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Note

X-Ray diffraction study on (1→3)-α-D-mannan dihydrate

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Some fungi produce branched heteropolysaccharides with a backbone chain of (1→3)-α-D-mannan. Fruiting bodies of edible mushrooms, *Auricularia auricullajudae* (Kikurage)[1,2] and *Tremella fuciformis* Berk (Shirokikurage)[3,4], for instance, contain D-glucurono-D-xylo-D-mannans in which the (1→3)-α-D-mannan backbones have short side-chains of D-glucuronic acid and D-xylose. A partially *O*-acetylated, linear (1→3)-α-D-mannan that shows anti-inflammatory properties[5] was extracted from the fruiting body of *Dictyophora indusiata* Fisch (Kinugasatake)[6].

Among homomannans, the crystalline conformation of (1→4)-β-D-mannan has been extensively studied by X-ray diffraction analysis[7,8] and by electron diffraction[9]. Two polymorphs of the D-mannan, known[7] as mannan I and mannan II, have been characterized. Both are nearly fully extended, two-fold helices and closely resemble cellulose in their crystalline behavior, with mannan I corresponding[9] to cellulose IV_{II} and mannan II to cellulose II. Our recent study of (1→3)-α-D-glucan revealed that it also possessed a chain conformation similar to that of cellulose[10]. Furthermore, the chain conformation of the (1→3)-α-D-mannan is predicted to be

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similar to that of the (1→3)-α-D-glucan, as D-glucose and D-mannose differ only in the disposition of the hydroxyl group at C-2: equatorial for the former and axial for the latter. X-Ray diffraction data for (1→3)-α-D-mannan are reported.

EXPERIMENTAL

Materials. — A polysaccharide, called T-2-HN and extracted with 70% aqueous ethanol from the fruiting body of *Dictyophora indusiata* Fisch., is a linear (1→3)-α-D-mannan containing about one *O*-acetyl group, at O-6 of the D-mannan, per two D-mannose residues (mol. wt., 6.2×10^5), and it dissolves[6] in water and Me_2SO. In contrast, a deacetylated D-mannan (T-2-HND), prepared by treatment of T-2-HN with 0.2M sodium hydroxide, is insoluble in water and Me_2SO, but dissolves in >2M sodium hydroxide[6]. Another linear (1→3)-α-D-mannan sample was supplied by Professor Akira Misaki of Osaka City University and Professor Mariko Kakuta of Konan Women's College. It is the backbone polysaccharide prepared by removing side chains (by mild Smith degradation) of a D-glucurono-D-xylo-D-mannan extracted from the fruiting body of *Tremella fuciformis*[4].

Methods. — The X-ray diffraction patterns were recorded by a flat-film camera with a Rigaku Geigerflex X-ray diffractometer, using Ni-filtered CuKα radiation generated at 40 kV and 15 mA. The irradiation at high relative humidities was performed in a helium atmosphere to prevent X-ray scattering caused by the presence of air. The density of samples was measured by flotation in solutions of carbon tetrachloride–*m*-xylene.

RESULTS AND DISCUSSION

A continuous film could not be obtained from the two pure (1→3)-α-D-mannan samples, T-2-HND or the mannan prepared by removing side chains of the D-glucurono-D-xylo-D-mannan. A similar situation occurred with the (1→3)-α-D-glucan prepared by removing side chains of the α-D-glucan produced[11] by *Streptococcus salivarius* TTL-LP1. For the glucan, we succeeded in obtaining a well oriented film by acetylation followed by solid-sate deacetylation of the triacetate. However, this procedure could not be used with the (1→3)-α-D-mannan samples, as they did not undergo acetylation, even under rigorous acetylation conditions using trifluoroacetic anhydride.

