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Molecular modeling of xanthan:galactomannan interactions

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X-ray diffraction patterns from stretched fibers of xanthan, guaran and the complex between the two are indicative of good orientation and reasonable crystallinity. The ordered structures of xanthan and guaran appear to be a 5-fold helix of pitch 47.4 Å and a 2-fold helix of pitch 10.3 Å, respectively. The diffraction pattern of the complex is a hybrid of those of the individual components. Both xanthan and guaran in the complex may adopt cellulose-like helices having a slightly longer pitch of 10.5 Å and form a non-coaxial duplex. Alternately, the complex may adopt a xanthan-like, coaxial, 5-fold, double helix in which one strand is xanthan and the other is guaran. The association of a pair of these hybrid helices can take place by direct cross linking of the carboxylate groups in the side chains of xanthan by divalent ions. The morphologies of these arrangements have now been visualized by computer modeling. © 1997 Elsevier Science Ltd

INTRODUCTION

Unlike proteins and nucleic acids, polysaccharides, also known as the Cinderella of biopolymers, have not only the potential to form a diversity of linkages between successive monosaccharide repeats in the main chain, but also the ability to accommodate mono- to oligosaccharide side chains as branches. The resulting three-dimensional structures are generally complex and unraveling them is rather tedious. Consequently, polysaccharide crystallography is relatively far less advanced than either protein or nucleic acid crystallography. For example, the molecular structure of the (1→6)-branched amylopectin molecule has been visualized not long ago (Imberty and Pérez, 1989) and that of a branch-on-branch glycogen molecule is not in sight. Although some reasonable X-ray data are available for the bacterial polysaccharide xanthan, which has a simple cellulose backbone but a trisaccharide side chain per cellobiose repeat, its molecular structure is yet elusive. A twenty year history in structural science has led scientists to accept the notion that xanthan forms a 5-fold helix of pitch 47.4 Å; a coaxially interwound double helix is apparently superior to a single helix, but the chirality of the helix and the polarity of the two chains, if it were a duplex, remain unresolved (Millane *et al.*, 1989; Chen, 1992). Since its discovery in the 1960s, xanthan is the most extensively used food hydrocolloid because of its very high viscosity in aqueous solutions being extremely stable over a wide range of temperature and pH and in the presence of various types and amounts of salts.

Galactomannans are plant polysaccharides displaying a mannan backbone with randomly substituted (1→6)-linked α-D-galactosyl units. The galactose:mannose (G/M) ratio varies from 0.3 in locust bean gum to 0.9 in fenugreek and intermediate values for other members in this family. In contrast to mannan, these polysaccharides are water soluble and form highly viscous solutions. The viscosity appears to be dependent on a number of factors such as molecular weight, degree of substitution, temperature and pH (Maier *et al.*, 1993). The availability of a spectrum of plant seed polysaccharides thus provides an excellent opportunity for the development of fine tuned food products by utilizing these galactomannans. X-ray investigations to date indicate that any of these galactomannans can form a 2-fold helix of pitch 10.3 Å similar to that of mannan itself (Chien and Winter, 1985). The galactosyl side chains stick out and have substantial conformational freedom.

The ultimate motive for the examination of mixed systems is the quest for cost effective blends having improved and/or product-oriented rheological properties useful for several industrial applications. This is a far

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superior, economical and practical approach that exploits synergism between inexpensive materials vis á vis the necessity to use an expensive ingredient already in vogue. Awaiting the discovery of yet another hydrocolloid followed by regulatory approval is both time consuming and costly, and hence not feasible. Under these circumstances, understanding the interactions between two polysaccharides upon physical mixing is desirable and helpful in order to rationalize the structure–function relationship of the complex at the molecular level.

In this context, several laboratories have reported interesting results from a series of physicochemical and X-ray studies during the last dozen years on the synergistic interactions of at least four major complexes, viz. κ-carrageenan:galactomannan, κ-carrageenan:glucomannan, agarose:glucomannan and xanthan:galactomannan systems. In every case, an anionic and a neutral polysaccharide are involved. No specific details on the molecular interactions have yet emerged. For the xanthan:galactomannan complex, the current picture is that the backbone of the xanthan helix associates with the naked region of the galactomannan chain as inferred from schematic drawings (Cairns *et al.*, 1986; Cheetham and Mashimba, 1991).

