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Molecular architecture of araban, galactoglucan and welan

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The debranched plant polysaccharide araban exhibits fat mimic properties. Among the *Rhizobium* family of bacterial polysaccharides, a galactoglucan, having a disaccharide repeating unit, is equally effective in root nodulation as the complex succinoglycan and similar polysaccharides having branched octasaccharide repeating units. The branched polymer welan, belonging to the gellan family of polysaccharides, has excellent affinity for calcium and shows very high viscosity in aqueous solution up to 130°C. The three-dimensional structures of these polymers have been determined to varying degrees by using X-ray diffraction and computer modeling methods. The structural details help to understand the molecular basis of the functional properties of these polysaccharides.

INTRODUCTION

Water-soluble polysaccharides often exhibit one or more commercially useful physical properties, such as viscosity and gelation. These properties are generally related to the underlying chemical structure. For example, the plant polysaccharide guaran, a galactomannan, derives its solubility and hence high viscosity due to random substitution of galactose side chains from the mannan backbone. Pectins which constitute the primary cell walls of plants form excellent gels in the presence of cations, due to intimate interactions between the carboxylate groups in the galacturonate residues and the ions (Walkinshaw & Arnott, 1981). Arabinan, a branched arabinose-rich polysaccharide, is associated with pectic substances in sugar beet. The enzymatically debranched polysaccharide, referred to as araban, consists of linear chains of 50–80 units of 1→5 linked α-L-arabinofuranosyl residues. This low molecular weight polysaccharide develops a spreadable gel-like structure and is a potential fat replacer in food applications (Cooper *et al.*, 1992).

Many legumes are agriculturally and economically important as food (e.g., soybean, beans, peanuts, cowpeas) and as forage crops (e.g., clover, alfalfa), and nitrogen is the nutrient which most commonly limits plant productivity. The symbiotic association of the bacteria in the *Rhizobia* family with legumes is mainly responsible for the biologically fixed nitrogen available for agriculture (Dazzo & Gardiol, 1984). Furthermore, the symbiosis reduces depletion of soil nitrogen, decreases the need for expensive nitrogen fertilizers which have to be produced with expensive natural gas, and cuts costs. There appears to be a selective or specific response between one bacterium and a plant cell. For example, *Rhizobium meliloti* nodulates alfalfa but not clover, and *Rhizobium trifolii* nodulates clover but not alfalfa. Biochemical evidence indicates that lectins, the carbohydrate-binding proteins in the host plant, interact with the carbohydrates on the bacterial cell surface in an extremely selective fashion (Dazzo, 1980). The bacterial polysaccharides have regular repeating sequences whose sizes can vary anywhere from two to ten or greater saccharide units. Their chemical and molecular structures are desirable in order to learn about the lectin–polysaccharide interactions.

The gellan family of bacterial polysaccharides, secreted by unrelated microorganisms, have in common a rhamnose/mannose-containing anionic tetrasaccharide-repeating motif in the main chain, but they contrast with each other with regard to substitution or branching (Moorhouse, 1987). The linear deacylated polysaccharide, called gellan, is well known for its cation-dependent gelation properties which can be explained at the molecular level in terms of the interactions between half-staggered double-helical gellan molecules (Chandrasekaran *et al.*, 1988). This is the first hydrocolloid to be FDA approved since xanthan gum in 1969 for use in icings, frostings and glazes, non-standardized jams and jellies. The branched polysaccharides, welan, S-657 and rhamsan, on the other hand, do not gel in aqueous solutions, but display very high viscosity up to about 150°C. The side chains in these polymers must in some way be responsible for inhibiting gelation. Welan, originally introduced to the oil field in 1985, has high affinity for calcium and is used as an anti-washout additive for underwater concrete placement. This polymer is further expected to handle challenging application needs of the construction industry.

One of the major goals of this study of polysaccharide structures is to understand the origin of the observed physical properties at the molecular level. Since the natural tendency of a polymer chain with regular repeating sequences is to adopt a helical structure, determination of the molecular morphology involves characterization of the helix. A knowledge of the molecular structure, i.e. the disposition of the atoms in the main chain, as well as in the side chain (if it is present), might not always be adequate to explain the functional properties. Experience shows that interactions between molecular helices, which are brought together by surrounding water molecules and cations, are responsible for the associative properties of the polymer chains; these in turn can be related to the observed solution behavior.

X-ray diffraction from single crystals is a direct and powerful experimental tool to study molecular structures at atomic resolution. Under crystallizing conditions, helical structures invariably tend to grow fast along the direction of the helix axis, and very slow in other directions. Consequently, it is seldom possible to achieve isotropic growth necessary for single crystals in the case of helix-forming polysaccharides. It is, however, possible to prepare polycrystalline and/or oriented specimens in the form of fibers or films. X-ray diffraction from fibers of the three polysaccharides, has been used to determine preliminary models for araban, and crystal structures for galactoglucan and welan. The important structural details are presented in the following sections.

