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CONFORMATION OF CRYSTALLINE TYPE III PNEUMOCOCCAL POLYSACCHARIDE

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ABSTRACT

The conformation of crystalline Type III pneumococcal polysaccharide, poly[(1→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*], has been studied by X-ray diffraction and stereochemical analysis The X-ray pattern, recorded at 80% relative humidity, led to a trigonal unit-cell, with $a = b = 1\,028$ nm, $c = 2\,77$ nm, and $\gamma = 120°$ Meridional reflections on the third and the sixth layer-lines show that the chain consists of a three-fold helix, with a repeat of 0 923 nm Conformational analysis, by the virtual-bond method, shows that one right-handed and two left-handed helices are equally favorable, on the basis of energies of isolated chains Packing of the three helix models in the unit cell, by contact-distance criteria, ruled out the possibility of the right-handed helical conformation One of the left-handed helices has intramolecular hydrogen-bonds O-3′ O-5 and O-2 O-6′ between the residues in the (1→4) linkage, and O-4′ O-5 between those in the (1→3) linkage In the other left-handed helix, the O-4′ O-5 hydrogen bond is replaced by an O-2 O-2′ hydrogen bond The choice between the two left-handed helix models is inhibited by the paucity of X-ray intensity data

INTRODUCTION

The importance of the pneumococcus Type III organism in establishing the relationship between heredity and DNA has been described[1,2] The immunological specificity of the polysaccharide itself was established by Avery and Heidelberger, as early as 1923 The chemistry of bacterial polysaccharides has advanced, in the last decade, to the extent of providing unambiguous data on the chemical constitution of these polymers[3,4] and the development of methods for the analysis of their crystalline structures has followed[5–8] Methods based on either the (ϕ,ψ) maps or the virtual-bond rotation[9] have been applied to the conformational analysis of complex

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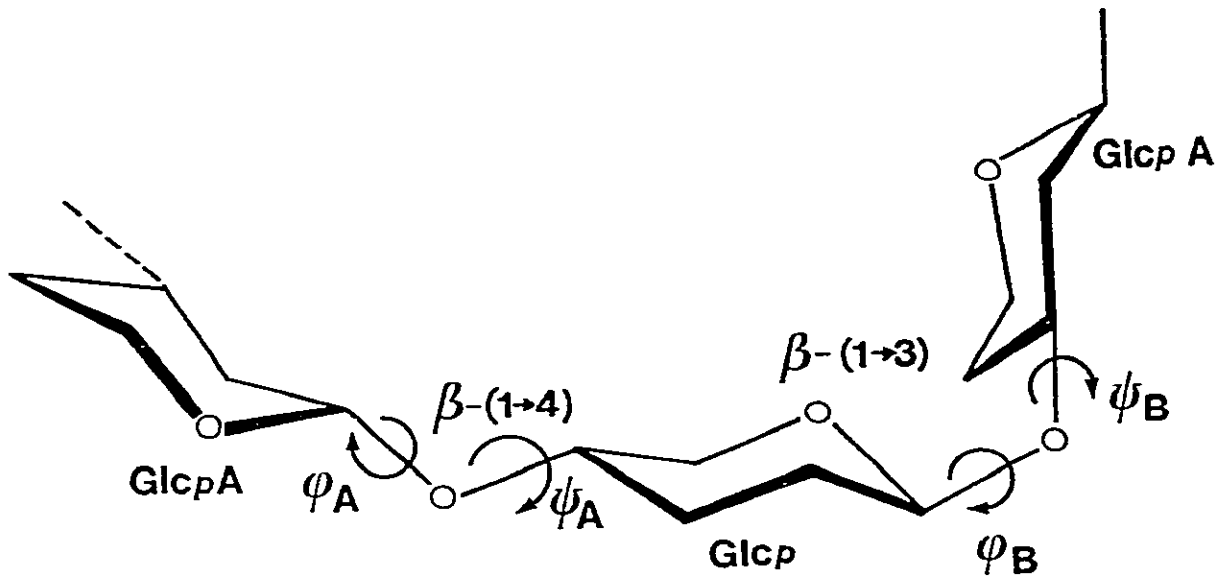


Fig 1 Schematic representation of the chemical structure of Type III pneumococcal polysaccharide The rotations around the glycosidic bonds, (ϕ_A,ψ_A) at the 1→4 linkage and (ϕ_B,ψ_B) at the 1→3 linkage, are shown

polysaccharides containing two or three residues in the repeating unit[6-8] The structural data accumulated from crystallographic studies on mono- and di-saccharides, and the stereochemical analysis of homopolysaccharides having various types of glycosidic linkage, have played a key role in such endeavors

