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X-RAY DIFFRACTION STUDY OF SULFURIC, NITRIC, AND HALOGEN ACID SALTS OF CHITOSAN

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ABSTRACT

Chitosan {poly-[(1→4)-β-D-glucosamine]}, the deacetylation product of chitin, is considered to be a promising bioresource, because, unlike chitin, it is The resulting salts were studied by X-ray fiber diffraction; salts formed with HNO_3, HBr, and HI each gave a distinct pattern that suggested retention of the two-fold, helical conformation of untreated chitosan. Patterns of salts prepared with HF, HCl, and H_2SO_4 differed substantially, showing helical repeats of 40.73 Å. Stereochemical analysis suggests that the last 3 salts form either left- or right-handed helices that have 8 residues in 3 turns. These 3 salts have identical unit-cell dimensions, despite differing anion sizes.

INTRODUCTION

Chitosan {poly-[(1→4)-β-D-glucosamine]}, the deacetylation product of chitin, is considered to be a promising bioresource, because, unlike chitin, it is soluble in dilute hydrochloric and some aqueous organic acids, and is a polyelectrolyte. The regular distribution of the aliphatic, primary amino group in the chain makes chitosan a first-rank chelating polymer for the transition metals[1]. For example, the polysaccharide can recover heavy metals from industrial waste-water. Because chitosan gives salts when it reacts with acids, it can be used to recover inorganic and organic acids as well.

Crystal structures of chitosan have been studied since the work of Clark and Smith[2] in 1937. They derived an orthorhombic unit cell with $a = 8.9$, $b = 17.0$, and c (fiber axis) = 10.25 Å. Averbach[3] found that the b axis shortened when chitosan was dried, and he proposed that water molecules are loosely bound between chitosan chains along the [010] direction. X-Ray diffraction measurements on complexes of chitosan with heavy metals led to a pendant model in which a metal ion

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is coordinated with an amino group of the D-glucosamine dimer[4]. Although some allomorphs have been found[2,5–8], the chitosan molecule has always been observed as a two-fold, helical structure similar to chitin or common cellulose.

The knowledge of chain conformations of the various salts may be helpful in application of chitosan to recovery of acids. We report herein the results of our studies of salts simply made with some common acids. Chitosan prepared from crab tendon[2], and called[6] "tendon chitosan", is ideal for the study as it is well oriented.

EXPERIMENTAL

Materials. — Chitosan was prepared from tendon chitin from the crab, *Paralichodes,* by *N*-deacetylation, that is, by stirring chitin in saturated sodium hydroxide under nitrogen for 45 h at 100°, followed by washing with water[2]. Immersing the resulting chitosan fiber in 3M H_2SO_4, 7M HNO_3, 6M HCl, 9M HBr, or 6M HI aqueous solution for 10–20 min, with stirring, gave the respective salts. For the hydrofluoric acid salt, the chitosan was placed in a mixture of 5M aqueous solution of HF (5 mL) and isopropyl alcohol (20 mL). The isopropyl alcohol was used to avoid dissolving the chitosan, which is otherwise soluble in HF solution. Each salt was washed with isopropyl alcohol and dried in air.

Methods. — The extents of formation for each salt were calculated from elemental analyses of the acid-treated specimens, which were dried under vacuum at 105°. Densities were measured by flotation in carbon tetrachloride–1,2-dibromoethane (ethylene bromide) for the HBr and HI salts, or CCl_4–*p*-xylene for the other salts. The X-ray diffraction patterns were recorded in a flat-film camera, using Ni-filtered CuKα radiation. Helium was used to reduce the air scatter; the relative humidity was kept high.

RESULTS AND DISCUSSION

Our chitosan from crab tendon showed a fiber pattern (see Fig. 1). All reflections could be indexed with an orthorhombic unit cell, with $a = 8.97$, $b = 17.05$, and c (fiber axis) = 10.17 Å; the cell is practically identical with that of Clark and Smith[2], who used lobster tendon. Elemental analyses (see Table I) indicated that >68% of the amino groups in chitosan had reacted to form salts. In each case, salt formation was also confirmed by the density values (see Table I) and the appearance of the fiber diagrams (see Fig. 2).

