Carbohydrate Polymers **13** (1990) 57–68

A Cellulose-Like Conformation Accessible to the Xanthan Backbone and Implications for Xanthan Synergism

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(Received 19 May 1989; revised version received 10 July 1989; accepted 18 July 1989)

ABSTRACT

Molecular modelling has been used to show that branching at the 3-position of every second glucose unit in the backbone of the xanthan molecule does not preclude the backbone from adopting a two-fold cellulose-like conformation. A stereochemically acceptable model of this type is defined with a pitch of 10·51 Å (slightly longer than that of cellulose). This is a model structure for the conformation of xanthan in cooperative interactions with galactomannans, that has been proposed as the molecular basis for the synergistic (gelling) properties of these two polysaccharides in solution.

INTRODUCTION

Xanthan gum is an anionic extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* that is widely exploited commercially (Sandford & Baird, 1983). It has a number of unusual rheological properties that include high viscosity and pseudoplasticity, that are relatively insensitive to temperature and pH. It is used as a thickening agent in the food industry, for mobility control in secondary and tertiary oil recovery, in petroleum drilling fluids, and in the paint, pharmaceutical and cosmetic industries.

The primary structure of xanthan is a $\beta(1 \rightarrow 4)$ linked D-glucose (cellulosic) mainchain with a trisaccharide sidechain attached to the 3-position of every second glucose, giving a pentasaccharide repeating unit (Fig. 1). The sidechain α- and β-linked mannose units are specifically and

Carbohydrate Polymers 0144-8617/90/$03.50

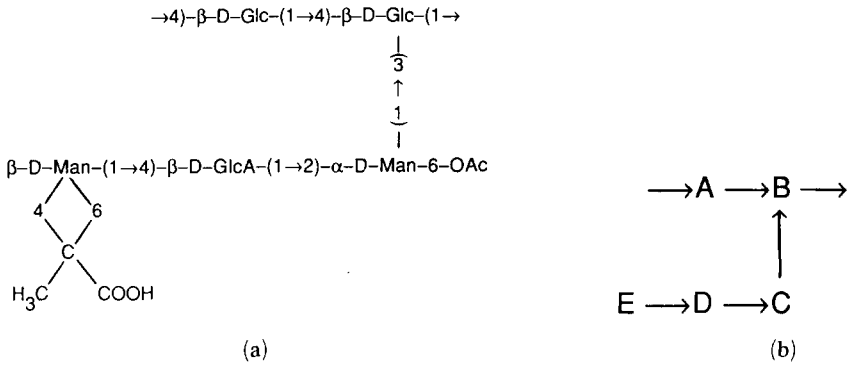

Fig. 1. (a) Chemical repeat of xanthan and (b) labelling of the sugar residues.

variably acetylated and pyruvylated respectively (Jansson *et al.*, 1975; Melton *et al.*, 1976). In aqueous solution, xanthan undergoes a thermally induced cooperative, conformational transition (Rees, 1972) that has been monitored by a number of physical techniques including optical rotation, viscometry, NMR, calorimetry and potentiometric titration (Rees, 1972; Morris *et al.*, 1977; Milas & Rinaudo, 1979). Although xanthan has been the subject of many physicochemical studies, the details of the ordered structure, particularly the number of chains involved, are still the subject of lively debate (Norton *et al.*, 1984; Milas & Rinaudo, 1986; Liu & Norisuye, 1988).

X-ray diffraction studies of xanthan show that in the solid state, it forms a helical structure with five-fold screw symmetry and a molecular repeat distance of 47 Å in hydrated fibers (Moorhouse *et al.*, 1977*a*; Okuyama *et al.*, 1980; Millane *et al.*, 1989). The diffraction data are insufficient however to define the molecular structure precisely, and determine the number of chains involved. There is evidence that the structure observed in the solid state corresponds to the ordered conformation in solution (Morris *et al.*, 1977). X-ray fibre diffraction studies of variant xanthan gums in which one and two of the sidechain terminal sugar units are absent show that these polymers adopt structures with helix pitch and symmetry identical to that of xanthan (Millane & Narasaiah, 1990; Millane *et al.*, 1989, 1990).