X-ray studies on oriented fibers of the sodium salt of Type III pneumococcal polysaccharide and the conformational analysis of the chain are described here The schematic illustration of a portion of the Type III polysaccharide is shown in Fig 1 It consists of a disaccharide repeating-unit, and is poly[(1→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*] Insofar as this contains alternating (1→3) and (1→4) linkages between β-D-glycosyl residues, it is similar to hyaluronic acid, which is poly[(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A]

EXPERIMENTAL AND METHODS

The samples of the sodium salt form of Type III pneumococcal polysaccharide were kindly provided by Prof M Heidelberger Films of the material prepared from aqueous solution gave an amorphous X-ray pattern Oriented fibers were prepared by drawing a concentrated polysaccharide-gel droplet between two glass rods, in a chamber maintained at 90% relative humidity (r h) The X-ray patterns were recorded in a vacuum camera with pinhole collimation, with the sample sealed in a capillary at 80% r h Patterns were also recorded for the same sample conditions in a Searle camera with toroidal-beam focussing The density of the sample conditioned at the same r h was found, by flotation in a density-gradient column containing carbon tetrachloride and xylene, to be 1 566 g/cm^3

The overall diffracted X-ray intensity was weak, indicating a relatively low degree of crystallinity However, the observed reflections were not arced, indicating good orientation The pattern showed one relatively strong, equatorial reflection,

and six layer-lines of diffraction were clearly visible While most diffraction spots were broad, a sixth layer-line arc at 0 418 nm was always very sharp

The Arnott–Scott geometry[10] was used for the residues in the conformational analysis The angle τ_A at the 1→4 linkage was taken to be 117 5°, and the calculations were repeated for selected cases having τ_A = 116, 118, and 119° The value of τ_B at the 1→3 linkage was allowed to vary, by using the virtual-bond method[9] The non-bonded interactions were calculated by the Lennard–Jones type 6-12 potential function, with values of atomic polarizabilities and effective number of electrons taken from Ketelaar[11] Van der Waals radii of 0 17, 0 15, and 0 12 nm, respectively, were used for C, O, and H atoms An intrinsic, three-fold torsional potential, with a barrier of 2 1 kcal mol^{-1}, was assigned to rotations ϕ and ψ The hydrogen-bond energy was estimated by using an inverse third-power expression[12] The (ϕ_A,ψ_A) = (0,0°) position was defined by the conformation in which the bonds C-1–H-1 and C-4′–H-4′ are coplanar with the glycosidic bonds[12] A similar definition holds for the position (ϕ_B,ψ_B) = (0,0°)

UNIT CELL AND SYMMETRY

The X-ray pattern from the Type III polysaccharide at 80% r h is shown in Fig 2 This pattern is similar in all respects to that recorded by Atkins (E D T Atkins, personal communication) The layer-line spacing is 2 77 nm Lack of meridional reflections except on the third and the sixth layer-lines indicates a three-fold helical conformation for the chain (with a rise per residue of 0 923 nm), and trigonal symmetry for the unit cell The observed *d*-spacings and the indices of