Salts of nitric, hydrobromic, and hydriodic acids. — The patterns from HNO_3, HBr, and HI salts (see the top 3 diagrams, Fig. 2) did not change with variation in humidity, even for a sample of the HNO_3 salt dried at 105° under diminished pressure. This suggests the absence of water in the crystalline salts. All diffraction spots of the HNO_3 and HBr salts were indexed with orthorhombic unit-cells (see Tables II and III). If 3 reflections not on the regular layer-lines are dis-

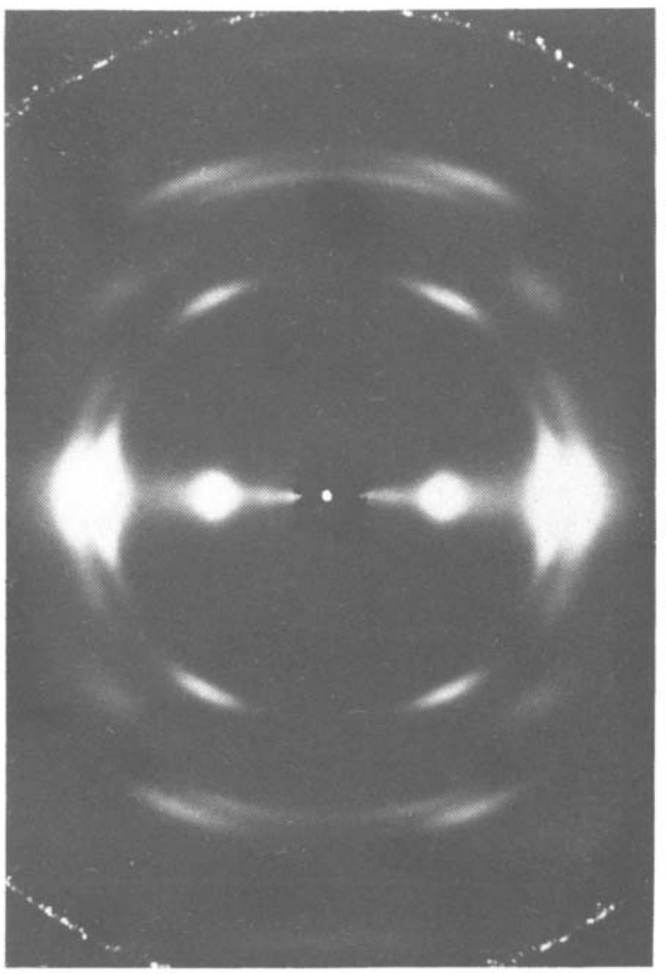

Fig 1 X-Ray diffraction pattern of chitosan prepared from crab-tendon chitin, at 100% relative humidity (Fiber axis is vertical)

regarded (to be discussed later), the HI salt may have the monoclinic cell shown in these Tables. There are poor agreements between calculated and observed density values for the HBr and HI salts (see Table II). The degree of salt formation and observed density of the original chitosan (1.44 g/cm^3) would lead to density values of respective samples to be 1.72 for HBr and 1.88 for HI salts. The latter is in good agreement with the density observed, but the former is still in poor agreement. This may suggest the presence of noncrystalline material in the sample of HBr salt.

The similarity of these three fiber axes and that of chitosan suggests that chitosan keeps its two-fold helical structure during formation of these salts. However, the *a* axes become larger and the *b* axes become shorter. This suggests that

TABLE I

DEGREE OF SALT FORMATION, AND DENSITY, FOR ACID-TREATED CHITOSAN

Acid	*% Formation*[a]	*Density (g/cm^3)*
H_2SO_4	69	1 53
HNO_3	90	1 56
HF	90	1 44
HCl	82	1 45
HBr	86	1 67
HI	78	1 86

