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Three-dimensional structure of guaran[1]

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Abstract

The galactosyl side chains were inadvertently fixed in the improper β-D configuration in a previously reported morphology of guaran [R. Chandrasekaran, A. Radha, and K. Okuyama, *Carbohydr. Res.* 306 (1998) 243–255]. This defect has now been rectified. In the correct α-D configuration, the 2-fold guaran helix is stabilized by periodic intramolecular hydrogen bonds between the galactosyl side chains and the mannan main chain. Ordered water molecules are responsible for the formation and association of sheets of guaran helices in the crystalline lattice. The crystallographic *R*-value is 0.14 for the revised model. The guaran molecular structure and packing arrangement are readily amenable to the entire galactomannan family.

Keywords: Guaran; Galactomannan; X-ray diffraction; Three-dimensional structure

1. Introduction

Since a recently published X-ray structure of guaran had erroneously incorporated the (1→6)-linked galactosyl side chains in the β-D instead of the correct α-D configuration, the related Tables 2–5 and Figs. 3–6 contain wrong details [1]. We have subsequently corrected this mistake and the revised structural results are presented in this paper. To conserve space, invariant information on X-ray data collection and methodology on structure determination found in [1] are not repeated here. Changes if any are indicated at appropriate places in the text.

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[1] Dedicated to Professor Roy Whistler, the pioneer in structural studies of guaran.

2. Experimental data

Same as described in [1].

3. Structure analysis

Same as described in [1].

4. Results

Molecular structure and packing arrangement.—The first task was to examine the feasibility of constructing a 2-fold helical galactomannan chain having the galactosyl side chain in the correct α-D configuration and yet resembling the previously reported morphology that provided a good X-ray

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fit [1]. Keeping the main chain as a rigid body, all three staggered domains around the C-5–C-6 bond at the 1→6 linkage were searched. Optimization of the non-bonded contacts by varying (χ_1, ϕ_2 and ψ_2) produced the desired geometry when χ_1 was in the trans domain. It had the side chain turned towards the reducing end so as to form a hydrogen bond with the main chain. This helix was positioned in the unit cell at $u = v = 0.25$ and the packing parameters μ and w given in [1] were used to generate the crystal structure having the space group *I*222. This initial arrangement showed some short contacts between the helices, but they could be relieved easily by small changes to the two variables in a preliminary refinement of the structure in which the helix was held as a rigid body. On the basis of water smeared scattering factors [2], the *R*-value for this initial crystal structure was 0.30 and it dropped to 0.23 when both main chain and side chain conformation angles were refined.

Table 1
Cartesian and cylindrical polar coordinates of a repeating unit of the guaran helix

