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CRYSTALLINE STRUCTURES OF POLY-D-MANNURONIC AND POLY-L-GULURONIC ACIDS

In a recent publication (1), we showed that alginic acid preparations enriched in D-mannuronic and L-guluronic acids gave different x-ray fiber diffraction photographs. We were able to correlate these x-ray fiber diagrams with poly-D-mannuronic acid and poly-L-guluronic acid, respectively, and present preliminary details of the two structures. We have now considered the x-ray photographs in greater detail and have supplemented the x-ray data with measurements on oriented films by polarized infrared spectroscopy. We can now present fuller accounts of the crystalline structures of these two substances including the nature of intra- and interchain hydrogen bonding.

Polymannuronic Acid

The orthorhombic unit cell with dimensions a = 7.58 Å, b = 10.35 Å (fiber axis), and c = 8.58 Å contains four monosaccharide units with space group $P2_12_12_1$. The monosaccharide units are in the energetically favorable C1 chain conformation and are arranged to form polysaccharide chains of 1 ⟶ 4 linked β-D- mannuronic acid. Fourier transforms were calculated for various positions of the monosaccharide unit within the unit cell together with rotations of the carboxyl group about the C_5–C_6 bond. Wire models (4 cm = 1 Å), constructed according to the best agreement between the measured and calculated structure factors, showed that several arrangements of intra- and interhydrogen bonding functions were possible. Of these possibilities, all but one were eliminated after consideration of the polarized infrared spectrum. Some of the principal bands of this spectrum and their assignments are given in Table I. Also given are the angles which the transition moments of these bands were calculated to make with the molecular axis (using the relationship $D_{\parallel}/D_{\perp} = 2\cot^2\theta$). The corresponding values calculated from the x-ray data are also given. Projections of the proposed structure are shown in Figure 1a and 1b.

It can be seen that the molecular chain of polymannuronic acid is similar to that found in other 1e, 4e linked hexosans (2,3). It is a flat ribbon-like molecule whose conformation appears to be stabilized by the formation of an intramolecular hydrogen bond between the $-O_3H$ of one unit and the ring oxygen atom (O_5) of the next sugar unit in the chain. The chains themselves are bonded into sheets by means of hydrogen bonds formed between the hydroxyl of the carboxyl group ($-O_6^SH$) and $-O_3$ in sugar units in parallel chains and between the axial $-O_2H$ and the carbonyl oxygen atom ($=O_6^D$) in antiparallel chains.

TABLE 1

Polymannuronic Acid: Infrared Band Assignments and Directions of Transition Moments with Respect to the Molecular Axis

Band frequency cm^{-1}	Assignment	Dichroic ratio $D_{\parallel}/D_{\perp}$	Calculated θ from $D_{\parallel}/D_{\perp}$ (degrees)	Calculated θ from model (degrees)
3557	OH stretch	$< 1/2$	> 63	65
3355	OH stretch	$> 5/1$	< 32	27
3300-2200	carboxylic acid OH	$\sim 1/1.5$	~ 60	58
1724	C=O stretch	1/1	55	54-56

Polyguluronic Acid

The x-ray fiber photograph of this substance indexes to an orthrohombic unit cell in which a = 8.60 Å, b = 8.72 Å (fiber axis), and c = 10.74 Å, also containing four monosaccharide units with space group $P2_12_12_1$. Fourier transforms were calculated as above for the 1 $\longrightarrow$ 4 linked α-L-guluronic acid unit in the IC chair form and again molecular models were constructed according to the best agreement between observed and calculated structure factors. Consideration of these models showed that although intra molecular hydrogen bonds could be formed between the equatorial $-O_2H$ of one unit and either oxygen of the carboxyl group of the next unit in the chain, no arrangement of the chains would allow the formation of intermolecular hydrogen bonds between chains unless water molecules were incorporated into the unit cell.