Although xanthan solutions exhibit 'weak gel' properties at low shear rates (that make it particularly suitable as a suspending agent), xanthan does not form true gels at any temperature or concentration. However, xanthan does gel when mixed with galactomannans such as carob, tara and enzymatically modified guar gums (Dea & Morrison, 1975; Dea *et al.*, 1977; Morris *et al.*, 1977). Many industrial applications of xanthan

exploit this synergistic behaviour with galactomannans. Since the galactomannans do not gel alone, this behaviour has been attributed to cooperative interactions between the unlike polysaccharides, resulting in mixed junction zones and thence network formation (Dea & Morrison, 1975; Dea *et al.*, 1977; Morris *et al.*, 1977). The degree of synergism (as measured by gel strength) increases with decreasing degree of galactose substitution of the galactomannan (Dea & Morrison, 1975; Morris *et al.*, 1977). This suggest that such cooperative interactions occur between xanthan and unsubstituted stretches of the galactomannan. Also, the strength of the interaction is sensitive to the galactomannan fine structure. Higher gel strengths are observed for galactomannans with higher proportions of unsubstituted blocks, and also for those that have frequent regions in which every second mannose is substituted with galactose (Morris *et al.*, 1977; McCleary, 1979; Dea *et al.*, 1986). Furthermore, some workers have reported that gel formation occurs only if the mixed polysaccharide solution is first heated above the xanthan order–disorder transition temperature, and then cooled (Cairns *et al.*, 1986, 1987; Cheetham & Mashimba, 1988). They interpret these results as showing that the interactions occur with xanthan that is not in the ordinary ordered or helix conformation, but one in which the backbone is presumably in an extended two-fold cellulose-like conformation.

Cairns *et al.* (1986, 1987) reported that X-ray fibre diffraction patterns obtained from oriented mixed xanthan/galactomannan gels contain, in addition to a typical xanthan pattern, a meridional reflection with a spacing of 5·2 Å as well as other reflections that correspond to those on diffraction patterns obtained from the galactomannan. They concluded that these additional reflections are due to regions in the specimen where there is a cooperative interaction between xanthan and the galactomannan in a cellulose-like (pitch = 10·4 Å) conformation.

The sidechain substitution at O3 of the cellulosic backbone of xanthan is known to restrict the conformational space accessible to the (1→4) linkage. Whether this restriction allows the backbone to adopt a two-fold cellulose-like conformation is clearly important to the viability of the proposal described above. This does not appear to have been addressed specifically although Perez and Vergelati (1987) recently analyzed the conformations accessible to xanthan by performing energy minimizations on an isolated chain. They found several families of stable conformers that would generate helices with the axial translation per residue varying between 1·21 Å and 10·13 Å, and the number of residues per helix turn varying from −4·9 to +2·4 (the minus sign denoting left-handed screw symmetry). An important conclusion of their study was that branching at the 3-position of every second glucose unit

'destroys the possibility of forming an extended 2_1 helical structure'. This conclusion is clearly incompatible with the above proposal for xanthan/galactomannan synergism. It is conceivable however that the xanthan backbone and the galactomannan could interact via a conformation that is similar to that of cellulose (or mannan), but deviates slightly from extract 2_1 screw symmetry. This would be consistent with the concept of the above proposal, but is not however consistent with the X-ray diffraction data whose interpretation does require an *exactly* repeating structure.

In view of these inconsistencies a stereochemical analysis was carried out to determine how close the xanthan backbone can approach the cellulose conformation. The modelling methods used are described in the next section, and the results and details of specific model structures described in the following section. The implications of these results for xanthan/galactomannan synergism and their relationship to results obtained from other studies are discussed in the final section.

METHODS

We used the linked-atom least-squares (LALS) technique (Arnott & Wonacott, 1966; Smith & Arnott, 1978) for steric refinement of molecular models. The use of this system has been described elsewhere and is not repeated here (Millane *et al.*, 1988). The pitch and helix symmetry of molecular models could be treated as variable parameters by applying appropriate restraints between atoms at each end of one helix repeat. Molecular models of an isolated chain were generated using standard pyranose ring conformations (Arnott & Scott, 1972). All hydrogen atoms, except hydroxyl group hydrogens, were included. The bond angles at the glycosidic oxygens were set to 116·5°. The acetate and pyruvate group geometries were obtained from Perez *et al.* (1985) and Moorhouse *et al.* (1977*b*), respectively. The sugar rings were treated as rigid entities and the variable parameters were the 10 conformation angles at the glycosidic linkages (4 in the mainchain and 6 in the sidechain), 8 conformation angles defining the side groups and, in some instances, the helix pitch and symmetry. Since the conformations accessible to the backbone are affected almost exclusively by the conformation at the $(1 \rightarrow 3)$ linkage, much of the analysis was performed using only the trisaccharide repeating unit consisting of the two glucose units and the α-linked mannose unit. This reduces the total number of conformational variables to 12.