Based on the X-ray diffraction patterns obtained from oriented fibers of xanthan, guaran and xanthan:-guaran, we have generated two distinct classes of molecular models for the xanthan:guaran complex. One of them is a non-coaxial duplex consisting of cellulose-like xanthan and guaran helices, each of pitch 10.5 Å. The other is a xanthan-like, 5-fold double helix of pitch 47.4 Å, in which a guaran chain intertwines around a xanthan chain. This is the first time some structural details have been visualized for any of the blends in relation to the observed synergism.

MATERIALS AND METHODS

Samples

Purified samples of xanthan from *Xanthomonas campestris* and guaran with a G/M ratio of about 0.6 were provided by Dr Talashek of The NutraSweet Kelco Company, San Diego. The xanthan sample was received in the potassium salt form. Oriented and polycrystalline fibers were stretched from a concentrated solution of each of them. For both samples, it was necessary to dissolve them in water heated to about 75°C and mixed thoroughly in order to obtain a homogeneous aqueous 0.5% solution.

The complex was prepared by mixing predetermined aliquots of the above hot solutions, the temperature of the mixture was raised to and maintained at about 95°C for an hour. Subsequently, the mixed solution was returned to room temperature, equilibrated overnight and then used to stretch oriented fibers.

X-ray diffraction

Diffraction patterns were recorded in a flat-film camera using nickel filtered CuKα radiation of X-rays of wavelength 1.5418 Å. The fiber-to-film distance was typically about 42 mm. During the X-ray exposure, the fiber was surrounded by helium atmosphere (to reduce air scattering) at 75% relative humidity.

Molecular modeling

The pentasaccharide repeat for xanthan and an idealized trisaccharide repeat for guaran are given in Fig. 1. In this formulation, xanthan has a cellobiose repeat in the main chain and a trisaccharide side chain which also contains acyl and pyruvyl substituents as shown. Guaran, on the other hand, has a mannobiose repeat in the main chain and a galactose in the side chain. This gives a G/M ratio of 0.5 which is close to the actual value of 0.6. The main chain disaccharide repeat, AB for xanthan and FG for guaran, is the asymmetric motif in relation to the helical parameters *n* and *h*. They are the number of such repeats per turn and axial rise per repeat, respectively, so that the pitch of the helix *P* is *nh*.

The Linked-Atom Least-Squares (LALS) program (Smith and Arnott, 1977) was used to generate helical structures consistent with the helix symmetry and pitch as observed in their X-ray diffraction patterns. The sugar rings with the standard $^4C_1$ geometry (Arnott and Scott, 1972) were used as the building blocks. The bond angle at each bridge oxygen atom was set at 116.5° in all the calculations. The major parameters refined were the (ϕ, ψ) conformation angles around the interglycosidic bonds, and the orientation of the hydroxyl, carboxyl, pyruvyl and acyl groups. In a (1→n) linkage when n = 2, 3 or 4, ϕ and ψ are the dihedral angles O5–C1–On–Cn and C1–On–Cn–Cn + 1, respectively. However, for the (1→6) linkage, they are given by O5–C1–O6–C6 and C1–O6–C6–C5. The hydroxymethyl (or carboxyl group)

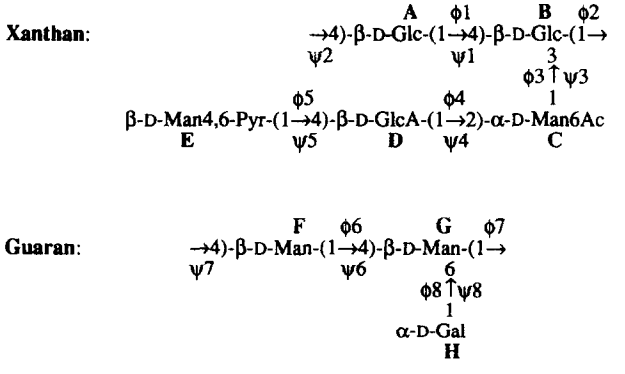

Fig. 1. Chemical structure of the repeating units of xanthan and guaran. Glc, GlcA, Man and Gal represent the pyranosyl form of glucose, glucuronic acid, mannose and galactose, respectively. The residues are labeled from A to H for convenience. All inter-glycosidic conformation angles are also marked.

orientation χ refers to C4–C5–C6–O6 (or O61). During the model building analysis, the main chain conformation angles were tethered to the corresponding low energy domains or to values in a known related structure; care was taken to insure that all the non-bonded contacts within the helix were stereochemically satisfactory and inter residue hydrogen bonds were facilitated whenever possible. The same criteria were followed while examining the interactions between helices as well. In general, the limits for a hydrogen bond (OH···O) were 2.5–3.0 Å for distance and 90–150° for the angle P—OH···O, where P is the precursor to the donor group.

