substituted (1→4)-β-D-xylans (*e.g.*, arabinoxylans) could be relevant to the function and properties of these polymers in their natural environments. Plant-seed galactomannans are found as food reserves in the endosperm of many leguminous plants where the galactomannan is laid down in abnormally expanded cell-wall tissue[28]. Galactomannans are therefore cell-wall polysaccharides, as are other substituted (1→4)-linked D-glycans (*e.g.*, xyloglucans, galactoglucomannans, arabinoxylans, glucuronoxylans). Such polysaccharides can be found in normal plant cell-walls in association with the linear polysaccharides cellulose, mannan, and glucomannan. These branched cell-wall components can bind strongly to cellulose[29,30] and this is probably an important factor in maintaining the strength and integrity of the cell wall.

Xanthan and related materials are extracellular polysaccharides from *Xanthomonas* species. These are pathogenic organisms which are responsible for blight diseases of several important crops. It has been proposed[24] that the specific binding between xanthan and a wide range of (1→4)-β-D-glycans, typically found as components of the plant cell-wall, is involved in host–pathogen recognition and attachment. The various extracellular polysaccharides from *Xanthomonas* species

all have the same basic structure but exhibit small structural differences. The present study indicates that the content of galactose and the distribution of D-galactosyl groups in galactomannans can have a significant effect on the interaction properties with xanthan. By analogy with the effect of structural variation of agarose polysaccharides on the interaction properties with galactomannans[9], it is reasonable to assume that the different *Xanthomonas* polysaccharides would exhibit differing binding properties with substituted (1→4)-β-D-glycans and this may play a role in the binding of pathogens.

The distribution of D-galactosyl groups along the mannan main-chain is a consequence of the *in vivo* synthetic mechanism, which most probably involves D-mannosyl and D-galactosyl transferases acting in concert, and would be most likely to vary slightly from species to species depending on structural differences around the active sites of the enzymes. Differences in the fine structures of these branched cell-wall polysaccharides would be expected to modify the interactions with cellulose, and the effect of fine structure on the interaction properties of galactomannans discussed here might be taken as a model for this phenomenon. This would account for the differences in fine structure between galactomannans having the same content of D-galactose from the seeds of different plant species. However, within a genetically stable species, the difference should be minimal and this had been demonstrated with the galactomannans from carob seed, where the galactomannan from several different varieties and from seed derived from numerous locations was studied. Although the contents of D-galactose varied slightly, and consequently so did the proportion of hot-water-soluble and cold-water-soluble fractions, the basic fine-structure remained the same[13]. Aspects of the likely mode of biosynthesis of galactomannan have been discussed separately[31].

ACKNOWLEDGMENTS

Part of this work was performed by Dr. B. V. McCleary as a visiting scientist at Unilever Research, Colworth Laboratory, and he thanks Unilever for financial support and the Nuffield Foundation for a Travelling Fellowship. We also thank Mr. J. Windust, Mrs. W. Shepperson, Mr. D. Cooke, and Mrs. E. Nurthen for valuable technical assistance.

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