RESULTS

A polytrisaccharide model was constructed with the backbone in the cellulose conformation (2_1 symmetry, i.e. the two glycosidic linkages in the mainchain conformationally identical, and pitch 10·34 Å; Woodcock & Sarko, 1980). This model contained over-short contacts between non-bonded atoms in the mannose unit and the main chain. Unacceptable non-bonded contacts were considered as those shorter than the inner limits defined by Ramachandran *et al.* (1963) that are listed in Table 1.

TABLE 1
Minimum Non-Bonded Interatomic Distances (in Å) for Stereochemically Acceptable Models (from Ramachandran *et al.*, 1963)

C—C	*C—O*	*C—H*	*O—O*	*O—O (HB)*[a]	*O—H*	*H—H*
3·00	2·70	2·20	2·70	2·50	2·20	1·90

[a]HB = hydrogen bonded.

For hydrogen-bonded oxygen atoms, the minimum distance accepted was 2·50 Å. These short contacts were not relieved when the two-fold symmetry was relaxed. The sidechain is therefore incompatible with the mainchain in the cellulose conformation. In order to explore conformations similar to cellulose, the molecule was modelled as a one-fold helix (since the repeating unit contains two, rather than one, glucose units) with different values of the pitch close to 10·34 Å. These refinements showed that models with a pitch between 10·51 and 10·53 Å are free of over-short contacts. Including the pitch as a variable during refinement produced a model with a pitch of 10·51 Å. Although the conformations at the (1→4) linkages were independent, they refined to identical values. The backbone therefore has 2_1 symmetry except that the hydroxymethyl groups adopt slightly different conformations if refined independently. The terminal two residues of the sidechain can be added to the trisaccharide repeat in this conformation without difficulty.

A 2_1 conformation is therefore sterically accessible to the xanthan backbone with a pitch slightly longer than that of cellulose. The conformation angles and atomic coordinates of this model are listed in Tables 2 and 3 respectively. Restrictions imposed by the pitch, and at the (1→3) linkage, mean that the angles at the mainchain glycosidic linkages and at the (1→3) linkage are well defined (within 3°) in the model. The side-

TABLE 2
Conformation Angles of the Xanthan Model with a 2_1 Backbone and Pitch of 10·51 Å, and 2_1 Cellulose with a Pitch of 10·34 Å

Conformation angle	*Type*	*Value (°)*	
		Xanthan	*Cellulose*
θ(O5A–C1A–O4B–C4B)	(1→4) Linkage	−107·8	−90·0
θ(C1A–O4B–C4B–C5B)		−133·2	−150·0
θ(C4A–C5A–C6A–O6A)	Hydroxymethyl groups	−96·2	−64·5
θ(C4B–C5B–C6B–O6B)		−89·8	−64·5
θ(C2B–C3B–O3B–C1C)	(1→3) linkage	−77·8	
θ(C3B–O3B–C1C–C2C)		−143·8	
θ(C4C–C5C–C6C–O6C)	Acetate group	53·5	
θ(C5C–C6C–O6C–C7C)		−179·4	
θ(C6C–O6C–C7C–C8C)		−101·4	
θ(O6C–C7C–C8C–H81C)		50·8	

TABLE 3
Cylindrical Polar Atomic Coordinates of the Trisaccharide Part of the Xanthan Structure with a 2_1 Backbone[a]