RESULTS

X-ray data

The diffraction patterns from oriented fibers of xanthan and guaran are similar to those in Fig. 2a and b, respectively. The xanthan pattern is diagnostic of a 5-fold helix of pitch 47.4 Å. There are a series of layer lines up to $l=15$. The superposition of continuous intensity on a few Bragg reflections which appear in the low resolution region is reminiscent of those originally reported by Moorhouse *et al.* (1977) and confirms that the helices in the fiber have only short range lateral organization. The guaran pattern shows some Bragg reflections out to a resolution of about 3.8 Å. The intensity distribution is consistent with a 2-fold helix of monosaccharide (or 1-fold helix of disaccharide) repeat in its backbone. The intensity data are by no means sufficient for conducting a detailed structure analysis in either case.

The diffraction pattern from a fiber of xanthan:-guaran (1:3 w/w) blend shown in Fig. 2c is not of a new specie. It is a composite of the individual patterns, in which the crystallinity and orientation of guaran are better than those inferred from Fig. 2b, and guaran outshines the xanthan component. Fibers could be equally well stretched from two other blends, (1:1) and (3:1), but their patterns were noisy. Hence, the modeling of the xanthan:guaran complex is mainly based on the diffraction in Fig. 2c only.

There are at least three different explanations that may fit this composite pattern. The first solution is in terms of two independent, non-associating phases of xanthan and guaran molecules in the specimen. While this is not ruled out, it is a trivial solution and provides little information on synergistic interactions. The second possibility is that both xanthan and guaran would adopt 1-fold helices, each of pitch 10.3 Å or slightly higher and interact with each other at a distance apart. The association between the two could be mediated by the main chains, side chains or a combination of both. We have generated a few models and classified

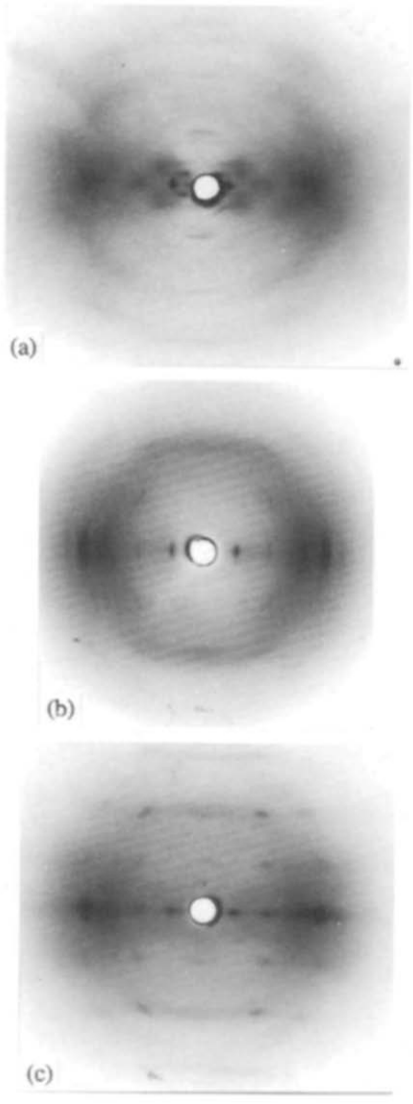

Fig. 2. X-ray diffraction patterns from stretched fibers of (a) xanthan, (b) guaran and (c) xanthan:guaran complex are indicative of structural organization.

them under type I. The third solution would require a 5-fold double helix of pitch 47.4 Å in which the xanthan and guaran chains are intertwined around the helix axis. The interaction in this type II would be more intimate than in type I.