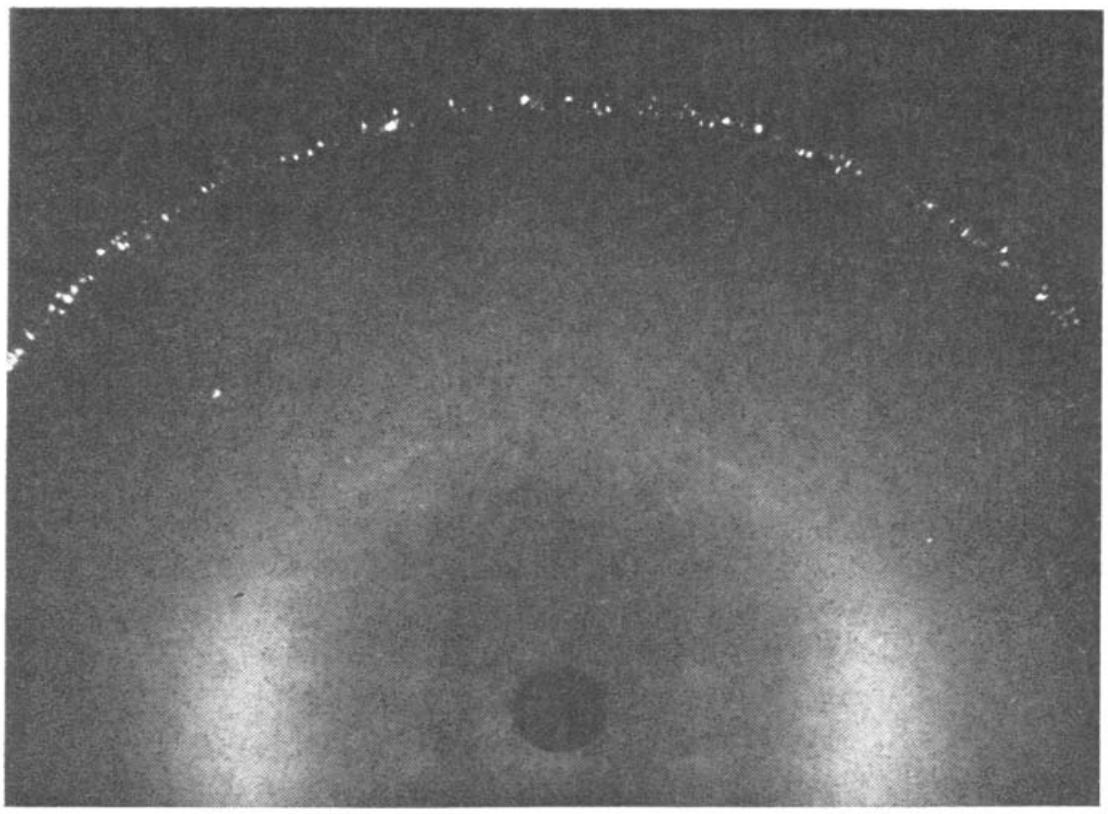


Fig 2 The X-ray pattern recorded at 80% relative humidity is shown with the fiber axis vertical The intense "dotted" outer ring is the NaF calibration reflection, 2 8 Å

TABLE I

CRYSTALLOGRAPHIC INTERPLANAR SPACINGS, MILLER INDICES, AND RELATIVE INTENSITIES FOR CRYSTALLINE TYPE III PNEUMOCOCCAL POLYSACCHARIDE

Reflection no	d *(nm)*	*Indices* (hkl)	*Intensity*[a]
1	0 875	101	s
2	0 765	102	m
3	0 480	105	m
4	0 418	106	s
5	0 515	110	m
6	0 510	111	s
7	0 435	201	w
8	0 419	202	w

[a]m, medium, s, strong, w, weak

the reflections based on the trigonal unit-cell, with $a = b = 1\ 028$ nm, $c = 2\ 77$ nm, and $\gamma = 120°$, are given in Table I The visual intensity-estimates are also given in this Table It is interesting that these unit-cell dimensions are close to the values of $a = b = 1\ 17$ nm and $c = 2\ 85$ nm reported by Winter *et al* [13] for hyaluronic acid

The calculated density, with six repeating units and 18 water molecules in the unit cell, is 1 54 g/cm^3, with 12 water molecules and 6 sodium ions it is 1 55 g/cm^3. With six repeating units in the unit cell and a three-fold screw-axis along the chain, the possible space-groups are $P3_1$, $P3_112$, $P3_121$, $P3_2$, $P3_212$, and $P3_221$ The first three space-groups require right-handed chirality for the 3(0 923) helix*, whereas the symmetry elements 3_2 in the last three denote left-handed 3(−0 923) helices The presence of two-fold axes normal to and intersecting the three-fold screw-axis of the chain, in the space groups $P3_112$ and $P3_212$, would lead to double-helical arrangement of the chains, the strands in the double helix being antiparallel to each other This situation can be ruled out because of the stereochemical untenability of such double helices in the extended conformations of the polysaccharide chains[13] This leaves four possible space-groups, two involving left-handed helices and the other two, right-handed helices

CONFORMATIONAL ANALYSIS

The virtual-bond method[9] has been used here to arrive at the favored conformation of the Type III polysaccharide Fig 1 shows that two sets of rotations, (ϕ_A,ψ_A) and (ϕ_B,ψ_B), are possible about the (1→4) and (1→3) linkages, respectively The two residues participating in one of the glycosidic linkages were taken as the repeating unit, namely, the polymer is a regular copolysaccharide and the repeating unit itself

*This notation refers to $n(\pm h)$, where n is the number of residues per turn and h is the rise per residue along the helix axis The negative sign refers to a left-handed helix