Atom	*r*	ϕ	*z*
O6A	3·412	37·10	2·377
H61A	2·663	−0·26	2·453
H62A	3·226	12·03	0·898
C1B	0·214	14·92	−5·886
C2B	1·436	35·18	−4·998
C3B	1·281	8·09	−3·624
C4B	0·121	174·36	−3·022
C5B	1·255	−145·66	−4·006
C6B	2·592	−161·91	−3·511
O2B	2·602	19·66	−5·623
O3B	2·297	25·64	−2·766
O4B	0·756	−93·90	−1·821
O5B	1·074	−180·00	−5·255
O6B	3·341	−143·38	−2·751
H1B	1·130	−58·84	−6·039
H2B	2·039	66·15	−4·891
H3B	1·927	−24·66	−3·715
H4B	1·139	117·78	−2·799
H5B	1·852	−111·00	−4·175
H61B	2·700	178·42	−2·893
H62B	3·248	−165·97	−4·370

TABLE 3 — *contd.*

Atom	*r*	*φ*	*z*
C1C	3·589	14·71	−2·946
C2C	4·264	13·91	−1·581
C3C	4·568	31·67	−0·979
C4C	5·544	39·03	−1·954
C5C	4·897	41·82	−3·311
C6C	5·882	45·87	−4·467
C7C	7·785	39·60	−5·503
C8C	7·607	32·94	−6·800
O2C	5·623	9·33	−1·736
O3C	5·272	28·68	0·238
O4C	6·170	50·65	−1·465
O5C	4·423	26·51	−3·741
O6C	6·940	37·20	−4·583
O7C	8·654	45·05	−5·368
H1C	3·725	−0·74	−3·420
H2C	3·782	3·31	−0·927
H3C	3·776	41·82	−0·772
H4C	6·417	32·72	−2·083
H5C	4·181	52·31	−3·201
H61C	5·329	46·85	−5·413
H62C	6·457	54·34	−4·244
H81C	6·550	32·31	−7·096
H82C	8·199	36·10	−7·619
H83C	8·060	25·77	−6·586

[a]Coordinates (r, ϕ, z) (in Å and degrees) are given for sugars B and C and the hydroxymethyl group of sugar A. Coordinates of the remaining atoms of sugar A are given by the transformation (r, $\phi + 180°$, z + 5·255 Å) applied to the coordinates of the corresponding atoms in sugar B. Coordinates of sequential trisaccharides are generated by adding multiples of 10·51 Å to z. The two-fold screw axis of the backbone (excluding the hydroxymethyl group) coincides with the z-axis.

chain (1→2) and (1→4) linkages are not particularly restricted and there are a variety of values these conformation angles can adopt. Therefore only the details of the trisaccharide part of the structure are listed in Tables 2–4. Two different conformations of the acetate group are sterically acceptable in this model, one of which is listed in Table 2. In the other, the conformation angles (as defined in Table 2) about C5-C6, C6-O6 and O6-C7 are in the (g-, t, g-) domains. Comparison of the backbone conformation angles with those of cellulose I (Woodcock & Sarko, 1980; Millane & Narasaiah, 1989) show that the differences are significant (Table 2). The hydrogen bonds in this model are listed in Table 4.

TABLE 4
Hydrogen Bonds in the Trisaccharide Repeat of the Xanthan Model with a 2_1 Backbone and Pitch of 10·51 Å

Donor	*Acceptor*	*Comment*	*Distance (Å)*	*Angle (°)*[a]
O6B	O2A	(1→4) linkage	2·64	97
O6A	O2B	(1→4) linkage	2·79	91
O3A	O5B	(1→4) linkage	3·10	100
O2B	O5C	(1→3) linkage	2·65	102
O3C	O6A	mainchain–sidechain	2·90	106

[a]The angle is P-D-A where D and A are the donor and acceptor atoms respectively, and P is the non-hydrogen atom bonded to D.

All of the intramolecular hydrogen bonds observed in cellulose I are present except one of the O3—O5 hydrogen bonds because of the mannose substitution at O3. This gives three hydrogen bonds stabilizing every two (1→4) linkages. There is also one hydrogen bond across the (1→3) linkage and one between the mannose unit and the mainchain. In this conformation, there are no interactions between the acetate group and the mainchain as are found in five-fold helical models of xanthan (Moorhouse *et al.*, 1977*a*). The sidechain (1→2) and (1→4) linkages can be stabilized by hydrogen bonds (O3C—O5D and O3D—O5E). We found that it is not possible however for the sidechain to fold alongside the mainchain as is possible for five-fold model structures (Moorhouse *et al.*, 1977*a*). The conformation of the structure is illustrated in Fig. 2 (with typical conformations at the sidechain (1→2) and (1→4) linkages that accommodate the hydrogen bonds mentioned above).