Type I models (1-fold helices)

Xanthan and guaran chains have been modeled using cellobiose and mannobiose, respectively, as their repeating units. The pitch had to be increased to 10.5 Å for consistency with Fig. 2c; also this is needed in order to accommodate the xanthan side chain. The final main chain and side chain conformation angles are listed in Table 1 and their molecular structures are shown in Fig. 3a for xanthan and Fig. 3b for guaran. The xanthan model has good resemblance to that previously reported (Millane and Wang, 1990). The two mono-

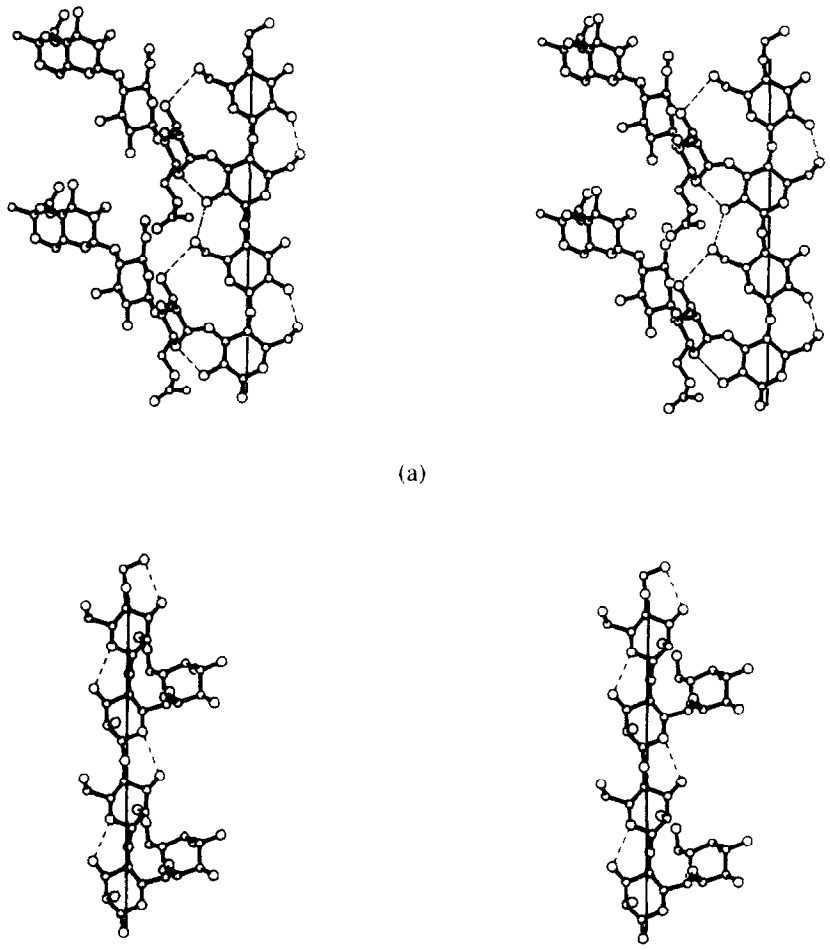

Fig. 3. Stereo view of two turns of the cellulose-like structures of (a) xanthan and (b) guaran. The length of the vertical line, which represents the helix axis, is $P = 10.5$ Å. The O3···O5 hydrogen bonds (broken line) are common to both. Main chain-side chain hydrogen bonds are present in xanthan, but not in guaran.

Table 1. Major conformation angles in cellulose-like models 1 to 3 (Type I) and double-helical model 4 (Type II) of the xanthan:guaran blend

Parameter	Type I	Type II	Parameter	Type I	Type II
$\phi 1$	−110	−79	χ_E	−51	−51
$\psi 1$	−131	−137	$\theta 1_C$	164	108
χ_A	−98	−68	$\theta 2_C$	79	80
$\phi 2$	−110	−92	$\theta 3_E$	−143	−147
$\psi 2$	−131	−97			
χ_B	−91	−160	$\phi 6$	−100	−92
$\phi 3$	93	113	$\psi 6$	−136	−97
$\psi 3$	161	147	χ_F	−91	−169
χ_C	180	−148	$\phi 7$	−100	−79
$\phi 4$	−65	−76	$\psi 7$	−136	−137
$\psi 4$	108	116	χ_G	−61	−94
χ_D	−74	−88	$\phi 8$	132	132
$\phi 5$	−122	−154	$\psi 8$	−99	−96
$\psi 5$	−143	−133	χ_H	−57	−164

The orientation of the acyl group in C is given by the dihedral angles $\theta 1$(C5–C6–O6–C7) and $\theta 2$(C6–O6–C7–O7); that of pyruvyl group in E by $\theta 3$(O6–C7–C8–O81).

saccharide conformations in a disaccharide repeat are quite similar among themselves and to those in cellulose and mannan structures. The prominent features are: the O3···O5 hydrogen bonds are preserved in the main chain; side chains are turned toward the non-reducing end. Since they are attached to alternate residues, they appear on the same side of the helix.