In contrast, the partially acetylated D-mannan, T-2-HN, which occurs naturally, gave a continuous film from its solution in Me_2SO. A strip of the film was stretched in 75% aqueous methyl alcohol at 60°. Subsequently, the stretched film was deacetylated by immersing it in 2M sodium methoxide–methyl alcohol overnight at room temperature, keeping the length of the film constant. The resultant D-mannan film gave a fiber pattern, but of very low crystallinity. When annealed in 70% aqueous isopropyl alcohol at 180° or higher (in a sealed bomb), the crystallinity was much improved and a well-defined fiber pattern was obtained at high

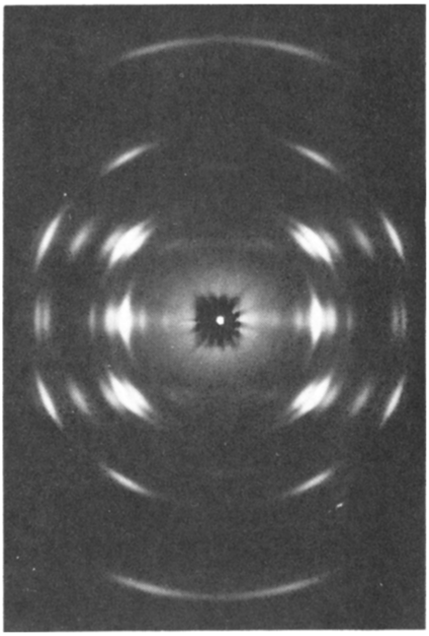

Fig. 1. X-Ray diffraction pattern of the (1→3)-α-D-mannan at 85% relative humidity. The fiber axis is vertical.

TABLE I

CRYSTAL DATA FOR THE (1→3)-α-D-MANNAN

Crystal system	Monoclinic
Space group	$P2_1$
Lattice parameters	
a (Å)	11.29
b (Å)	18.12
c (fiber axis) (Å)	8.40
γ (degrees)	79.17
Density	
ρ (obs.) (g/cm^3)	1.50
ρ (calc.) (g/cm^3)	1.53
In the unit cell	
Number of mannose residues	8
Number of water molecules	16
Number of chains	4
Helix parameters[a]	
n	2
h (Å)	4.20

[a] n: Number of mannose residues per turn. h: Advance per residue along the helix axis.

TABLE II

OBSERVED SPACINGS AND INTENSITIES FOR THE (1→3)-α-D-MANNAN

h	k	l	Spacings (Å)		Intensity[a]
			Calc.	Obs.	(Obs.)
1	0	0	11.09	11.16	w
1	2	0	7.68	7.81	m
1	$\bar{2}$	0	6.38	6.38	vs
2	1	0	5.60	5.61	s
2	0	0	5.55		
1	$\bar{3}$	0	4.87	4.86	m
0	4	0	4.45	4.43	vw
1	4	0	4.43		
2	$\bar{3}$	0	3.72	3.68	s
3	0	0	3.70		
3	2	0	3.67		
3	$\bar{1}$	0	3.49	3.50	s
1	$\bar{5}$	0	3.22	3.20	m
3	$\bar{2}$	0	3.21		
2	$\bar{4}$	0	3.19		
3	$\bar{3}$	0	2.90	2.92	m
2	$\bar{6}$	0	2.43	2.45	vw
4	5	0	2.42		
5	$\bar{1}$	0	2.15	2.13	m
3	$\bar{6}$	0	2.13		
1	$\bar{8}$	0	2.11		
0	1	1	7.60	7.70	m
1	0	1	6.70	6.82	m
1	2	1	5.67	5.61	s
1	$\bar{2}$	1	5.08	5.05	vs
2	1	1	4.66	4.63	s
2	0	1	4.63		
2	3	1	3.96	3.92	s
0	4	1	3.93		
1	4	1	3.92		
2	$\bar{2}$	1	3.87		
2	$\bar{3}$	1	3.40	3.38	vs
3	0	1	3.38		
3	2	1	3.36		
3	$\bar{1}$	1	3.22	3.23	m
1	$\bar{5}$	1	3.01	3.00	m
3	$\bar{2}$	1	3.00		
2	$\bar{4}$	1	2.98		
1	6	1	2.83	2.82	vw
0	6	1	2.80		
1	7	1	2.47	2.46	w
4	4	1	2.47		
3	6	1	2.45		
5	2	1	2.18	2.17	vw
5	1	1	2.18		
4	6	1	2.17		
3	$\bar{6}$	1	2.06	2.06	m
0	0	2	4.20	4.16	m
1	0	2	3.93	3.89	vw
1	1	2	3.89		
1	$\bar{2}$	2	3.51	3.46	vs
0	3	2	3.43		
2	1	2	3.36	3.33	w
2	0	2	3.35		
1	$\bar{3}$	2	3.18	3.15	m
1	4	2	3.05	2.99	w
2	$\bar{2}$	2	3.02		
2	$\bar{3}$	2	2.78	2.77	vw
3	0	2	2.78		
3	2	2	2.76		
3	$\bar{1}$	2	2.69	2.67	w
3	3	2	2.66		
2	$\bar{4}$	2	2.54	2.52	m
3	4	2	2.52		
4	5	2	2.10	2.09	w
3	8	2	1.87	1.86	vw
4	7	2	1.86		
5	5	2	1.85		
5	$\bar{2}$	2	1.85		
2	$\bar{8}$	2	1.76	1.76	vw
3	$\bar{7}$	2	1.76		
0	2	3	2.67	2.67	m
1	$\bar{1}$	3	2.67		
0	3	3	2.53	2.52	vs
1	3	3	2.51		
2	1	3	2.51		
2	0	3	2.50		
2	3	3	2.38	2.38	m
0	4	3	2.37		
1	4	3	2.37		
0	5	3	2.20	2.18	vw
3	$\bar{1}$	3	2.19		
3	3	3	2.17		
3	$\bar{5}$	3	1.80	1.81	vw
4	$\bar{3}$	3	1.80		
0	0	4	2.10	2.08[b]	
0	1	4	2.09		
1	0	4	2.06	2.06	m
1	1	4	2.06		
0	2	4	2.04		
1	$\bar{2}$	4	2.00	1.97	s
0	3	4	1.98		
1	3	4	1.97		
2	1	4	1.97		
2	0	4	1.96		
2	2	4	1.95		
1	4	4	1.90	1.88	vw
2	$\bar{2}$	4	1.89		
1	5	4	1.81	1.81	vw
0	5	4	1.81		