POLYSACCHARIDE FIBER PREPARATION

The secret of success in obtaining good quality X-ray data, suitable for polysaccharide structure analysis, is to induce axial, as well as lateral, organization of the long chain molecules as much as possible within the diffracting specimen. This can be achieved during the process of slow evaporation of a saturated solution of the polymer under controlled experimental conditions of temperature, humidity, co-solute, pH, etc. The basic principle is to reduce entropy and thus increase the extent of three-dimensional ordering of the polymer molecules in a condensed state, be it a single crystal or not. The most commonly used method is to prepare a fibrous specimen from a polysaccharide solution, whose concentration is typically of the order of 2 mg/ml. A few drops of the solution are placed in the gap of approximately 2 mm between the beaded ends of two thin glass rods, whose other ends are clamped in the threaded rods of a fiber puller (Fig. 1) and allowed to dry by gradually reducing the initial humidity of 100% surrounding the sample to about 80 or 75% over a period of 2–4 h. The humidity is controlled by placing a small plastic well filled with about 2 ml of water, or the appropriate salt solution, in the fiber puller. A rubber O-ring fits into a groove in the top of the circular chamber. It is greased and a circular glass plate set over the top to provide a tight seal. By observation under a low power stereo-microscope, the sample is periodically thoroughly mixed with a hypodermic syringe needle, and the glass rods are moved back and forth slowly by turning of the knurled heads near either end of the fiber puller. Once it reaches a semi-solid state, the gap between the glass rods is gradually increased so that the specimen stretches without breaking and becomes a fiber, having dimensions of 5 mm or more length and about 0.5 mm thickness. The fiber is then equilibrated in the puller at the desired humidity for a few hours. Finally, the glass rods with attached fiber are carefully removed from the puller and clamped in a small fiber holder, which can then be transferred to a flat film pin-hole camera for X-ray exposure.

X-RAY DIFFRACTION ANALYSIS

The quality of a diffraction pattern is largely determined by the extent of molecular organization in the fiber. For example, if the fiber consists of microcrystallites having random orientations, the resulting powder pattern will have a series of concentric rings. In the case of a fiber composed of a bundle of polymer chains with no lateral organization except that their long axes are parallel to each other, the pattern will exhibit continuous intensities on layer lines, whose vertical spacings are related to the pitch and symmetry of the helix. On the other hand, when the specimen is both crystalline and oriented such that the molecular axes are nearly parallel, the pattern will show sharp Bragg reflections on layer lines. In addition to the crystal structure analysis, such patterns can often lead us to locate cations and water molecules associated with the polymers in difference Fourier maps. The procedures for intensity measure-

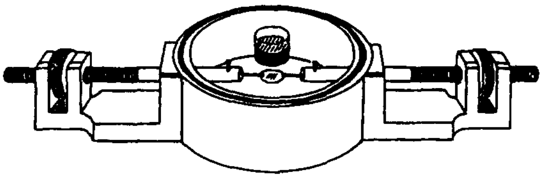


Fig. 1. The circular chamber of the fiber puller has dimensions of approximately 4″ diameter and 1″ depth. The solution in the small plastic well controls relative humidity in the chamber. Stretching of the polymer sample held in the gap at the center between the glass rods is achieved by rotating the knurled heads near either end.

ment, molecular modeling and refinement of molecular and/or crystal structures against the X-ray data are the same as previously reported (Chandrasekaran, 1989; Chandrasekaran & Thailambal, 1990*a*).

CONFORMATION OF ARABAN HELIX

Araban dissolves in hot water at about 95°C with mechanical stirring and forms a slightly cloudy solution. It is possible to prepare intact and slightly stretched fibers at room temperature and 75% relative humidity. Instead of the familiar layer lines diagnostic of oriented specimens, the diffraction pattern (Fig. 2) consists of a series of sharp concentric rings. Attempts to improve orientation, by stretching the fibers at different conditions of temperature and relative humidity, were not successful.

All the 12 rings in the diffraction pattern can be indexed on the basis of a monoclinic unit cell of dimensions $a = 5.6$, $b = 6.1$, $c = 8.7$ Å and $\gamma = 90°$. According to the fiber density of 1.53 g/cc, the unit cell can accommodate two arabinosyl units. This implies that if the araban chain adopts a helical conformation, it should correspond to a 2-fold helix. Although the pitch of the helix is not readily apparent in the diffraction pattern, it has to be one of the three cell edges, *a*, *b* or *c*.

A schematic representation of the (1→5) linked disaccharide unit of α-L-Ara-α-L-Ara is shown in Fig. 3. Assuming a rigid C2-*endo* furanose ring geometry adopted from the crystal structures of β-D-arabinosyluracil (Sherfinski & Marsh, 1974) and β-D-arabinosylcytosine (Tougard & Lefebvre-Soubeyran, 1974), sterically possible helical conformations were examined as a function of the three major dihedral angles, (φ, ψ) around the glycosidic bonds and χ around C4–C5. Because of the 1→5 linkage, there is considerable variation in the virtual bond distance O5···O5 in the arabinosyl unit, from 4.8 to 5.3 Å. Disaccharide contact maps were computed as a function of φ and ψ at 10° intervals, corresponding to each of the three staggered domains for χ = 60°, 180° and −60°, respectively. The center of the allowed regions were used as starting points to generate the required helical conformations.

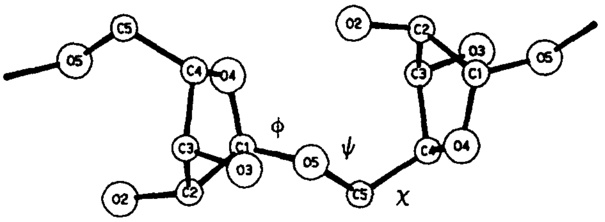


Fig. 3. Schematic representation of a 1→5 linked disaccharide, made up of two α-L-arabinosyl units, and its main variable conformation angles φ, ψ and χ.

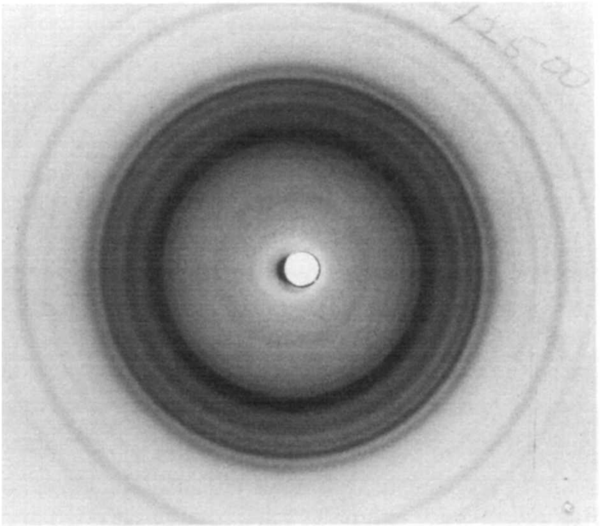


Fig. 2. X-ray diffraction pattern from a fibrous specimen of araban using CuKα radiation of wavelength 1.5418 Å. The sharp concentric rings indicate the presence of randomly oriented microcrystallites of araban in the specimen.