These two molecular structures have been treated as rigid bodies and examined for association for parallel and antiparallel modes, by adjusting their orientations and translations relative to the helix axis. The most probable lateral separations between their helix axes were identified with related structures known in the literature. Consequently, if the helices are 7.9 Å apart, based on the parallel packing arrangement for cellulose I (Gardner and Blackwell, 1974; Sarko and Muggli, 1974), then the xanthan:guaran association would involve atoms in the main chains alone. This, model **1**, is shown in Fig. 4. Note that their side chains are on opposite sides.

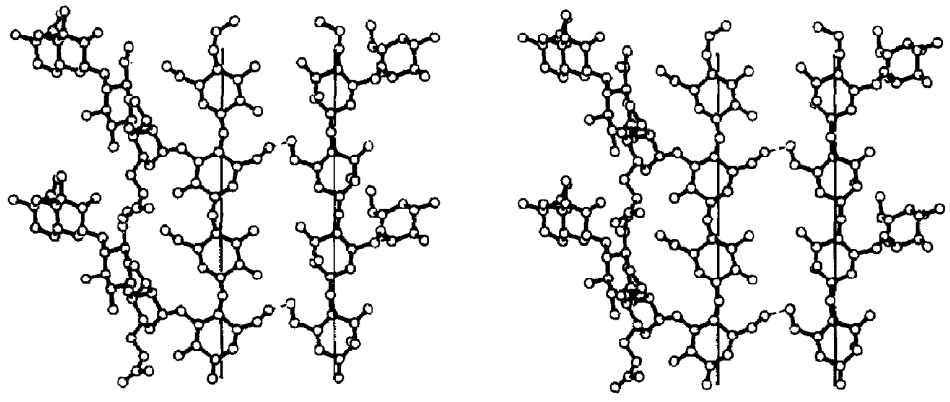

Fig. 4. Stereo view of two turns of **1** showing the parallel association of xanthan (open bonds) with guaran (filled bonds) stabilized by main chain–main chain O6F···O6A hydrogen bonds. Their helix axes (vertical lines of length $2P$) are 7.9 Å apart.

Common to the reported unit cell dimensions and positions of xanthan (Moorhouse *et al.*, 1977) and guaran (Chien and Winter, 1985) molecules in their cells is the interhelix separation of 17.8 Å. If the two helices in **1** are drawn apart to this distance and their orientation and translation (i.e. packing) parameters adjusted, then they are too far to interact; at an intermediate distance of about 12.5 Å, however, the side chains of the polymers in the resulting model **2** are in juxtaposition to form side chain–side chain hydrogen bonds (O3D···O3H) as shown in Fig. 5. If one of the chains in **2** is inverted, then it is possible to re-optimize the packing parameters and obtain model **3** (Fig. 6) that displays guaran main chain hydrogen bonding with the acetyl group of xanthan.

It was not possible to achieve favorable interactions between xanthan main chain and guaran side chain by altering the packing parameters in the parallel or antiparallel mode.

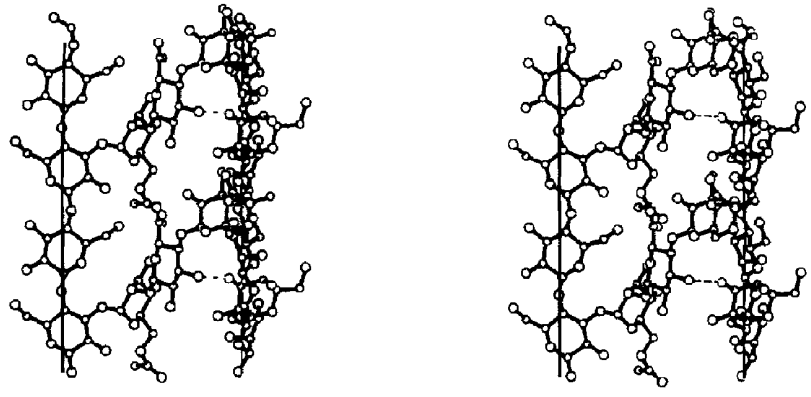

Fig. 5. Stereo view of two turns of **2** also showing the parallel association of xanthan with guaran. The complex is stabilized by side chain–side chain O3D···O3H hydrogen bonds. Their helix axes (vertical lines of length $2P$) are 12.5 Å apart.