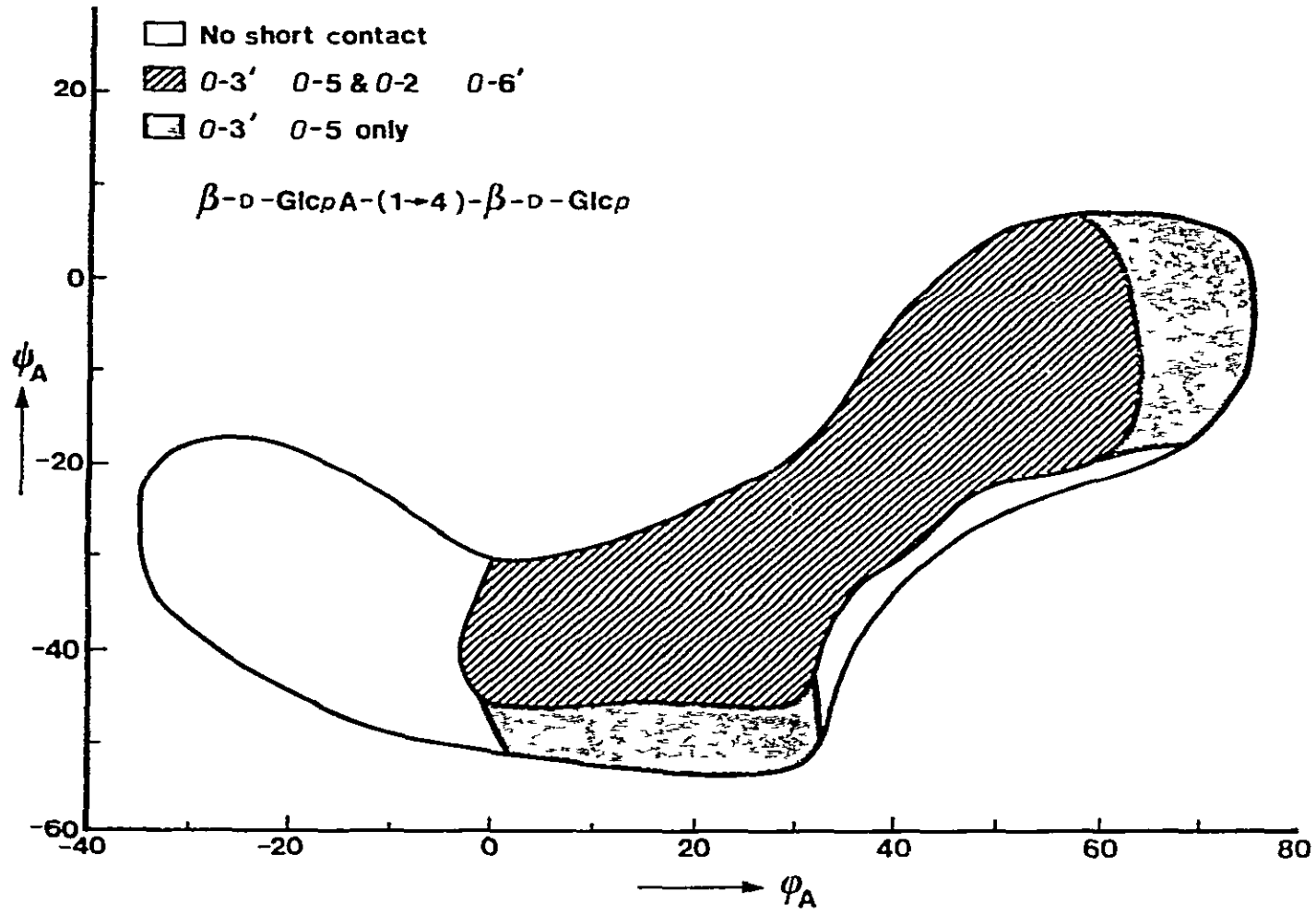


Fig 3 The (ϕ_A,ψ_A) conformational map for the disaccharide β-D-Glc*p*A-(1→4)-β-D-Glc*p* The allowed area and the hydrogen-bonded conformations are shown

acquires flexibility because of rotations at one glycosidic linkage, say (ϕ_A,ψ_A) Several favorable conformations of the two residues in the repeating unit were selected on the basis of steric constraints, presence of intra-unit hydrogen bonds, and so on With each conformation for the repeating unit thus selected, the virtual-bond method was applied for exploring the conformational space allowed for the second type of linkage For the Type III polymer, either [β-D-Glc*p*A-(1→4)-β-D-Glc*p*] or [β-D-Glc*p*-(1→3)-β-D-Glc*p*A] can be taken to be the repeating unit As the presence of the O-3′ O-5 intramolecular hydrogen-bond with its restrictive conformational influence is well established for (1→4)-linked β-D-Glc*p* units[5-8], the former (cellobiouronic acid) was taken to be the repeating unit in the present calculations

The conformational map for the disaccharide β-D-Glc*p*A-(1→4)-β-D-Glc*p* is shown in Fig 3 as a function of ϕ_A and ψ_A The hydroxymethyl group was kept in the *t* position† and the C-6–O(carbonyl) bond was so placed as to eclipse the C-5–H-5 bond[13] The areas that permit (*i*) O-3 O-5 intramolecular hydrogen-bonding only, (*ii*) O-3′· O-5 and O-2 O-6′ intramolecular hydrogen-bonds simultaneously, and (*iii*) no hydrogen bond at all, are shown in Fig 3 The conformations that give rise to O-2 O-6′ intramolecular hydrogen-bonds alone are not considered here