Preliminary results suggest that there may be at least three different 2-fold helical conformations for araban. The first model corresponds to the −60° domain for χ and has 8.7 Å (= *c*) as its pitch (Fig. 4) and is stabilized by intrachain O2···O4 hydrogen bonds. Packing

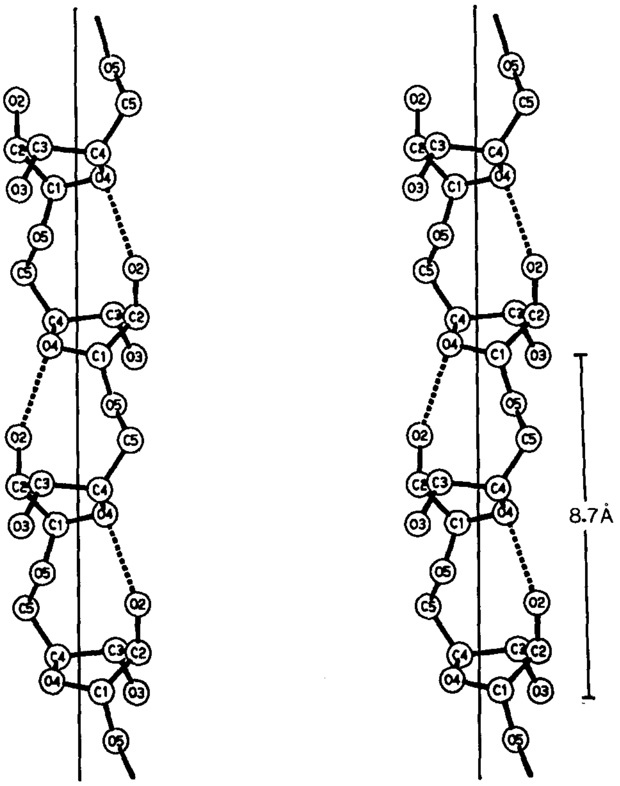


Fig. 4. A stereo view of one of the possible 2-fold helical structures of araban. Intra-chain hydrogen bonds are drawn in dashed lines and hydrogen atoms are omitted for clarity in this and subsequent figures showing molecular structures.

arrangement of this helix in the unit cell (Fig. 5) indicates that neighboring chains separated by 5.6 Å along the *a*-axis are connected by interchain hydrogen bonds of the type O2···O3 and O3···O4. Such hydrogen bonded sheets, separated by 6.1 Å along the cell edge *b*, are however held together only by van der Waals interactions. An interesting feature of this structure is that atom O2 is involved in bifurcated intra- and inter-chain hydrogen bonds.

The second molecular model for araban also has a pitch of 8.7 Å, but it corresponds to the *trans* domain for χ. While the third model retains the same *trans* domain for χ, the conformation angles ϕ and ψ are different such that the pitch of the helix is reduced to 6.1 Å (= *b*). The structural details of these two and other possible models are currently under investigation and will be reported elsewhere.

It is most probable that each of these helical conformations forms microcrystallites in the fibrous specimen. Their random orientations are mirrored by the concentric rings in the diffraction pattern. The X-ray experiments and molecular models from this study are consistent with the existence of different crystalline forms for araban, similar to the polymorphism commonly observed in fat molecules such as triacylglycerides (Hagemann, 1988). Thus, it is tempting to suggest that the ability to adopt several crystalline allomorphs and the propensity to form microcrystals might confer upon araban the potential for fat mimic properties as reported by Cooper *et al.* (1992).

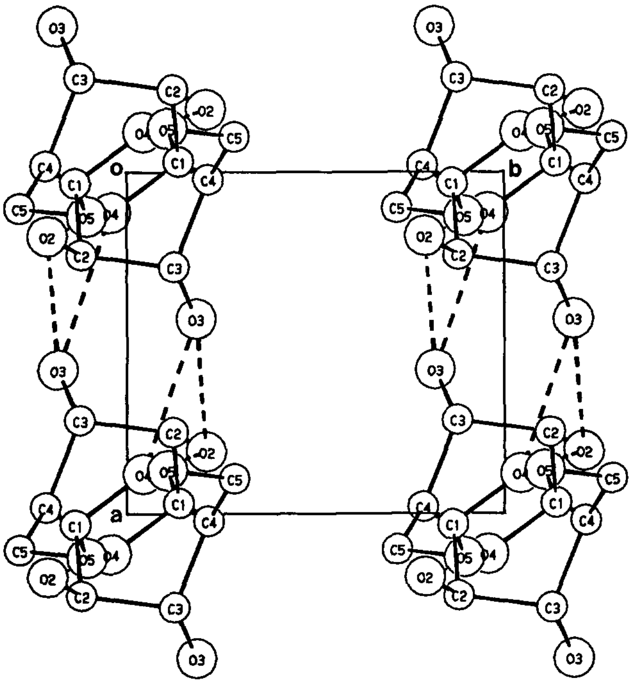
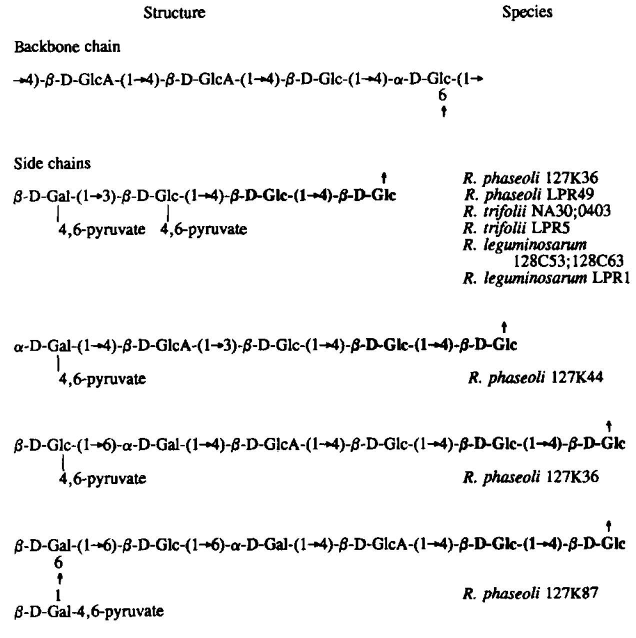