Group	Atom	*x*(Å)	*y*(Å)	*z*(Å)	*r*(Å)	ϕ(°)
Mannose	C-1	0.0243	0.3352	1.1883	0.3360	85.86
	C-2	−0.4912	1.4096	2.1367	1.4927	109.21
	C-3	0.1535	1.1959	3.4976	1.2057	82.68
	C-4	−0.0300	−0.2387	3.9751	0.2405	−97.15
	C-5	0.4918	−1.2134	2.9247	1.3092	−67.94
	C-6	0.2811	−2.6609	3.3151	2.6757	−83.97
	O-1	−0.6852	0.4535	0.0000	0.8217	146.50
	O-2	−1.9107	1.3361	2.2037	2.3315	145.04
	O-3	−0.4301	2.0872	4.4498	2.1310	101.64
	O-4	0.6851	−0.4535	5.1900	0.8216	−33.50
	O-5	−0.2212	−0.9759	1.7010	1.0006	−102.77
	O-6	0.2868	−3.5227	2.1777	3.5344	−85.34
	H-1	1.1064	0.4800	1.0542	1.2061	23.45
	H-2	−0.2356	2.4029	1.7393	2.4145	95.60
	H-3	1.2291	1.4172	3.4328	1.8759	49.07
	H-4	−1.1018	−0.4199	4.1434	1.1791	−159.14
	H-5	1.5703	−1.0532	2.7793	1.8908	−33.85
	H-61	1.0716	−2.9711	4.0143	3.1584	−70.17
	H-62	−0.6765	−2.7618	3.8469	2.8434	−103.76
Galactose	C-1	−0.9240	−4.1179	1.8475	4.2203	−102.65
	C-2	−0.9895	−5.4813	2.5232	5.5699	−100.23
	C-3	0.1009	−6.4206	2.0312	6.4214	−89.10
	C-4	0.1154	−6.4688	0.5090	6.4698	−88.98
	C-5	0.1835	−5.0501	−0.0461	5.0534	−87.92
	C-6	0.1407	−5.0120	−1.5591	5.0140	−88.39
	O-1	0.2868	−3.5227	2.1777	3.5344	−85.34
	O-2	−0.8847	−5.3133	3.9323	5.3865	−99.45
	O-3	−0.1261	−7.7323	2.5505	7.7334	−90.93
	O-4	−1.0662	−7.1002	0.0204	7.1798	−98.54
	O-5	−0.9394	−4.2914	0.4291	4.3930	−102.35
	O-6	1.2712	−5.6628	−2.1377	5.8038	−77.35
	H-1	−1.7510	−3.4566	2.1452	3.8748	−116.87
	H-2	−1.9674	−5.9433	2.3228	6.2605	−108.32
	H-3	1.0782	−6.0706	2.3949	6.1657	−79.93
	H-4	0.9888	−7.0442	0.1685	7.1133	−82.01
	H-5	1.1193	−4.5729	0.2804	4.7079	−76.25
	H-61	−0.7817	−5.4961	−1.9123	5.5514	−98.10
	H-62	0.1057	−3.9664	−1.8989	3.9678	−88.47
Water	*W*1	−0.9349	−9.5929	1.2137	9.6383	−95.57
	*W*2	1.5146	−9.5625	2.4677	9.6817	−81.00
	*W*3	−2.3137	−3.3558	−1.3937	4.0761	−124.59
	*W*4	2.3138	−7.7100	−1.3937	8.0497	−73.30

The cylindrical polar coordinates of the next repeat in the helix are r, $\phi + 180°$, $z + 5.19$ Å.
The cartesian coordinates of the first repeat of helices I to IV in the unit cell are:

I $0.25a + x, 0.25b + y, 0.1343c + z$ II $0.25a - x, 0.75b + y, 0.3657c - z$
III $0.75a + x, 0.25b - y, 0.3657c - z$ IV $0.75a - x, 0.75b - y, 0.1343c + z$

Additional repeats can be generated by applying the helix symmetry to each of them.

Water molecules in the crystal structure.—As mentioned in [1], normal atomic scattering factors [3] were used in this section onwards, both for computing the difference Fourier maps in search of water molecules, and for further structure analysis. The first map showed a water molecule *W*1 in the vicinity of atoms O-3 and O-4 of the side chain. Refinement of its position gave a better X-ray fit ($R = 0.23$) for the augmented crystal structure. Likewise, second through fourth maps, each based on the previously updated crystal structure, led to identifying three more water molecules, *W*2 to *W*4, one per map in the side chain region. An important observation was that both *W*3 and *W*4 were in special positions; *W*3 on the dyad along the *b*-axis ($u = w = 0$), and *W*4 at the intersection of the three crystallographic dyads ($u = 0.5$, $v = w = 0$), their respective occupancies being 0.5 and 0.25. Together, this translates to 2.75 water molecules per monomer repeat of the galactomannan helix, although the measured fiber density (1.48 g/cm^3) could account for up to 3.7. However, no additional water molecules were in sight in the fifth and final difference map.

A last round of crystal structure refinement with flexible sugar rings and variable thermal parameter provided smooth convergence and improved the *R*-value to 0.14 for the 20 observed reflections and for 4 out of 15 unobserved reflections. Neither the molecular and packing parameters of the polysaccharide chain, nor the water positions moved significantly from their respective values prior to this refinement. The final atomic coordinates of this model are given in Table 1. The observed and calculated structure amplitudes are in Table 2.