†This corresponds to the *tg* notation used by various authors See ref 8 for correlation of the notations

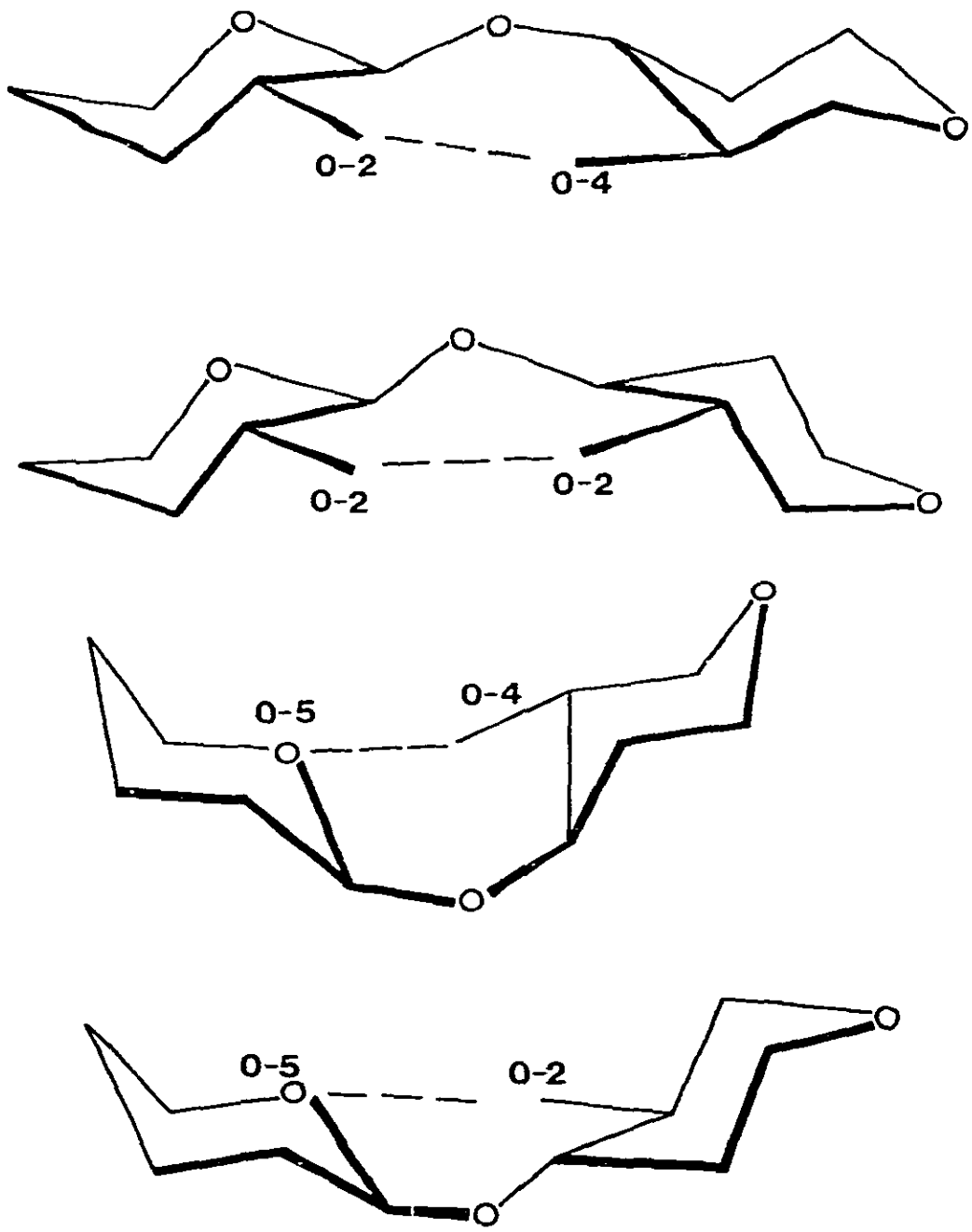


Fig 4 The four possible types of hydrogen bond between β-D-glucopyranosyl residues participating in a 1→3 linkage are shown schematically

For the cellobiouronic acid repeating-unit in the Type III polysaccharide, the vector joining O-3 of the Glc*p*A residue and O-1 of the Glc*p* residue defines the virtual bond The length of the virtual bond depends on the values chosen for (ϕ_A,ψ_A). The conformations ϕ_A,ψ_A in Fig 3, which allow the O-3′ ·O-5 hydrogen bond, with or without the additional O-2 O-6′ hydrogen bond, were chosen for the virtual-bond analysis With the repeating-unit thus defined, conformational calculations were performed, for both right- and left-handed helices, as described before for homopolymers[9]