Fig. 5. One of the probable crystal packing arrangements of araban helices which is stabilized by interchain hydrogen bonds (dashed lines) across *a*-axes, and van der Waals forces across *b*-axes.

ANATOMY OF THE *RHIZOBIUM MELILOTI* GALACTOGLUCAN

The extracellular polysaccharides in the *Rhizobia* family, whose repeating units are given in Fig. 6, have been the targets of several scientists around the world because of their importance in nitrogen fixation. Despite their complex chemical structures, the repeating units of these polysaccharides have in common the same tetrasaccharide in the backbone and the 6-linked cellobiosyl unit in the side chain. The length of the side chain varies between 4 and 7 sugar units. There is a pyruvyl group attached to the terminal sugar residue in the side chain. In one type, there is a second pyruvyl in the penultimate sugar residue in the side chain. The O-acetyl substitution pattern, which is different in various species and strains, appears to be determined by the bacterial genome (Canter Cremers *et al.*, 1991) and does not relate to the host specificity (O'Neill *et al.*, 1991). The conservation of the hexasaccharide as part of the building block in all these polysaccharides reveals that the secondary structure of the polyhexasaccharide should be fairly robust. The remaining sugar units in the side chains might only have minor influence on the geometry of the main chain helix. On the other hand, longer side chains which are more flexible might substantially interfere with the lateral organization of the polymer molecules.

This is exactly what happens in the case of the *Rhizobium trifolii* polysaccharide as inferred from its



Structure | Species

Backbone chain

→4)-β-D-GlcA-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-α-D-Glc-(1→
6
↑

Side chains

β-D-Gal-(1→3)-β-D-Glc-(1→4)-**β-D-Glc-(1→4)-β-D-Glc** ↑
4,6-pyruvate (on Gal), 4,6-pyruvate (on Glc)

R. phaseoli 127K36
R. phaseoli LPR49
R. trifolii NA30;0403
R. trifolii LPR5
R. leguminosarum 128C53;128C63
R. leguminosarum LPR1

α-D-Gal-(1→4)-β-D-GlcA-(1→3)-β-D-Glc-(1→4)-**β-D-Glc-(1→4)-β-D-Glc** ↑
4,6-pyruvate (on Gal)

R. phaseoli 127K44

β-D-Glc-(1→6)-α-D-Gal-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-**β-D-Glc-(1→4)-β-D-Glc** ↑
4,6-pyruvate (on Glc)

R. phaseoli 127K36

β-D-Gal-(1→6)-β-D-Glc-(1→6)-α-D-Gal-(1→4)-β-D-GlcA-(1→4)-**β-D-Glc-(1→4)-β-D-Glc** ↑
6
↑
1
β-D-Gal-4,6-pyruvate

R. phaseoli 127K87

Fig. 6. The repeating units of the extracellular polysaccharides in the *Rhizobiaceae* family have in common an acidic tetrasaccharide in the backbone and can be arranged into four major groups depending on their side chain structures. The 6-linked cellobiosyl unit in the side chains (bold face) is also common to all the polysaccharides (adapted from McNeil *et al.*, 1986).

fiber diffraction pattern shown in Fig. 7(a). Molecular organization in fibers is similarly poor in the case of a succinoglycan from *Agrobacterium radiobacter*, which also has a complex octasaccharide repeating unit (Fig. 8(a)). Succinoglycans also belong to the *Rhizobiaceae* polysaccharide family, are regularly substituted with pyruvyl and/or succinyl groups and implicated in the *Rhizobium*-legume symbiosis (McNeil *et al.*, 1986; Carlson *et al.*, 1986), but the best diffraction pattern (Fig. 7(b)) to date provides very little information on their secondary structures. The intensity distribution and layer line spacing in Fig. 7(a) are compatible with a 5-fold helix of pitch 48.9 Å for the *Rhizobium* extracellular polysaccharide, and those in Fig. 7(b) can be interpreted in terms of a 1-fold helix of pitch 18.8 Å for the succinoglycan. Further molecular details for both polysaccharides will remain unknown until much better diffraction patterns can be produced.