The accessible conformations can be further assessed by examining hard-sphere contacts maps of the (1→4) and (1→3) linkages. These maps were constructed using the distances listed in Table 1 with the O—O distance being taken as 2·50 Å to allow for possible hydrogen bonds, and are shown in Fig. 3. The hard-sphere map for an isolated β-D-Glc-(1→4)-β-D-Glc linkage (Fig. 3(a)) shows that two-fold cellulose chains with pitches between 10·30 Å and 10·54 Å are accessible. Adding a (1→3) linked α-D-mannose (with the (1→3) linkage conformations listed in Table 2) to the non-reducing glucose has no effect on this map. However, adding it to the reducing residue restricts the conformational space available at this linkage (Fig. 3(a)) and the range of pitches accessible is reduced to 10·48–10·54 Å. The range of pitches accessible to the xanthan structure used is somewhat smaller than this since the hydroxymethyl groups were not included in the calculation of the hard-sphere

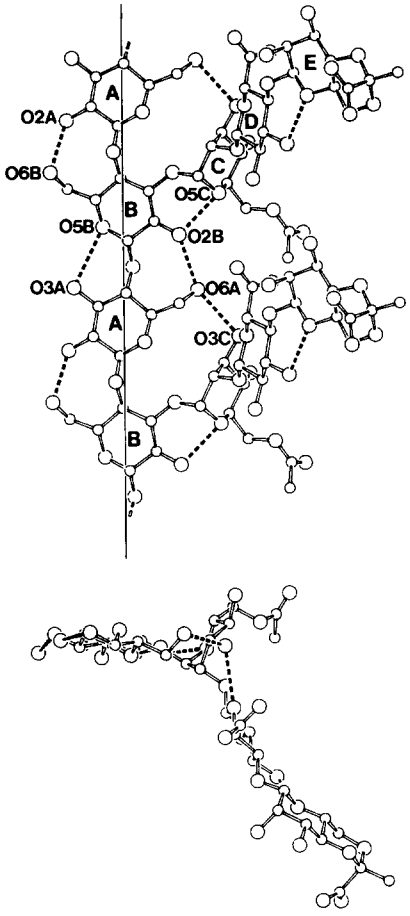

Fig. 2. Views of the xanthan structure with a 2_1 backbone, normal to and along the helix axis. The broken lines denote hydrogen bonds.

maps as their conformations are variable (they were, of course, included in the refinements described above).

The hard-sphere map for an isolated β-D-Glc-(3$\leftarrow$1)-α-D-Man linkage is shown in Fig. 3(b). Adding (1$\rightarrow$4) linked glucose residues (with the (1$\rightarrow$4) linkage conformations listed in Table 2) to each end of the glucose and calculating a new map shows that the conformational space available at the (1$\rightarrow$3) linkage is substantially reduced when the glucose is part of a cellulose chain. The conformations of the (1$\rightarrow$3) linkage for the xanthan models used, of various pitches, are shown in Fig. 3(b). Inspection of the maps in Fig. 3 shows that the conformation angles in the (two-fold) xanthan model can vary by only 1° at the (1$\rightarrow$4) linkage and 3° at the (1$\rightarrow$3) linkage.