Theoretical calculations on β-D-Glc*p*-(1→3)-β-D-Glc*p* by Sathyanarayana and Rao[14] showed that four types of hydrogen bond are possible between (1→3)-linked glucosyl residues These are O-2 O-2′, O-4′ O-5, O-2′ O-5, and O-2 O-4′, shown schematically in Fig 4 The last two types are simultaneously possible in the extended conformations of (1→3)-β-D-glucan[14] The studies of Bluhm and Sarko[15] on lentinan showed that the O-2 O-2′ intramolecular hydrogen-bond is possible

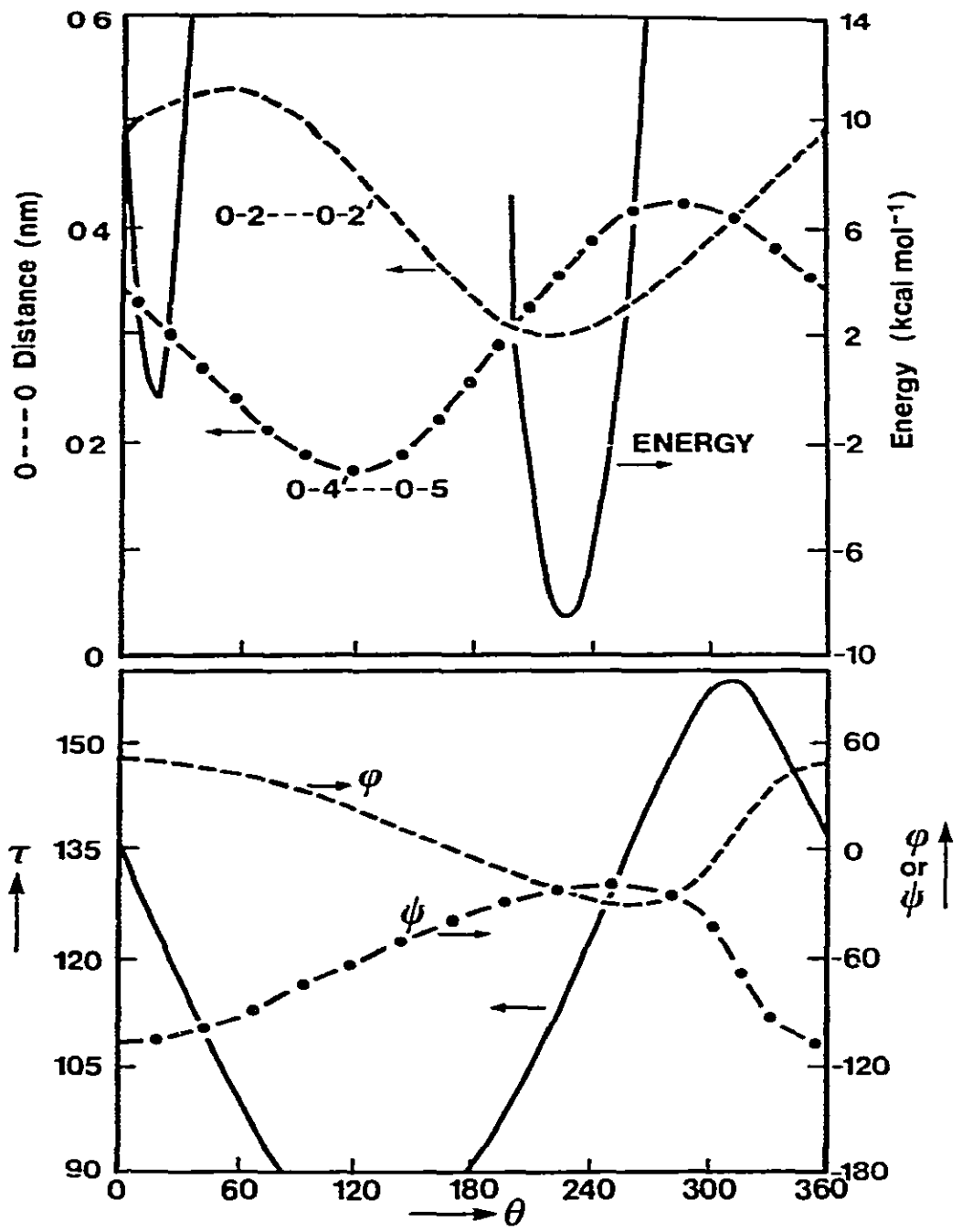


Fig 5 Results of the virtual-bond analysis are shown for the right-handed 3(0 923) helix, with $(\phi_A,\psi_A) = (10,-40°)$ The values of τ (left ordinate), and ϕ and ψ (right ordinate) are plotted as a function of θ, the rotation about the virtual bond, in the lower half of the Figure The energy curve and the types of hydrogen-bond and their distances are shown in the upper half

for both right- and left-handed helices of (1→3)-β-D-glucan The crystal structure of laminarabiose[16] shows the presence of an O-4′· O-5 hydrogen-bond