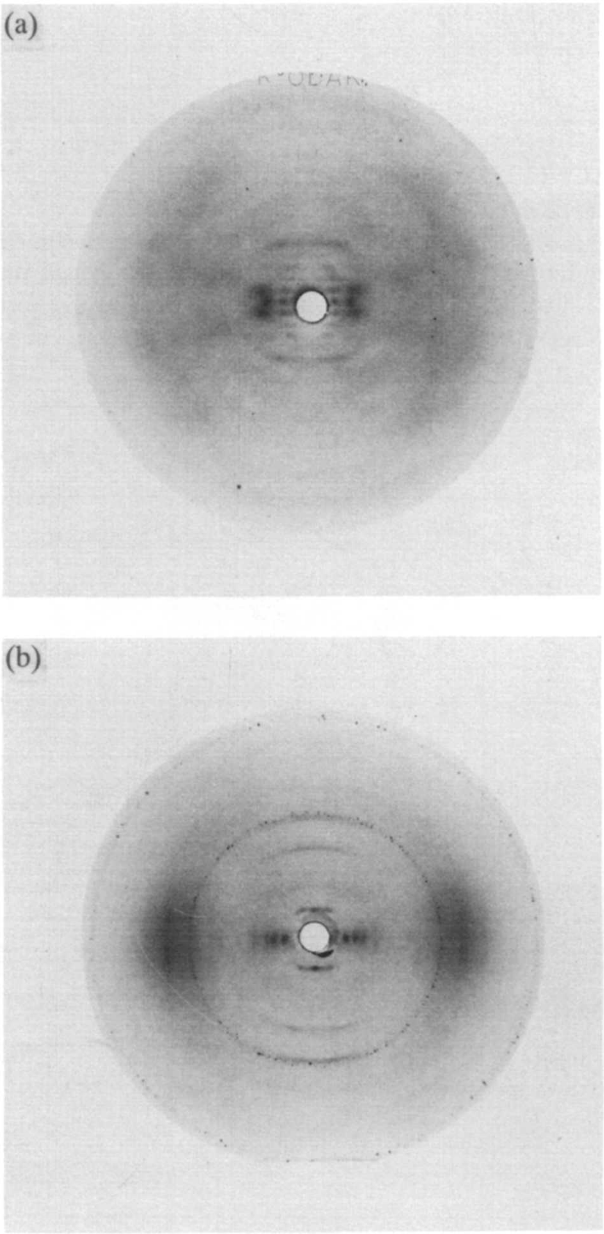


Fig. 7. X-ray diffraction patterns from partially oriented fibers, but having some small range lateral organization of polymer molecules, of the potassium salts of (a) extracellular polysaccharide from *Rhizobium trifolii* and (b) succinoglycan.

Simple *Rhizobium* polysaccharides such as the galactoglucan (Fig. 8(b)), having a disaccharide repeating unit, can also be equally effective in the root nodulation process in alfalfa (Glazebrook & Walker, 1989) as the complex polysaccharides mentioned above. During the biosynthetic pathway of succinoglycan, the *Rhizobium meliloti* strain YE-2 produces the galactoglucan which contains Glc:Gal:pyruvyl:acetyl in the ratios 1:1:1:1 (Zevenhuisen & Faleschini, 1991). Common to succinoglycan and galactoglucan are the β-D-glucose-(1→3)-D-galactose linkage and the pyruvyl and acetyl substitutions. This implies significant structural roles for the substituents in the root infection process. Elucidation of its molecular architecture has become possible from an analysis of the diffraction pattern (Fig. 9) obtained from a polycrystalline and well oriented fiber of the potassium salt of the polysaccharide.

The spots in the pattern can be indexed on the basis of an orthorhombic unit cell of dimensions a = 14.5, b = 9.8 and c = 15.9 Å. The galactoglucan molecule forms a 2-fold helix of pitch 15.9 Å consistent with the meridional reflections on the even layer lines. The helix has a right-handed twist (Fig. 10) and both acetyl and pyruvyl substituents occur on the periphery. The twist between successive glycosyl bridge oxygen atoms, which are both in the interior of the helix, are 66° across D-glucose and 113° across D-galactose and, consequently, there are no direct intrachain hydrogen bonds across either bridge oxygen atom. The closely related structures of 1→3 linked polymers such as β-D-xylan (Atkins & Parker, 1969; Atkins *et al.*, 1969) and β-D-glucan (curdlan) (Fulton & Atkins, 1980) also do not have intrachain hydrogen bonds and hence are very flexible. The galactoglucan helix is, however, stabilized by two water molecules $W1$ and $W2$ which form bridges across oxygen atom O3B between residues A and B. While the

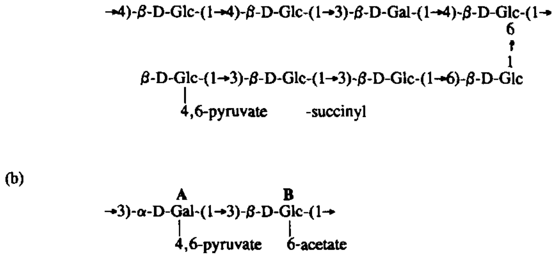


Fig. 8. Repeating units of (a) succinoglycan and (b) galactoglucan have in common the 1→3 linkage between glucose and galactose units and the pyruvyl and acetyl substitutions.

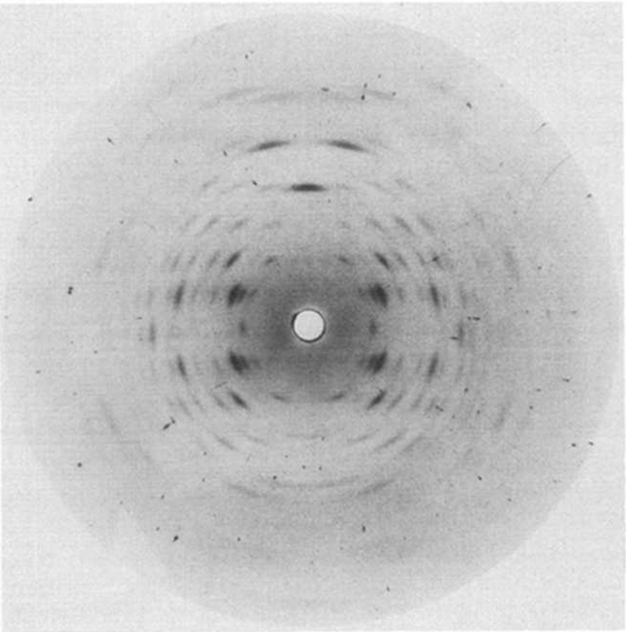


Fig. 9. X-ray diffraction pattern from a well oriented and polycrystalline fiber of the potassium salt of galactoglucan. Fiber tilt is 11° to record the meridional spot on the 4th layer line.