Morphological features.—As expected, the main chain retains a fully extended mannan-like conformation. The major conformation angles of the helix are listed in Table 3 along with those of mannan I and mannan II [4] for comparison. Notable is that χ_1 is in the same trans domain as in mannan I, but not in mannan II. Using helix I (at $u = v = 0.25$) as the reference, a set of 25 attractive interactions, less than 3.1 Å, in the crystal structure

Table 2
Observed and calculated structure amplitudes for guaran for (a) even and (b) odd layer lines

a

h *k*	*l* = 0	2	4
0 0	M *[1238]*	M *[26]*	M *[62]*
0 2	215 *229*	(52) *(56)*	[80] *[76]*
1 1	[62] *[2]*		
0 4	217 *232*	255 *181*	227 *163*
1 3	104 *86*	115 *93*	[229] *[146]*
0 6 1 5	359 *348*	219 *182*	
2 0 2 2	702 *724*	[122] *[108]*	
1 7 2 4 0 8	326 *311*		
2 6	[162] *[123]*		

b

h *k*	*l* = 1	3
0 1	42 *3*	137 *94*
0 3	[45] *[41]*	[68] *[13]*
1 0	[56] *[4]*	
1 2	223 *199*	268 *207*
0 5 1 4	79 *73*	[127] *[85]*
2 1 1 6		
0 7 2 3	225 *239*	191 *216*
2 5 1 8 0 9	(169) *(200)*	318 *294*
2 7	(127) *(188)*	[267] *[93]*
3 0 3 2	(161) *(211)*	
1 10 3 4	207 *255*	
0 11 2 9	194 *193*	
3 6	[193] *[160]*	

In each box, the observed amplitude is given in the first line and the calculated amplitude in the next line in *italics*. The curved and square brackets respectively refer to the below threshold refections included in and rejected from the least-squares refinement. M refers to meridional reflection. The calculated structure amplitudes include a temperature factor with $B = 3.0$ Å^2.

Table 3
Major conformation and bridge bond angles (and e.s.d.) in degrees in guaran and mannan helices[a]

Parameter	Guaran	Mannan I	Mannan II	Remarks
ϕ_1(O-5M–C-1M–O-4′M–C-4′M)	−98(2)	−90	−88	$\beta(1\rightarrow4)$
ψ_1(C-1M–O-4′M–C-4′M–C-5′M)	−143(2)	−149	−153	$\beta(1\rightarrow4)$
χ_1(C-4M–C-5M–C-6M–O-6M)	158(3)	180	−23	Hydroxymethyl
τ_1(C-1M–O-4′M–C-4′M)	117(1)	117	117	Bridge
ϕ_2O-5G–C-1G–O-6M–C-6M)	149(2)			$\alpha(1\rightarrow6)$
ψ_2(C-1G–O-6M–C-6M–C-5M)	−111(3)			$a(1\rightarrow6)$
χ_2(C-4G–C-5G–C-6G–O-6G)	−63(6)			Hydroxymethyl
$\tau_2$2(C-1G–O-6M–C-6M)	116(2)			Bridge

[a] The letters M and G, and symbol ′ refer to mannose, galactose and the reducing end, respectively.

are given in Table 4. They are sequentially numbered in the second column for easy identification. As shown in Fig. 1, the familiar O-3H···O-5 hydrogen bond (1) links adjacent mannosyl residues and gives structural stability. By adopting a *gt* orientation, i.e., gauche to O-5 and trans to C-4 ($\chi_1 = 158°$), atom O-6 of the backbone is 3.5 Å from the helix axis and the galactosyl side chain turns towards the reducing end such that its atom O-3 at $r = 7.7$ Å describes the periphery of the helix. The conformation angles ϕ_2 and ψ_2 (Table 3) are such that atom O-5 of the galactosyl residue is at hydrogen bonding position (2) from atom O-3 of the adjoining mannosyl residue in the reducing end. This creates a bifurcated hydrogen bond from atom O-3M. Since χ_2 is in the gauche minus domain, atom O-6G is turned away from the backbone of the helix. Overall, the galactomannan chain appears to have a sheet-like structure of width almost 15.4 Å from left to right as in Fig. 1.