[a] Abbreviations: vs, very strong; s, strong; m, medium; w, weak; and vw, very weak. [b] Observed by tilting to the corresponding θ value.

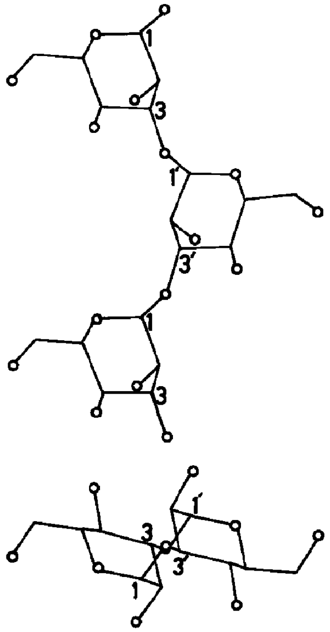

Fig. 2. Projections perpendicular to and along the helix axis of a conformation of the (1→3)-α-D-mannan.

relative humidity (Fig. 1). When the mannan film was X-rayed under vacuum immediately after drying at 105° under diminished pressure, a very diffuse fiber pattern was obtained. This suggests the presence of water molecules in the crystal.

All 47 visible reflections on the 0th, 1st, 2nd, 3rd, and 4th layer-lines could be indexed in terms of a monoclinic unit-cell with dimensions: a = 11.29; b = 18.12; c = 8.40 Å; and γ = 79.17° (compare Tables I and II). The volume of this cell and the observed density are in good agreement with the density calculated for 8 D-mannose residues and 16 water molecules per unit cell.

The presence of (002) and (004) reflections (Table II) suggests that a two-fold screw axis along the c-axis is present and that the $P2_1$ space group may be assigned. The value of h, the advance per residue along the helix axis, of 4.20 Å (Table I), is close to the virtual bond-length of ~4.3 Å typical for α-D-mannose. This indicates that the (1→3)-α-D-mannan molecule is almost fully extended along the fiber axis.

The present results support the prediction that the chain conformation of (1→3)-α-D-mannan is similar to that of (1→3)-α-D-glucan, as the values of the helix parameters for the former, n = 2 and h = 4.20 Å, are very similar to those[11] for the latter, n = 2 and h = 4.22 Å. Thus, by using the atomic coordinates for the D-glucan[10], we may propose a chain conformation for the D-mannan (Fig. 2).

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