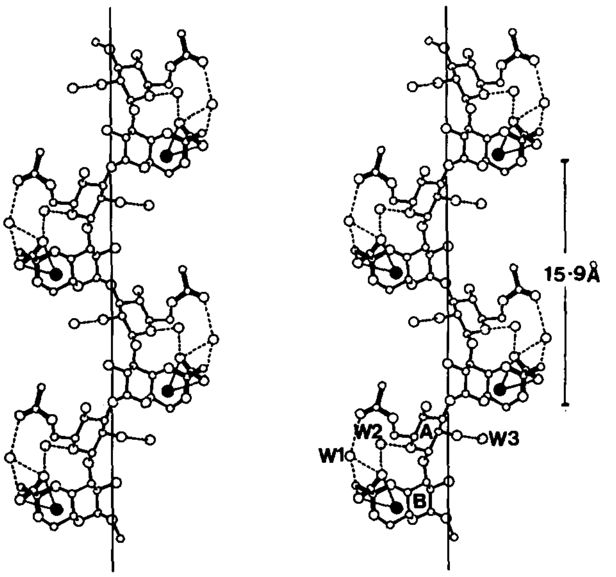


Fig. 10. Stereo view of two turns of the 2-fold galactoglucan helix. The pyruvyl and acetyl substituents (thick bonds) on the periphery are responsible for intra-chain hydrogen bonds (dashed lines). The three water molecules (*W*1, *W*2 and *W*3) and the potassium ion (filled circle) per repeating unit are important for helix stability and interchain interactions.

exterior water molecule *W*1 connects the acetyl and pyruvyl groups, the interior water molecule *W*2 connects a pyruvyl oxygen atom to a nearby glucose ring oxygen. However, there is no such water bridge across the other oxygen atom O3A. This suggests that the main chain itself is perhaps flexible. A potassium ion occurs in the vicinity of each carboxylate group for charge balance and its coordination shell accommodates six ligands drawn from three surrounding helices.

Two helices are packed in the unit cell antiparallel to each other, one at the corner and the other at the center, and are related by 2_1 axes parallel to the *a* and *b* axes as shown in Fig. 11. There is also a third water molecule *W*3 whose special role is to link the acetate group of one helix with oxygen atom O2 in the glucose unit of a second helix. Also the carboxylate groups of neighboring polysaccharide chains are connected by potassium and water molecule *W*1 via a series of $\cdots COO^- \cdots K^+ \cdots W \cdots COO^- \cdots$ interactions, progressing roughly in a direction parallel to the *b*-axis. These interactions are responsible for the associative properties of the galactoglucan chains.

STRUCTURAL ROLES OF CALCIUM IONS, ACETYL GROUPS AND SIDE CHAINS IN WELAN

Welan has a pentasaccharide repeating unit (Fig. 12) and corresponds to the simplest of the branched polysaccharides in the gellan family. The acetyl substitution is about 80% and rhamnose/mannose ratio in the side chains is approximately 2.

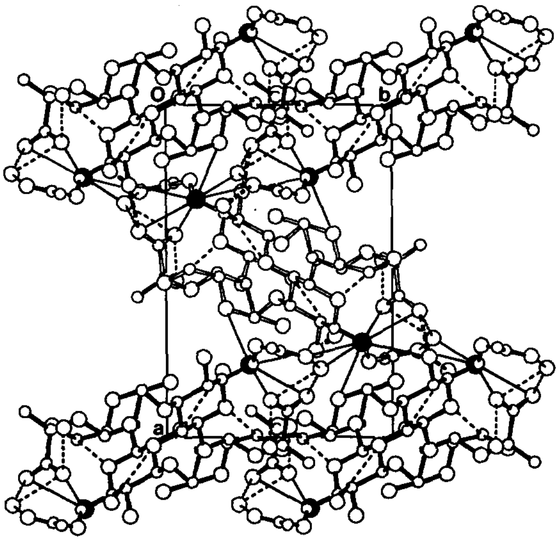
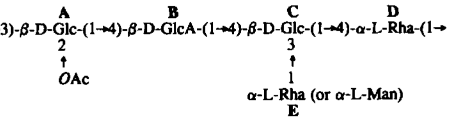


Fig. 11. The packing arrangement of one up (at each corner, filled bonds) and one down (at the center, open bonds) galactoglucan molecules in the unit cell showing intra-chain (thin dashed lines) and inter-chain hydrogen bonds (thick dashed lines). Thin lines connect the potassium ion (filled circle) to its ligands.



$$\rightarrow 3)\text{-}\underset{\substack{2\\ \uparrow\\ O\mathrm{Ac}}}{\overset{\mathbf{A}}{\beta\text{-D-Glc}}}\text{-}(1\rightarrow 4)\text{-}\overset{\mathbf{B}}{\beta\text{-D-GlcA}}\text{-}(1\rightarrow 4)\text{-}\underset{\substack{3\\ \uparrow\\ 1\\ \alpha\text{-L-Rha (or }\alpha\text{-L-Man)}\\ \mathbf{E}}}{\overset{\mathbf{C}}{\beta\text{-D-Glc}}}\text{-}(1\rightarrow 4)\text{-}\overset{\mathbf{D}}{\alpha\text{-L-Rha}}\text{-}(1\rightarrow$$

Fig. 12. The pentasaccharide repeating unit of welan.