[a]Calculated from the elemental analysis

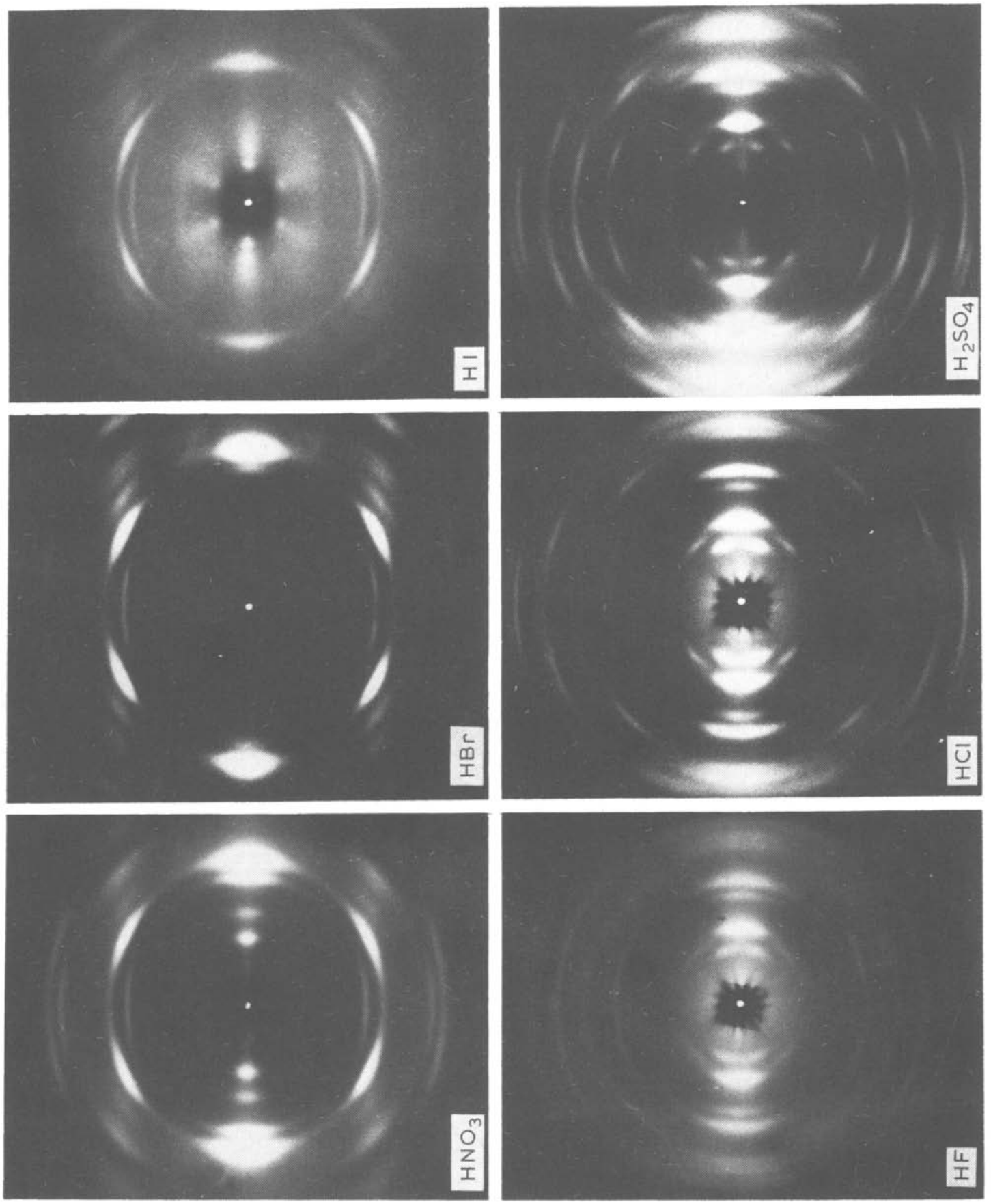

Fig 2 Fiber patterns of the chitosan salts of various acids indicated on each photo (Upper three were obtained under vacuum, and lower three, at 100% relative humidity.)

TABLE II

CRYSTAL DATA FOR CHITOSAN HNO_3, HBr, AND HI SALTS

Property	*HNO_3*	*HBr*	*HI*
Crystal system	orthorhombic	orthorhombic	monoclinic
Lattice parameters			
a (Å)	11.18	11 87	12.58
b (Å)	16.40	14 72	14.88
c (fiber axis) (Å)	10.40	10 42	10.45[a]
γ (degrees)			102.27
Density			
ρ (calc) (g/cm^3)	1.56	1.77	2.00
ρ (obs) (g/cm^3)	1.56	1.67	1.86
In the unit cell			
Number of residues	8	8	8
Number of chains	4	4	4
Helix parameters[b]			
n	2	2	2
h (Å)	5.20	5 21	5.23

[a]The fiber-axis length is for the backbone chitosan chain of the salt. [b]n: Number of salt residues per turn. h Advance per residue along the helix axis.

salt formation causes removal of the water molecules that are loosely bound along the [010] direction, and that reaction of the anions with the amino groups enlarges mainly the a axes.