Experimentally, the presence of water in the unit cell was supported by the measured density of 1.59 g/cc (calculated density with one water molecule per sugar unit = 1.60 g/cc) and the observed deterioration of the x-ray fiber diffraction diagram on intensive drying. (The unit cell then has dimensions a = 7.7 Å, b = 8.7 Å, and c = 10.6 Å, i.e., there is a shrinkage of about 1 Å in the "a" dimension which indicates that water is required to maintain the crystalline structure; see also Ref. 4). Unfortunately, polarized infrared measurements have not yet provided clear-cut results about hydrogen bonding apart from evidence for a parallel hydrogen bond at 3500 cm^{-1} and for perpendicular dichroism for both the hydroxyl and the carbonyl stretching frequencies of the carboxyl group. Figure 2a and b show projections of the structure we propose. This structure is the first example of a diaxially linked (1a, 4a) polymer. The

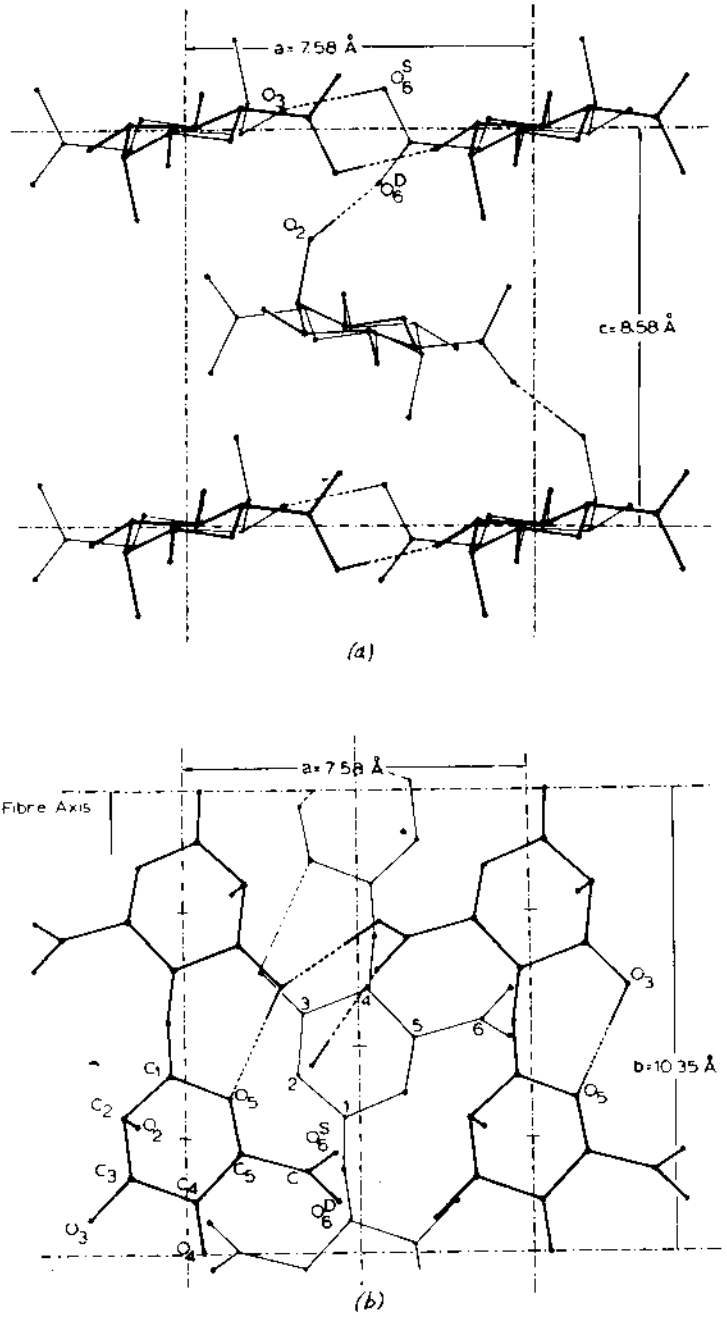

Fig. 1. Proposed crystalline structure of polymannuronic acid. (a) Projection down fiber axis showing interchain hydrogen bonding. (b) Projection in (a b) plane showing both intra- and interchain hydrogen bonding.