Of the various (ϕ_A,ψ_A) conformations used for the repeating unit, twelve of them permitted the O-4′ · O-5 *or* the O-2 O-2′ hydrogen bond between the residues participating in the 1→3 linkage, for the right-handed helix Nineteen (ϕ_A,ψ_A) conformations allowed such hydrogen bonds for the 3(-0 923) helix None of the (ϕ_A,ψ_A) conformations gave rise to the O-2′· O-5 or the O-2···O-4′ hydrogen-bond across the 1→3 linkage Fig 5 shows the results of the virtual-bond analysis for the best 3(0 923) helix. The relevant parameters are listed in Table II The energy curve in Fig 5 includes the non-bonded, torsional, and hydrogen-bond energies between the residues in the 1→4 linkage and between those in the 1→3 linkage It may be seen that the lowest minimum occurs at $\theta = 231°$, with $\tau_B = 115\,2°$ and

TABLE II

PARAMETERS DEFINING THE BEST MODELS FOR RIGHT-HANDED HELICES

	Across (1→4) linkage	*Across (1→3) linkage*
Model 1	$\phi_A,\psi_A = 10,-40°$	$\phi_B,\psi_B = 47,-103°$
	$\tau_A = 117\,5°$	$\theta = 28°$
	O-3′ O-5 0 29 nm	$\tau_B = 117\,7°$
	O-2 O-6′ 0 29 nm	O-4′ O-5 0 29 nm
		C-1 C-4′ 0 26 nm
		C-1 O-4′ · 0 26 nm
		O-5 H-4′ 0 20 nm
		H-1 O-4′ 0 19 nm
Model 2	$\phi_A,\psi_A = 10,-40°$	$\phi_B,\psi_B = -27,-23°$
	$\tau_A = 117\,5°$	$\theta = 231°$
	O-3′ O-5 0 29 nm	$\tau_B = 115\,2°$
	O-2 O-6′ 0 29 nm	O-2 O-2′ 0 297 nm

$(\phi_B,\psi_B) = (-27, -23°)$ The O-2 O-2′ hydrogen-bond distance is 0 297 nm The secondary minimum that occurs at $\theta = 28°$, with $\tau = 117\,7°$, $(\phi_B,\psi_B) = (47,-103°)$, has an O-4′ O-5 hydrogen-bond of length 0 29 nm However, the internal energy for this conformation is high compared to the model having $\theta = 231°$, because of short contacts (see Table II) These are between the residues in the 1→3 linkage Similarly, short contacts between such residues are responsible for the high-energy state of the conformation having $\theta = 200°$, although both O-4′ O-5 and O-2 O-2′ intramolecular hydrogen-bonds (both of length 0 3 nm) are simultaneously possible For the right-handed helix, the conformations having O-4′ · O-5 hydrogen-bonds always involve short contacts between the residues joined by the 1→3 linkage Hence, Model 2 in Table II, with an O-2 O-2′ hydrogen-bond across the 1→3 linkage, is the only permissible conformation for the 3(0 923) helix In hyaluronic acid, this hydrogen bond does not occur because of the presence of an acetamido group at C-2 in one of the residues This feature, coupled with the presence of short contacts in all conformations that give rise to the O-4′ O-5 hydrogen-bond, led Winter *et al* [13] to reject the right-handed helix model for hyaluronic acid

The results of the virtual-bond analysis for the best left-handed helices are shown in Figs 6 and 7 For the results shown in Fig 6, $(\phi_A,\psi_A) = (30,-20°)$, and the parameters characterizing the conformation (Model 3) are given in Table III The minimum in energy occurs at $\theta = 137°$, with $\tau_B = 114\,8°$. The O-4′ O-5 hydrogen-bond distance is 0 26 nm, with no short contacts, whereas the O-2–O-2′ distance is beyond the hydrogen-bonding limit The internal energy is very high, because of short contacts in conformations where possibilities of O-2 · O-2′ hydrogen-bonding exist

On the other hand, Fig 7 and Table III show that when $(\phi_A,\psi_A) = (50,0°)$