The odd-order layer-lines of the patterns of the HNO_3 and HBr salts are not found for the HI salt, formally reducing the c axis to half that of the other patterns. However, the pattern is diffuse, indicating disorder. Furthermore, 3 off-layer spots (see Table IIIC) could be indexed by doubling the a and b axes and multiplying the c axis by 8 (c = 83.6 Å). This may indicate that the iodine ion is arranged in a 16-fold helical array; a similar result was found for amylose–iodine[9].

Salts of hydrofluoric, hydrochloric, and sulfuric acids. — In contrast to the foregoing salts, the fiber patterns of the HF, HCl, and H_2SO_4 salts (see Fig. 2, lower row) at 100% humidity were much sharper than those taken *in vacuo*. Moreover, the a and b axes decreased. This indicates the presence of water molecules in the crystal structures. Despite the different sizes of the anions, the patterns are very similar, except for the sharpness of the patterns. Apparently, the 3 acid salts have similar crystal structures, and the anions are not arranged in regular positions in the crystals. Some arrows observed around the center of patterns of both the HF and the HCl salts may suggest small crystallites; those of the latter disappeared when the HCl salt was annealed (see Fig. 3).

TABLE III

OBSERVED SPACINGS AND INTENSITIES FOR CHITOSAN HNO_3, HBr, AND HI SALTS

hkl	*Spacings (Å)*		*Int [a] (obs)*	*hkl*	*Spacings (Å)*		*Int [a] (obs)*
	Calc	*Obs*			*Calc*	*Obs*	
(A) HNO_3 salt							
010	16 40	16 05	vw	012	4 96	5 12	m
110	9 24	9 02	s	112	4 53	4 42	s
120	6 61	6 62	m	022	4.39		
130	4 91	4 91	s	202	3.81	3 94	w
040	4 10	4 25	vs	132	3 57	3 51	m
330	3 08	3 08	s	222	3 45		
				312	2 98	2 99	w
001	10.40	10 24	w				
011	8 78	8 77	vw	013	3.39	3.35	m
021	6 44	6 50	vw	023	3 19	3 20	m
041	3 82	3 88	w	223	2 77	2 78	w
341	2.67	2 67	w	313	2 51	2 48	w
411	2 66						
				114	2 50	2 50	m
(B) HBr salt							
010	14 72	14 81	w	132	3 42	3 41	w
110	9 24	8 94	vw	042	3 01	2 99	s
030	4.91	4 79	m	242	2 68	2 68	vw
300	3.96	4 18	vs	152	2 51	2 50	vw
400	2 97	3 00	s				
430	2 54	2 57	vw	113	3 25	3 24	w
				023	3 14	3 13	vw
101	7.83	7.86	vw	303	2 61	2.66	vw
301	3 70	3 74	vw				
				004	2 61	2 61	m[b]
012	4 91	4 98	m	114	2 51	2.50	m
022	4 25	4 40	s	124	2.41	2 42	m
202	3 92	3.93	w	134	2 26	2 22	w
032	3 57	3 60	w				
(C) HI salt							
010	14 54	15 18	s	012	4 92	5.03	m
110	10 56	10 39	m	$1\bar{1}2$	4 46	4 45	s
$1\bar{1}0$	8.54	8 26	m	212	3 98	4 02	m
230	4 27			202	3.98		
$2\bar{2}0$	4 27	4 30	s	132	3 56	3 54	w
$1\bar{3}0$	4 21			032	3 55		
420	3 08			$3\bar{1}2$	3 04	3.07	m
400	3 07	3 07	m	$3\bar{2}2$	2 78	2 75	w
340	3 06						
				004	2.61	2 61	s[b]
		15 73	s[c]	$1\bar{1}4$	2.50	2.52	w
		10 82	m[c]	024	2.46	2 43	m
		8 53	m[c]	124	2 45		
				$1\bar{3}4$	2 22		
				314	2 22	2 21	w
				304	2 20		