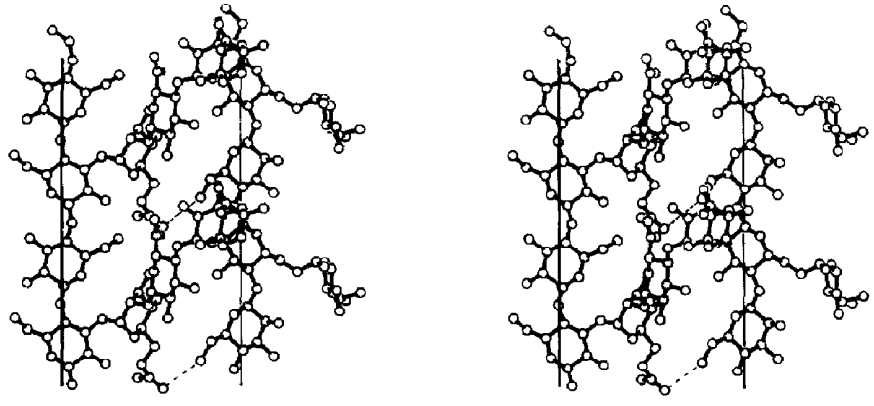

Fig. 6. Stereo view of two turns of **3** showing the antiparallel association of xanthan with guaran. The complex is stabilized by main chain–side chain O6F···O7C hydrogen bonds. Their helix axes (vertical lines of length $2P$) are 12.5 Å apart.

Type II models (5-fold helices)

Although the fine details of the structure of xanthan are still debated (Moorhouse *et al.*, 1977; Millane *et al.*, 1989; Chen, 1992), there appears to be a marginal edge for the antiparallel, right-handed, 5-fold double helix. Using this as a basis, our first task was to build a hybrid helix that incorporated only a cellulose chain coiling around a mannan chain that corresponds to the xanthan:guaran skeleton devoid of side chains. After minor adjustments in conformation angles, the equatorial 2OH groups in one strand were in harmony with the axial 2OH groups in the other. The resulting xanthan-like cellulose:mannan helix is shown in Fig. 7. This hybrid helix exhibits O6···O3 hydrogen bonds from mannan to cellulose. The second task was to include the respective side chains in a stereochemically acceptable fashion through fine tuning of the additional conformation angles. The final xanthan:guaran model **4** is depicted in Fig. 8. Its conformation angles are listed in Table 1. This duplex is further stabilized by periodic hydrogen bonds involving both main and side chains. The terminal pyruvyl groups are farther away from the helix axis than the acyl groups. The galactosyl units of guaran, situated radially in intermediate positions, are able to interact with the acyl groups and generate a series of O4···O7 hydrogen bonds. This provides robustness to the hybrid helix.