Guaran–guaran interactions.—Related by the crystallographic 222 symmetry, the four helices in the unit cell are pointing alternately up (I and IV) and down (II and III) and located at intervals of $a/2$ and $b/2$. As shown in Fig. 2, I and II, separated by $b/2$ (15.42 Å) form a sheet structure. So do III and IV. Within the sheet, the secondary hydroxyl groups of the side chains of adjacent helices are linked by three hydrogen bonds (3, 4, and 5). The two sheets, $a/2$ (4.63 Å) apart along the *a*-axis, are antiparallel. Although absent in the analogous mannan II crystal structure, there is one intersheet hydrogen bond (6) directly between the main chains of guaran. In addition, the galactosyl units are engaged in three intersheet hydrogen bonds (7, 8 and 9). This network of hydrogen bonds, some of which are bifurcated, involving the hydroxyl groups in the side chains, is critical for stability of the galactomannan crystal structure.

Water bridges.—Each of the four water molecules has two or more links to the galactomannan helices. *W*1 is linked to four of the side chain hydroxyl groups (10–13). The same trend is found for *W*4 located in a special position equidistant from four symmetry mates of atom O-6G (22 to 25). *W*2, on the other hand, has two of its four ligands as side chain oxygen atoms (14, 15) and the other two as surrounding water molecules (16, 17). Finally, due to its special position, *W*3 is linked to atoms O-3M (18) and O-5G (19) in one helix and their symmetry mates in another (20, 21). The 16 water bridges are responsible for strengthening the

Table 4
Attractive interactions within and among guaran helices and those involving water molecules[a]

Type	Interaction	Atom X	Atom Y	X...Y (Å)	Precursor P	P-X...Y (°)
Intrachain	1	O-3M	O-5M	2.76	C-3M	104
	2	O-3M	O-5G	2.85	C-3M	124
Intrasheet	3	O-2G	O-3G(II,0-1)	2.73	C-2G	109
	4	O-3G	O-3G(II,0-1)	2.49	C-3G	110
	5	O-4G	O-4G(II,0-1)	3.08	C-4G	119
Intersheet	6	O-2M	O-3M(III,−10)	2.58	C-2M	152
	7	O-2G	O-2G(III,−10)	2.87	C-2G	80
	8	O-4G	O-4G(IV,−1-1)	2.78	C-4G	160
	9	O-6G	O-6G(III)	2.56	C-6G	114
Water bridges	10	O-2G(II,0-1)	*W*1	2.50	C-2G(II,0-1)	161
	11	O-3G	*W*1	2.43	C-3G	124
	12	O-4G	*W*1	2.77	C-4G	102
	13	O-6G(II,0-1)	*W*1	2.89	C-6G(II,0-1)	72
	14	O-2G(II,0-1)	*W*2	2.73	C-2G(II,0-1)	118
	15	O-3G	*W*2	2.46	C-3G	124
	16	*W*1	*W*2	2.75		
	17	*W*2	*W*2(III)	3.10		
	18	O-3M	*W*3	3.09	C-3M	76
	19	O-5G	*W*3	2.47	C-5G	113
	20	O-3M(III,-10)	*W*3	3.09	C-3M(III,-10)	76
	21	O-5G(III,-10)	*W*3	2.47	C-5G(III,-10)	113
	22	O-6G	*W*4	2.41	C-6G	127
	23	O-6G(II,0-1)	*W*4	2.41	C-6G(II,0-1)	127
	24	O-6G(III)	*W*4	2.41	C-6G(III)	127
	25	O-6G(IV,0-1)	*W*4	2.41	C-6G(IV,0-1)	127

[a]Unless otherwise labeled in parentheses, all atoms belong to helix I. The two arabic numerals in parentheses refer to *a* and *b* translations, respectively.

attractive interactions within as well as between the sheets formed by the galactomannan helices.