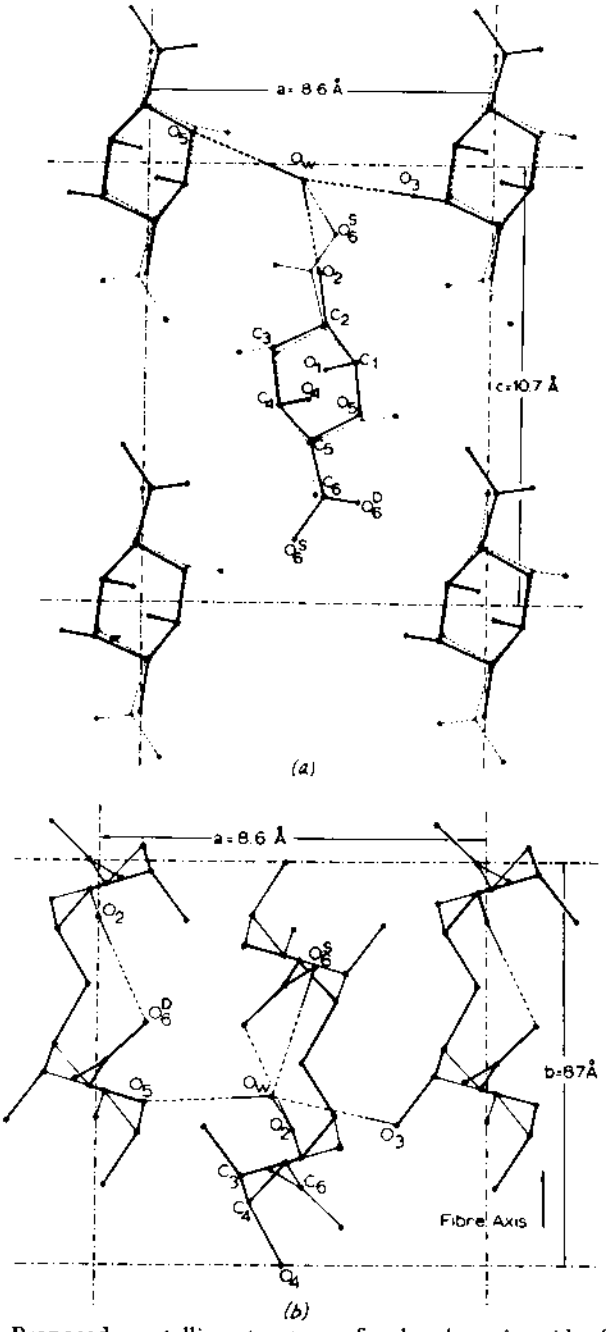

Fig. 2. Proposed crystalline structure of polyguluronic acid. (a) Projection down fiber axis. (b) Projection in the (a b) plane. Note the monosaccharide is in the 1C chain conformation. Only one water molecule (Ow) is shown to avoid confusion.

molecular chain has a rod-like conformation stabilized, in our model, by an intramolecular hydrogen bond between $-O_2H$ and $=O_6^D$ in adjacent units. The interchain bonds are more complicated than in the case of polymannuronic acid and involve water molecules. A water molecule is shown in such a position that it functions twice as a hydrogen bond donor and twice as an acceptor, the hydrogen bonds so formed being in the range 2.7 Å - 2.9 Å. In accord with density measurements and to preserve symmetry we require four water molecules in the unit cell.

Current ideas on the biological occurrence of alginates suggest that they are largely copolymers made up of blocks of the type $(-M-)_n$, $(-G-)_n$, and $(-M-G-)_n$ occurring within the same molecule (5). It has also been noted that alginates enriched in mannuronic acid may be more characteristic of intercellular regions and primary cell wall tissue, whereas guluronic acid rich alginates appear to be more characteristic of tissues where greater stiffness is required, e.g., *Laminaria hyperborea* stipes (6,7). There seems little doubt that part of this stiffness must arise from the well-known selectivity of $1 \longrightarrow 4$ linked L-guluronic acid units for divalent ions such as calcium which leads to the formation of rigid gels (8), but it may also be partly inherent in the chain. In this respect it can be seen qualitatively that the rod-like conformation of the polyguluronic acid chain might be less flexible than the ribbon-like polymannuronic acid molecule.

It is hoped that consideration of the structures of the salts of these substances (currently being carried out) will enable better correlations between molecular structures and properties to be made.

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