There has been little success in obtaining good diffraction patterns from the monovalent salts of welan (Chandrasekaran, 1992), but those from the calcium salt form are exceptional as judged from a typical pattern shown in Fig. 13. Sharp Bragg reflections up to 11 layer lines can be indexed with a trigonal unit of dimensions $a = 20.8$ and $c = 28.7$ Å which are about 5.0 and 0.6 Å larger than the respective values in gellan (Chandrasekaran *et al.*, 1988). Although the gellan-like intensity distribution in Fig. 13 is indicative of a gellan-like double helix as the most probable structure for welan, considerable lateral expansion of its unit cell relative to that of gellan is strong evidence that their packing arrangements are different.

The crystal structure of welan has been developed and refined starting with the molecular structure previously proposed from a computer modeling study (Lee & Chandrasekaran, 1991). The results indicate that welan forms a gellan-like double helix as shown in Fig. 14. The polysaccharide chain is a 3-fold left-handed helix of pitch 57.4 Å, which is 1.1 Å larger than that of potassium gellan. While some of the backbone conformation angles differ up to 20° from those of gellan in order to accommodate the acetyl group, the side chain and a slightly larger pitch, the side chain rhamnose/mannose adopts the same folded conformation (Lee & Chandrasekaran, 1991), and forms hydrogen bonds with the carboxylate group in the main chain. This agreement between prediction and experimental results is gratifying. The double helix retains, and is stabilized by, the gellan-like interchain O6C···O62B hydrogen bond. There are modest conformational changes for the hydroxymethyl and carboxylate groups relative to gellan so as to establish favorable interactions with the neighboring welan double helices, as well as with the water molecules and calcium ions located around the double helices.

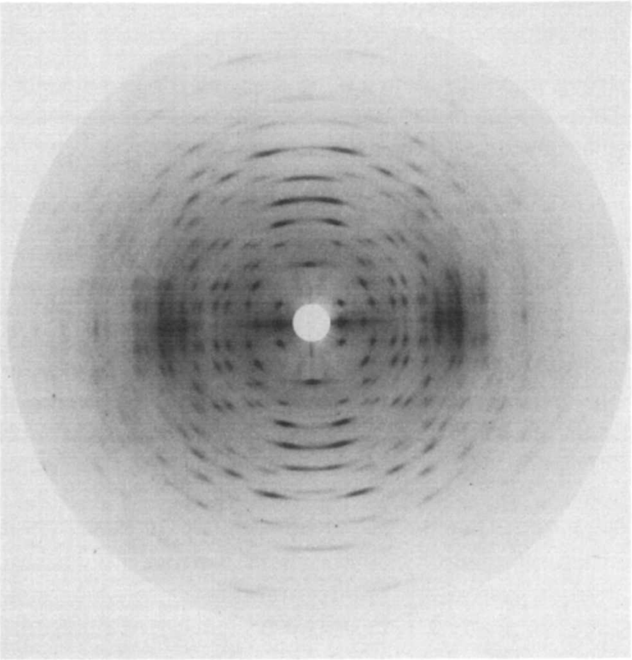


Fig. 13. X-ray diffraction pattern from an extremely well oriented and polycrystalline fiber of the calcium salt of welan.

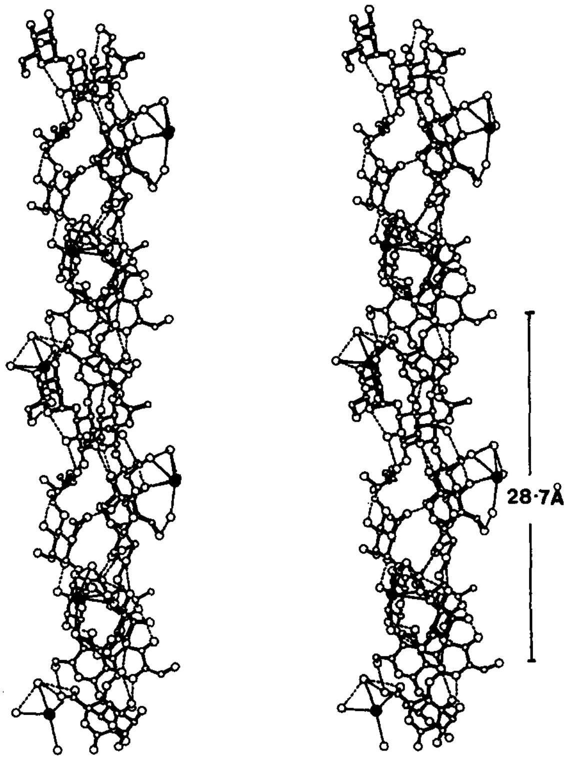


Fig. 14. Stereo view of about one pitch long welan double helix, and the associated calcium ions (filled circles) and water molecules (open circles). Hydrogen bonds within a chain (thin dashed lines) and between the chains (thick dashed lines) are shown along with the ligands connected to the calcium ion by thin lines. Side chains and acetyl substituents are accentuated in thick bonds.

Consistent with the measured fiber density of 1.49 g/cc, there is sufficient room for three welan double helices to pass through the unit cell, compared with two in the case of gellan. Double helices I and II at (2/3, 1/3) and (1/3, 2/3), respectively, are positioned antiparallel to each other, as in the potassium gellan crystal structure (Chandrasekaran *et al.*, 1988); the third double helix III at (0, 0) has the same polarity as double helix I. Inter double helical separation of 12.0 Å in welan is 2.9 Å greater than in gellan and this is necessary in order to accommodate the side chain. This packing arrangement shown in Fig. 15 is made possible by intimate interactions between pairs of carboxylate groups, one from each double helix. These interactions can be classified into three distinct types depending on the nature of involvement of the calcium ion. The first type involves double helices I and II which are not connected by calcium ions, but side chain···side chain hydrogen bonds tie them together. This is a good example for illustrating the positive influence of side chain interactions towards lateral organization of the double helices.