[a]Abbreviations: m, medium; s, strong; vs, very strong; vw, very weak; w, weak [b]Observed by tilting to the corresponding θ value [c]The reflections do not fit to the monoclinic cell with $c = 10\ 45$ Å

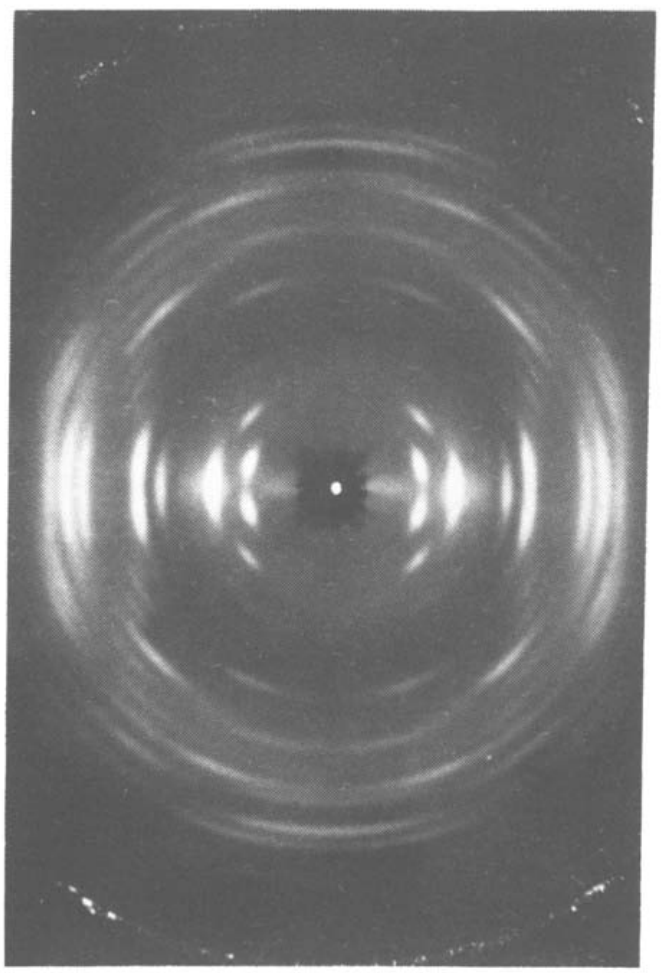

Fig. 3 Fiber pattern of the annealed chitosan·HCl salt at 100% relative humidity

After the chitosan·HCl salt had been annealed in 80% isopropyl alcohol–water mixture at 160° in a sealed bomb, a very sharp pattern was observed (see Fig. 3). No other change in the crystal structure was observed. The 43 reflections on 17 layer-lines were indexed with a monoclinic unit-cell, with $a = 13.81$, $b = 16.33$, and $c = 40.73$ Å, and $\gamma = 96.46°$ (see Table IV). The c axis length corresponds to 8 D-glucosamine residues in an extended conformation; meridional reflections are observed on the 8th, 12th, and 16th layer-lines. The volume of this cell (9.127 Å^3) and the observed density (see Table I) lead to the presence of 4 chitosan·HCl chains plus an undefined amount of water that depends on the humidity. These conclusions apply to the HF and H_2SO_4 salts as well. It may be that there are cavities in the helices that can accomodate even the largest of these anions, and that variable amounts of water make final adjustment, with more water compensating for the smaller anions.

The absent meridionals suggest that the helix has 4-fold symmetry; however, a simple 4-fold helix could extend only to 1/2 the observed distance. A non-integral helix is more in keeping with the monoclinic symmetry than a simple, 4-fold helix would be.