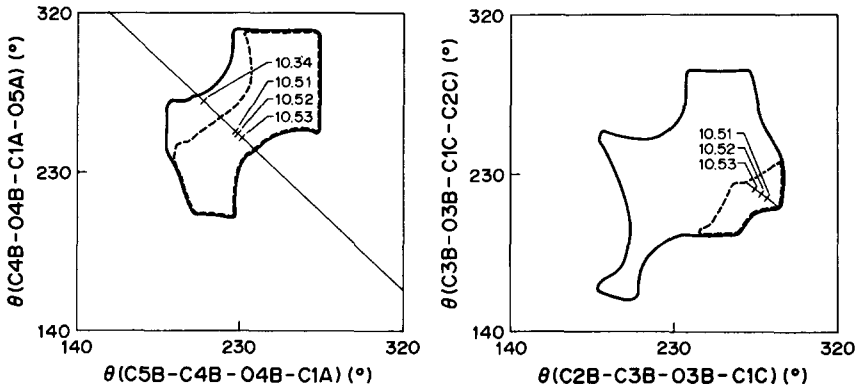

Fig. 3. Hard-sphere maps of the linkages in xanthan (see text). (a) Backbone (1→4) linkage alone and with a mannose attached to the non-reducing glucose (———), and with a mannose attached to the reducing glucose (– – –). The diagonal line denotes combinations of the conformation angles that give a backbone with 2_1 symmetry, and the points marked on the line show the conformation angles for particular values of the pitch (in Å). (b) The (1→3) linkage alone (———) and with the glucose replaced by a glucose trisaccharide (– – –). The conformations at the (1→3) linkage for the refined two-fold models for the backbone (for different pitches) are shown. These conformations lie approximately on a straight line (as shown) although the significance of such a locus is not clear.

DISCUSSION

The results show that a 2_1 structure is sterically accessible to the xanthan backbone but that the pitch must be extended (from the cellulose value) to at least 10·51 Å. This is a small extension (0·09 Å/sugar) but results in a pitch that is close to the maximum (10·54 Å) for a cellulose chain. The virtual bond (O1—O4) distance for β-D-glucose is 5·46 Å. We have used fixed sugar rings in this analysis, but relaxing the rings would only marginally expand the accessible conformations. Similarly, relaxing the minimum non-bonded distances (Table 1) by, say, 0·2 Å, only slightly expands the range of accessible conformations (the minimum pitch becomes 10·47 Å). This does allow different acetate group conformations however, one of which accommodates an O6C—O2B hydrogen bond. The backbone retains the intramolecular hydrogen bonds observed in cellulose although one of them is a little long. Since the minimum non-bonded distances listed in Table 1 cannot be considered inviolable, it is probably possible to construct an acceptable model similar to the one described here but with a pitch of say 10·40 Å, (quite close to that of cellulose) by relaxing the sugar rings and accepting a few slightly shorter non-bonded distances.

Although the two-fold model described here is sterically accessible, a five-fold structure is the only one that has been observed in the solid state. This is so for different salts, humidities, pyruvate contents, and truncation of the sidechain. The five-fold structure apparently has a lower free energy than the two-fold model described here. This could be related to the ability of the sidechain in the five-fold conformation to fold down alongside and interact with the mainchain, which is not possible with the two-fold model.

The two-fold model described here is however a candidate for the xanthan conformation in the proposed xanthan/galactomannan interactions described above. The meridional reflection with a spacing of 5·2 Å reported on diffraction patterns from mixed xanthan/galactomannan oriented fibres (Cairns *et al.*, 1986, 1987) would have to have a spacing of 5·26 Å to be consistent with the model described here. Although Cairns *et al.* (1986, 1987) gave no error estimates for their measurements (a disturbingly common practice), it is unlikely that they could distinguish between these two spacings. The model described here is therefore compatible with the type of model proposed from their X-ray data. It is conceivable that the xanthan/galactomannan cooperative interactions are sufficiently favourable energetically to lock the xanthan chain in this two-fold conformation rather than in the more commonly observed five-fold conformation of xanthan in isolation. In other words, although the two-fold model probably does not represent an energy minimum for xanthan in isolation, it may represent an energy minimum for the xanthan/galactomannan complex.

We have shown that a two-fold cellulose-like conformation is sterically accessible to the backbone of xanthan gum. This does not prove the correctness of the proposed mechanism of synergistic gelation of xanthan/galactomannan mixtures, but it does show that such a mechanism is sterically feasible. This structure may also be used as a model for recognition in certain host–pathogen interactions (Dea *et al.*, 1977).

ACKNOWLEDGMENTS

The authors are grateful to the US National Science Foundation for support (DMB-8606942 to RPM) and Deb Zerth for word processing.

REFERENCES

Arnott, S. & Scott, W. E. (1972). *J. Chem. Soc., Perkin Trans. II*, 324–35.
Arnott, S. & Wonacott, A. J. (1966). *Polymer*, **7**, 157–66.