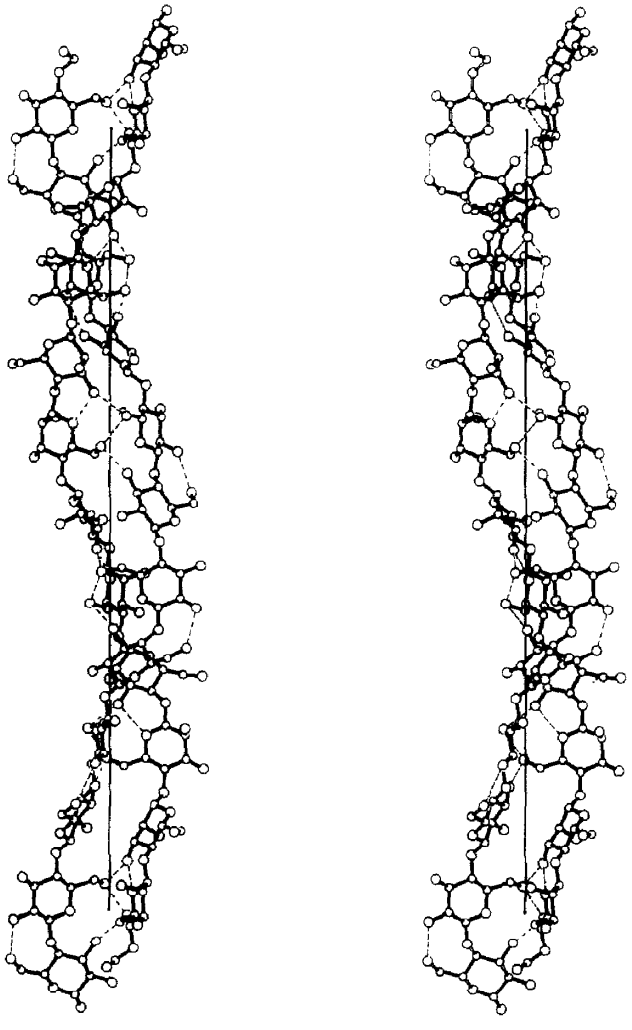

Fig. 7. Stereo view of one turn of a right-handed, double-helical model showing the main chains of xanthan and guaran intertwined in an antiparallel mode. The helix axis (vertical line) corresponds to a length of $P = 47.4$ Å. A series of hydrogen bonds are present both within and between the chains.

DISCUSSION

Cairns *et al.* (1986) were the first to report the X-ray diffraction patterns of xanthan:carob blend and suggest possible interactions between the mannan backbone and helical xanthan in a schematic fashion. Millane and Wang (1990) subsequently modeled xanthan in an extended form, very similar to that in Fig. 3a, and alluded to its feasibility in cooperative interactions with galactomannans. On the basis of optical rotation studies and space filling models, Cheetham and Mashimba (1991) illustrated a cartoon of xanthan:locust bean gum:xanthan sandwich containing ribbon structures and some interchain hydrogen bonds. However, none of these studies included a molecular model of the xanthan:galactomannan complex. Our efforts have so far produced not one, but at least three models, **1** to **3**, which can fill in the spatial details. The association between the two cellulose-like structures can be mediated whether they are aligned with the same or opposite polarity. The lateral separation between them can be about 7.9 Å as in **1** or 12.5 Å as in **2** and **3**. Within this spread, there is a spectrum of attractive interactions through the backbone atoms and the functional groups in the side chains.

That the blends were prepared by heating the mixtures at an elevated temperature of 95°C is in support of melting of the initial preferred structures of either or both components. This, we believe, should facilitate the formation of a new structure for the complex upon cooling. Models **1**–**3** are valid only if the native 5-fold xanthan double helix could unwind and separate into two intermediate cellulose-like chains in order for one of them to associate with a structurally similar galactomannan chain. Likewise, model **4** would rely on the validation of galactomannan's ability to adopt a 5-fold helical structure resembling that of native xanthan. What we have shown is that such a possibility is stereochemically feasible (Fig. 8). The two strands in this model are antiparallel, and a parallel mode is equally possible.

One or more of the four models for the xanthan:guaran blend presented above are quite appropriate for a galactomannan of any general side chain composition. Although the cooperative interactions we have visualized might not exist over long ranges,