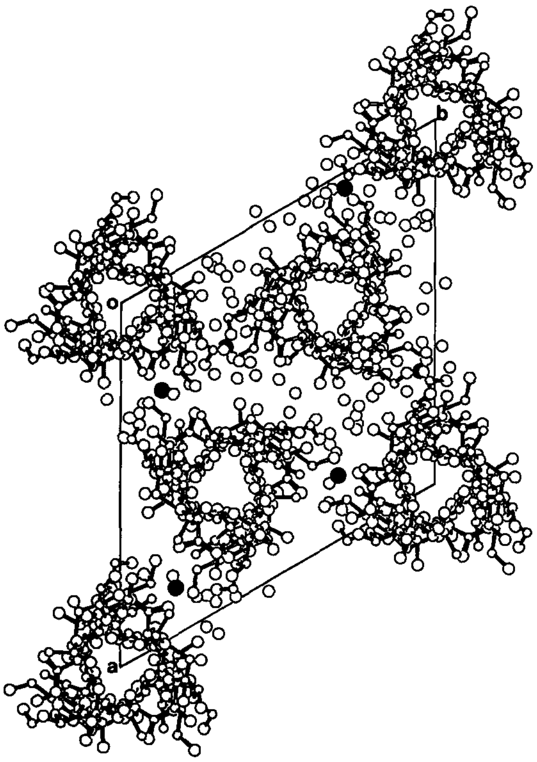


Fig. 15. A *c*-axis projection of the packing arrangement of three welan double helices, calcium ions (filled circles) and water molecules (open circles) in the unit cell. The carboxylate groups (filled bonds) are involved in the association of the double helices.

The second type involves a calcium ion (Ca1) which directly crosslinks the carboxylate groups of double helices II and III as shown in Fig. 16. This arrangement is in good agreement with that proposed for a hypothetical model of the calcium gellan structure (Chandrasekaran & Thailambal, 1990*b*). As expected, the calcium ion is located between, and not within, the two double helices and is surrounded by three ligands from each double helix. There are also four water molecules close to this ion. The third type of interaction involves a water molecule together with a calcium ion (Ca2) to crosslink the carboxylates of double helices I and III which are aligned parallel to each other. The resulting $\mathrm{COO^-\cdots Ca^{2+}\cdots W\cdots\cdots COO^-}$ interactions would be weaker than the direct $\mathrm{COO^-\cdots Ca^{2+}\cdots COO^-}$ interactions between double helices II and III, but stronger than the side chain···side chain interactions between double helices I and II. It appears that this water molecule partly shields the charge on Ca2 such that the two calcium ions effectively balance the charge on three carboxylate groups in their surroundings. Further details have been reported elsewhere (Chandrasekaran *et al.*, 1994).

The simultaneous occurrence of weak, medium and strong intermolecular interactions in the crystal structure implies that each type might be responsible for certain useful rheological properties of welan. For example, the strong interactions might be relevant for concrete mix, while the medium and weak interactions for viscous solutions at low and high temperatures, respectively.

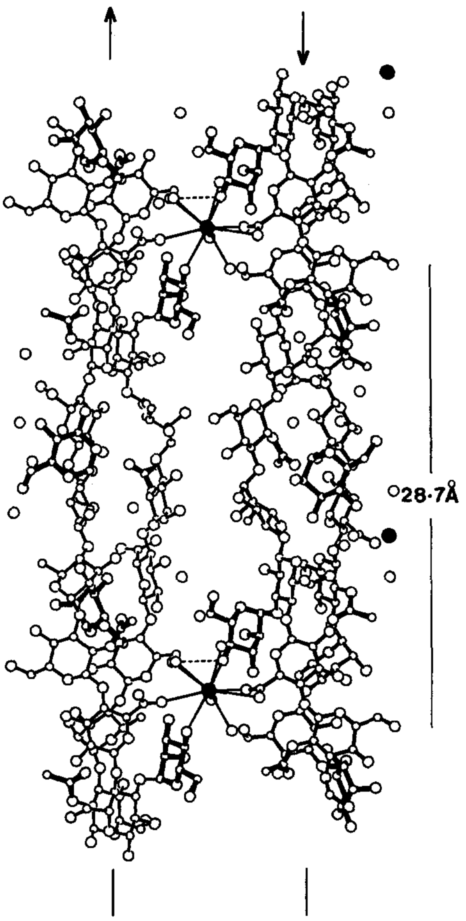


Fig. 16. A side view of a pair of welan-double helices, up (left) and down (right), showing the calcium ion coordination involving the side chains (filled bonds) and carboxylate groups from both double helices. There is an approximate 2-fold symmetry, perpendicular to the plane of paper, about the ion which is linked to its ligands by thin lines.

CONCLUSIONS

The plant polysaccharide araban is able to form microcrystallites and exists in at least three crystalline allomorphs. Its molecule adopts a 2-fold helical structure whose conformation is different in each allomorph. Some of the structural characteristics derived from this diffraction study might be relevant to explain the potential for araban as a fat replacer. The galactoglucan from *Rhizobium meliloti* also forms a 2-fold helix whose structural rigidity via intrachain hydrogen bonds and ability to associate via potassium ions and water molecules are derived from the acetyl and pyruvyl substitutions. These groups might therefore be equally important in the intimate interactions between galactoglucan and the root hairs of leguminous plants which are implicated in the *Rhizobium*-legume symbiosis. The branched polymer welan in the gellan family of polysaccharides forms a gellan-like double helix. The intermolecular association is brought about by calcium ions, water molecules and side chains. While the shielding of carboxylate groups by the side chain enhances molecular stability, the extent of involvement of the calcium ion in the crosslinking of carboxylate groups in neighboring welan double helices appears to be correlated with the observed rheological properties.

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