Cairns, P., Miles, M. J. & Morris, V. J. (1986). *Nature* (London), **322**, 89–90.
Cairns, P., Miles, M. J., Morris, V. J. & Brownsey, G. J. (1987). *Carbohydr. Res.*, **160**, 411–23.
Cheetham, N. W. H. & Mashimba, E. N. M. (1988). *Carbohydr. Polym.*, **9**, 195–212.
Dea, I. C. M. & Morrison, A. (1975). *Adv. Carbohydr. Chem. Biochem.*, **31**, 241–312.
Dea, I. C. M., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A. & Price, J. (1977). *Carbohydr. Res.*, **57**, 249–72.
Dea, I. C. M., Clark, A. H. & McCleary, B. V. (1986). *Carbohydr. Res.*, **147**, 275–94.
Jansson, P. E., Kenne, L. & Lindberg, B. (1975). *Carbohydr. Res.*, **45**, 275–82.
Liu, W. & Norisuye, T. (1988). *Int. J. Biol. Macromol.*, **10**, 44–50.
McCleary, B. V. (1979). *Carbohydr. Res.*, **71**, 205–30.
Melton, L. D., Mindt, L., Rees, D. A. & Sanderson, G. R. (1976). *Carbohydr. Res.*, **46**, 245–57.
Milas, M. & Rinaudo, M. (1979). *Carbohydr. Res.*, **76**, 189–96.
Milas, M. & Rinaudo, M. (1986). *Carbohydr. Res.*, **158**, 191–204.
Millane, R. P. & Narasaiah, T. V. (1989). *Polymer*, **30**, 1763–7.
Millane, R. P. & Narasaiah, T. V. (1990). *Carbohydr. Polym.*, **12**, 315–21.
Millane, R. P., Chandrasekaran, R., Arnott, S. & Dea, I. C. M. (1988). *Carbohydr. Res.*, **182**, 1–17.
Millane, R. P., Narasaiah, T. V. & Arnott, S. (1989). In *Biomedical and Biotechnological Advances in Industrial Polysaccharides*, ed. V. Crescenzi, I. C. M. Dea, S. Paoletti, New York, pp. 469–78.
Millane, R. P., Narasaiah, T. V. & Wang, B. (1990). In *Gums and Stabilisers for the Food Industry 5*, ed. G. O. Phillips, D. J. Wedlock & P. A. Williams. IRL Press, Oxford, in press.
Moorhouse, R., Walkinshaw, M. D. & Arnott, S. (1977*a*). *ACS Symp. Ser.*, **45**, American Chemical Society, Washington, DC, pp. 90–102.
Moorhouse, R., Winter, W. T., Arnott, S. & Bayer, M. E. (1977*b*). *J. Mol. Biol.*, **109**, 373–91.
Morris, E. R., Rees, D. A., Young, G., Walkinshaw, M. D. & Darke, A. (1977). *J. Mol. Biol.*, **110**, 1–16.
Norton, I. T., Goodall, O. M., Frangou, S. A., Morris, E. R. & Rees, D. A. (1984). *J. Mol. Biol.*, **175**, 371–94.
Okuyama, K., Arnott, S., Moorhouse, R., Walkinshaw, M. D., Atkins, E. D. T. & Wolf-Ullish, C. (1980). *ACS Symp. Ser.*, **141**, American Chemical Society, Washington, DC, pp. 411–27.
Perez, S. & Vergelati, C. (1987). *Int. J. Biol. Macromol.*, **9**, 211–18.
Perez, S., Vergelati, C. & Tran, V. H. (1985). *Acta Crystallogr.*, **B41**, 262–7.
Ramachandran, G. N., Ramakrishnan, C. & Sasisekharan, V. (1963). In *Aspects of Protein Structure*, ed. G. N. Ramachandran. Academic Press, New York, pp. 121–35.
Rees, D. A. (1972). *Biochem. J.*, **126**, 257–73.
Sandford, P. A. & Baird, J. (1983). In *The Polysaccharides*, Vol. 2, ed. G. O. Aspinall. Academic Press, New York, pp. 411–90.
Smith, P. J. C. & Arnott, S. (1978). *Acta Crystallogr.*, **A34**, 3–11.
Woodcock, C. & Sarko, A. (1980). *Macromolecules*, **13**, 1183–7.