We studied a number of possible conformations for the chitosan·HCl chain, using the computer program, PS 79 (refs. 10 and 11). The atomic coordinates of a molecule of β-D-glucosamine were derived from the structure of α-D-glucosamine hydrochloride by Chu and Jeffrey[12]. The distance between O-1 and O-4 (virtual bond length) of our model of β-D-glucosamine is 5.53Å. Neither simple, 8-fold

TABLE IV

OBSERVED SPACINGS AND INTENSITIES FOR CHITOSAN · HCl SALT AT 100% RELATIVE HUMIDITY

hkl	*Spacings (Å)*		*Int [a] (obs)*	*hkl*	*Spacings (Å)*		*Int [a] (obs)*
	Calc	*Obs*			*Calc.*	*Obs*	
020	8 11	7 87	vs	008	5.09	5 08	vw
$1\bar{2}0$	7 36			$1\bar{1}8$	4 63	4 60	m
200	6.86	6.86	vw	118	4 53		
120	6 66			$2\bar{2}8$	3.75	3 75	w
210	6 08	6 24	vw	038	3 71		
$2\bar{2}0$	5 56	5 53	s				
030	5 41			$2\bar{2}9$	3 51	3.51	w
$4\bar{1}0$	3 44	3 47	m	039	3.47		
400	3 43			229	3.35	3 32	m
$2\bar{5}0$	3.07			139	3 31		
$4\bar{3}0$	3 06	3 04	m				
420	3 04			$1\bar{1}10$	3.83	3 82	m
				1110	3.77		
$1\bar{1}1$	10 72	10 69	vs	2210	3 15	3 13	m
221	4 93	4 91	vs	1310	3 12		
131	4 82						
$1\bar{4}1$	4 00	3 89	m	$1\bar{2}11$	3.31	3.32	s
$3\bar{3}1$	3 69	3.66	s				
$2\bar{4}1$	3 66			0012	3 40	3 40	vw[b]
				$1\bar{1}12$	3 25	3.24	m
$1\bar{1}3$	8.60	8 53	s	1112	3.21		
				2212	2 80	2.79	w
$2\bar{2}4$	4 88	4 85	w	1312	2 78		
034	4 78			3212	2 53	2.55	w
224	4 47	4 46	w	$1\bar{1}13$	3 02	2 99	s
134	4 38			1113	2 99		
$3\bar{3}4$	3 48	3 46	m	$1\bar{2}13$	2.88	2 89	s
$2\bar{4}4$	3 46			1313	2.63	2 63	w
				3213	2 42	2 43	w
$1\bar{1}5$	6.57	6 61	vw				
225	4.24	4 18	w	$1\bar{2}14$	2 71	2 71	w
135	4 17						
$3\bar{3}5$	3 37	3 34	w	$1\bar{1}15$	2 64	2 63	w
$2\bar{4}5$	3 35			1115	2 62		
155	2.88	2 87	m	2015	2 53	2.52	vw
$2\bar{5}5$	2 87			1215	2.52		
$4\bar{3}5$	2 86			$2\bar{1}15$	2 51		
425	2 85						
				0016	2 55	2 55	vs[b]
$1\bar{1}6$	5 79	5 67	vw	$1\bar{2}16$	2 41	2 40	m
116	5.61			2016	2.39		
$1\bar{1}7$	5 16	5.05	vw				
117	5 02						
$2\bar{2}7$	4 02	4 00	s				
037	3 96						
227	3.78	3.75	w				
137	3.73						

[a,b]Refer to the footnotes in Table III

helices nor two simple 4-fold helices of either handedness were able to attain glycosidic angles in the range of 110° to 125°. Both left- and right-handed helices with 8 residues in 3 turns were feasible. This corresponds to $n = 2.67$, $h = 5.09$, within allowed zones on conformational maps of cellulose[13]. These hydrofluoric, hydrochloric, and sulfuric acid salts are thus the first reported structures of chitosan that are not 2-fold helices. The possibility of (0012) has to be explained in a final analysis.

Chitosan recovered by deacidification of each salt. — The 6 salts of chitosan were readily deacidified by immersing each salt in M sodium hydroxide for 10 min with stirring, followed by washing with water. The resulting chitosans showed, however, fiber patterns of mixtures of "tendon chitosan" (see Fig. 1) and "annealed chitosan" (orthorhombic, $a = 8.24$, $b = 16.48$, $c = 10.39$ Å) reported previously[6], in which we found that by raising the annealing temperature, the proportion of the latter polymorph increased; it is easy to find from the equatorial reflections. The chitosan from the HNO_3, HBr, HI, or HCl salt is, in large part "tendon chitosan", whereas, that from the HF or the H_2SO_4 salt contains a significant proportion of "annealed chitosan" polymorph. The different actions of these acids towards chitosan cannot at present be explained.

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