The extensive network of hydrogen bonds promoted by the ordered water molecules, shown in Fig. 3, demonstrates that the peripheral galactosyl side-chains have a dominant influence on the lateral organization of the galactomannan helices. Consequently, depletion of the side chains would

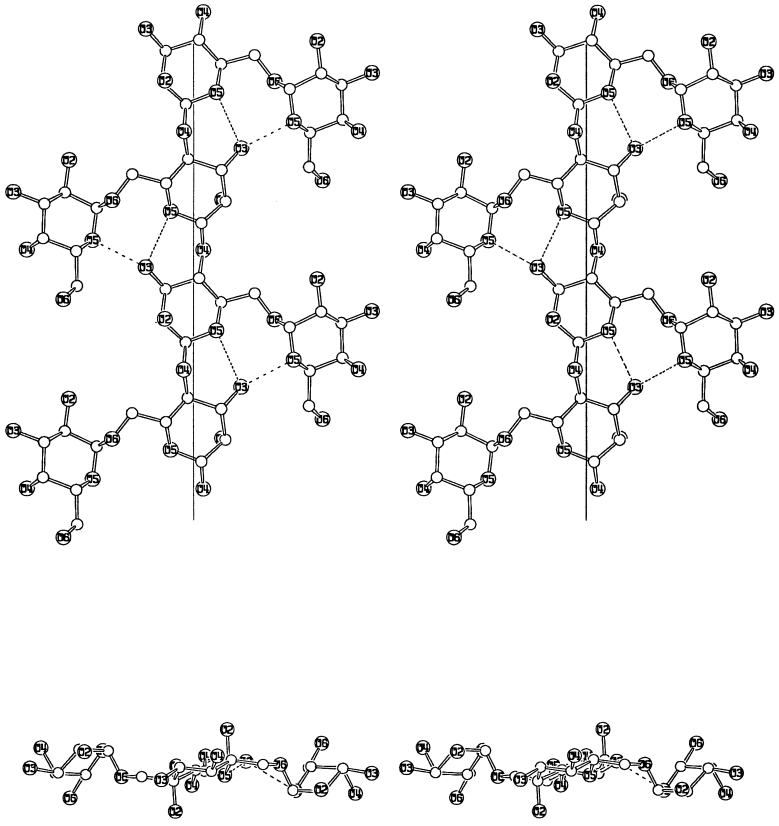

Fig. 1. Two mutually perpendicular stereo views of the guaran helix. Two turns are shown at the top. The helix is stabilized by a series of hydrogen bonds (dashed lines) involving both main and side chain atoms. The vertical line, helix axis, measures 2*c*. An axial projection of one turn at the bottom shows the flat nature of the galactomannan chain whose thickness is only 4 Å.