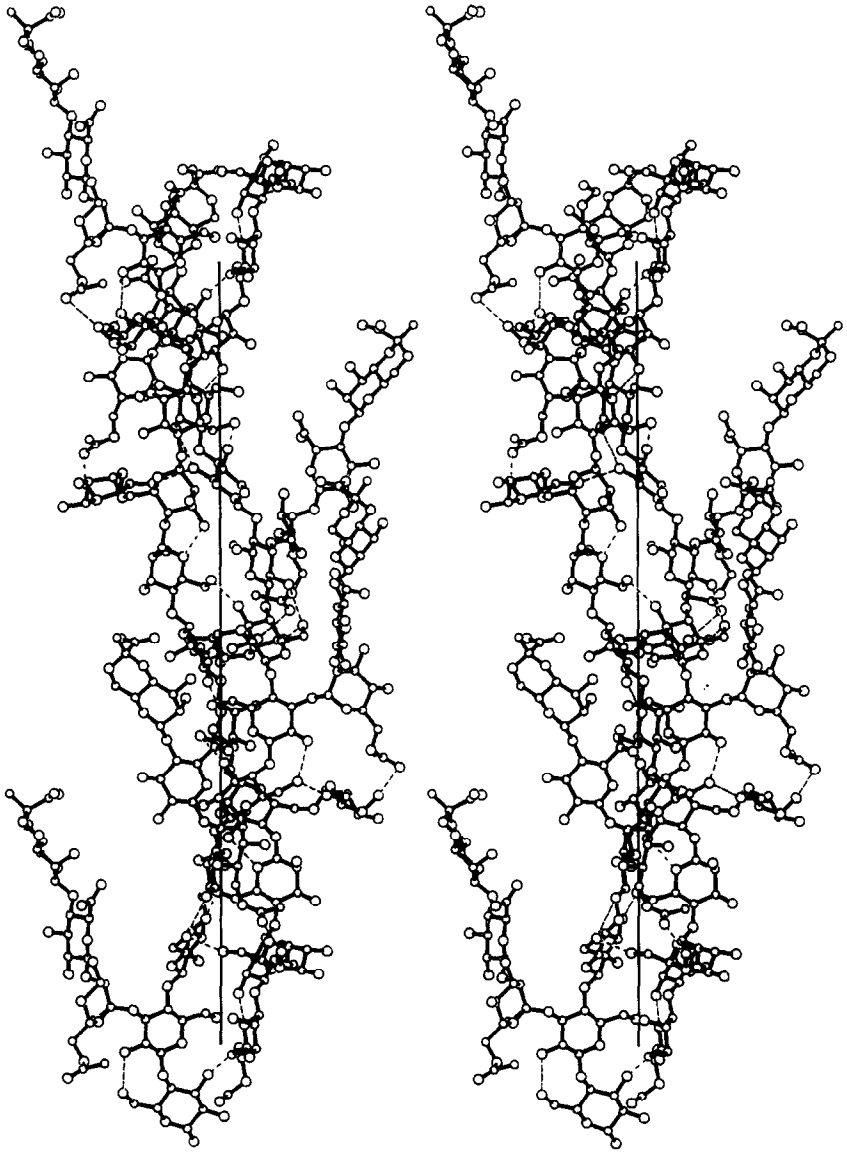

Fig. 8. Stereo view of one turn of **4** displaying the side chains on the periphery. Note the side chain–side chain O4H···O7C hydrogen bonds on the helix surface between the two polymers.

they are quite probable for short segments. The preference for one over the other would greatly depend on the exact side chain sequence in the galactomannan. These synergistic interactions certainly would influence the rheological properties such as viscosity, gelation or texture as observed by different research groups (Dea *et al.*, 1977; Doublier and Llamas, 1991; Shatwell *et al.*, 1991a, b; Tako, 1991; Fernandes, 1995). A common feature in these models is the occurrence of the carboxylate group of the pyruvyl moiety in the periphery of the xanthan chain. Two hybrid helices as in **4** can readily associate via these carboxylate groups, at a lateral separation of 17.3 Å between the helices, mediated by a divalent cation such as calcium. Such interactions are vital for the onset of gelation as inferred from the intermolecular associations in the gel-forming polysaccharides gellan (Chandrasekaran *et al.*, 1988) and welan (Chandrasekaran *et al.*, 1994). Thus, **4** is an attractive candidate for explaining at least in part the observed gel formation between xanthan and locust bean gum (Cheetham and Mashimba, 1991).

If removal of the pyruvyl groups from **4** would abolish or weaken the association between two of these helices, detaching the acetyl groups would result in the disappearance of the side chain–side chain O4···O7 hydrogen bonds causing instability to the helix itself. This underscores the importance of the structural roles of these two groups in native xanthan. This is also consistent with our inability to get X-ray diffraction patterns similar to Fig. 2c for the blends prepared by mixing guaran with xanthan samples devoid of either or both of these two groups.

Finally, it is quite conceivable that some sections of the electron (Lundin and Hermansson, 1995) and opti-

cal (Schorsch *et al.*, 1995) micrographs of the xanthan:-galactomannan mixtures might correspond to one or more models presented above. Also, **1**, **3** and **4** could readily represent xanthan:glucomannan blends which have been examined by X-ray (Brownsey *et al.*, 1988) and are known to display synergism in their rheological properties (Shatwell *et al.*, 1991c).

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