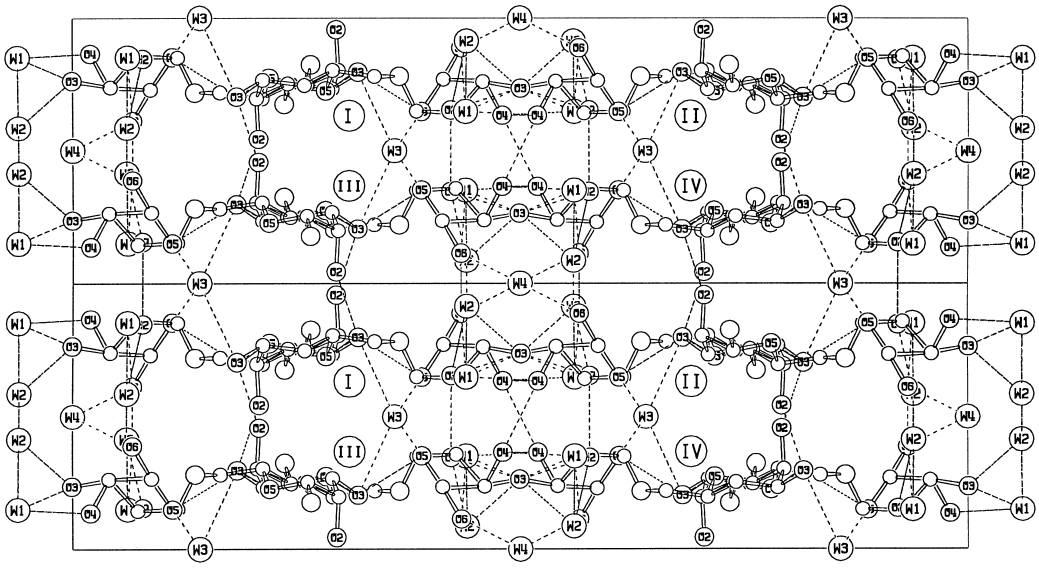

Fig. 2. Packing arrangement, as viewed down the *c*-axis, of the galactomannan helices in two unit cells (2*a* is down and *b* across the page). Both direct and water-mediated hydrogen bonds (dashed lines) involving the hydroxyl groups (labeled) of mannose and galactose are responsible for association of the sheets of helices in pairs within and between unit cells.

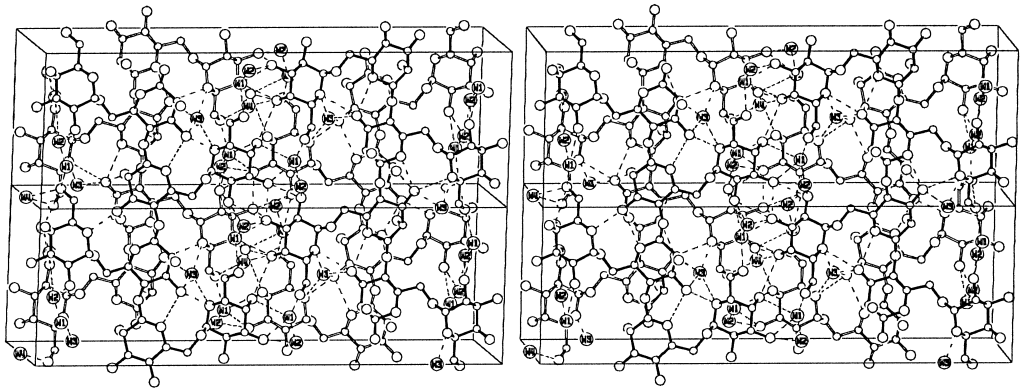

Fig. 3. A stereo view, roughly normal to the *c*-axis, of the dense packing and intermolecular interactions of helices in two unit cells. The sheet behind (open bonds) is *a*/2 from the sheet in front (filled bonds). Hydrogen bonds (dashed lines) between helices and those involving water molecules (labeled) are extensive. While there is considerable stacking between the front and back mannan chains, the galactosyl residues, surrounded by water molecules, form a column in the middle and display little overlap.

lead to disorganization of the ordered water molecules and hence disruption of crystallinity.

5. Discussion

The space group riddle.—Same as in [1].

Relationship to mannan II.—Same as in [1] except perhaps some minor difference in the fine details of Fig. 7b in Ref [1]. It is sufficient to say that the revised structure does not alter the previous conclusions.

Hydration of galactomannan helices.—Same as in [1], since the panels in Fig. 8 in Ref [1], barring four (new) instead of two (old) water molecules, would nearly resemble the same if the revised structure is used to redraw them.

Acknowledgements

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References

[1] R. Chandrasekaran, A. Radha, and K. Okuyama, *Carbohydr. Res.*, 306 (1998) 243–255.
[2] R. Chandrasekaran and A. Radha, *J. Biomol. Struct. Dynamics*, 10 (1992) 153–168.
[3] Anon, *International Tables for X-ray Crystallography*, Vol. IV, Keynoch Press, England, 1974, pp. 99–101.
[4] R. Chandrasekaran, *Adv. Carbohydr. Chem. Biochem.*, 52 (1997) 311–439.