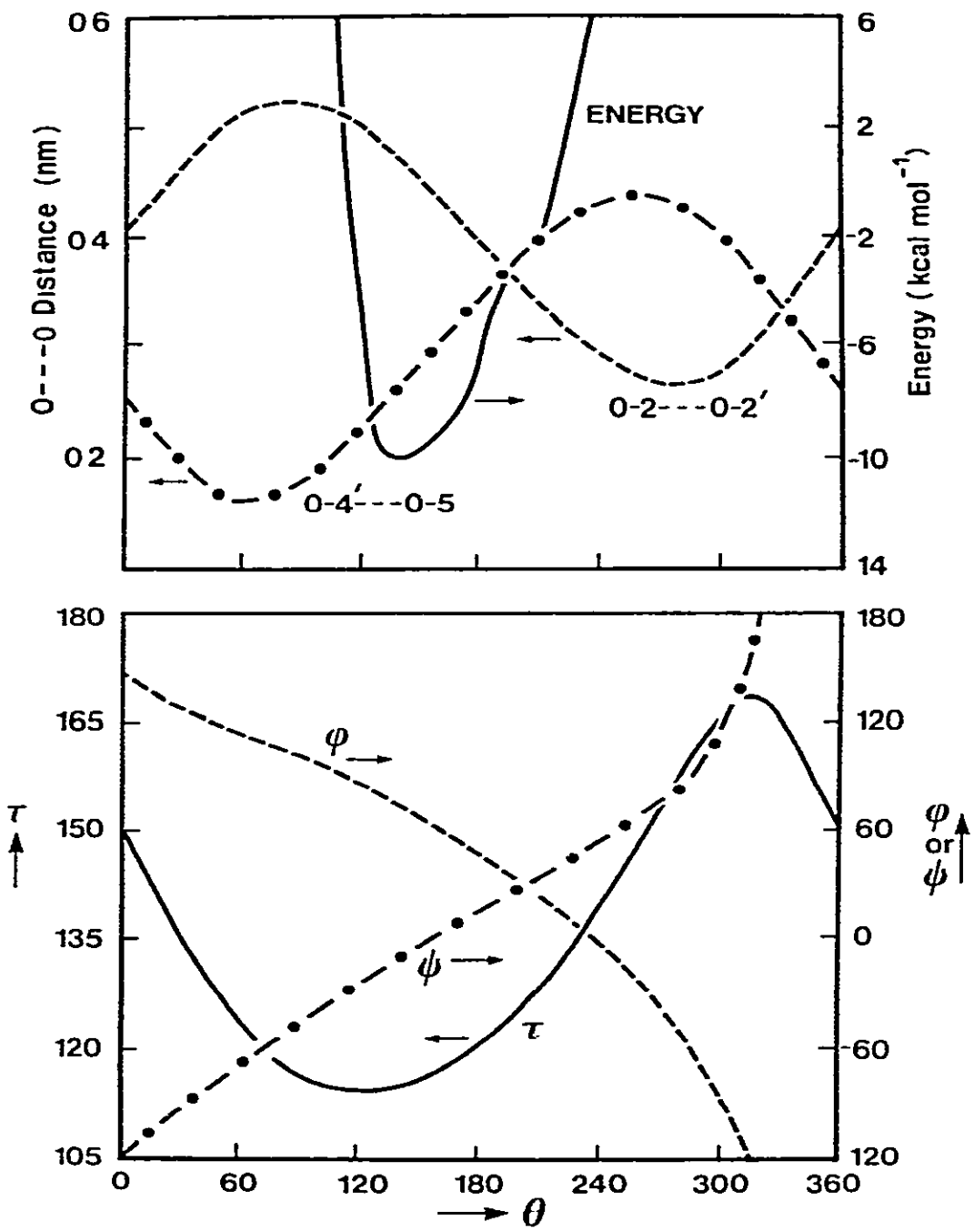


Fig 6 Same as Fig 5, but for the 3(-0 923) helix, with $(\phi_A,\psi_A) = (30,-20°)$

(Model 4), O-2 O-2′ hydrogen-bonding is possible, without short contacts, for $\theta = 232°$ with $\tau_B = 114\,8°$. Here, the O-4′–O-5 distance is too large for hydrogen bonding The values of the energies at the minimum in Fig 6 and 7 are the same Thus, there exist two equally favored conformations for the left-handed Type III helix, one having the O-2 O-2′ hydrogen bond and the other, the O-4′ O-5 bond Simultaneous occurrence of both hydrogen bonds is not possible, even after adjustment of the τ_A value in the region of 116–119°

Comparing Figs 5–7, the energies at the minima for the right- and the two left-handed helices are about the same Thus, there are three, equally stable helices to choose from The choice was simplified in the analysis of the conformation of hyaluronic acid, as the O-2 O-2′ hydrogen bond is precluded because of the acetamido group This ruled out all of the models except the one similar to Model 3 in Table III The values of (ϕ_A,ψ_A) and (ϕ_B,ψ_B) calculated from the results of Winter *et al*[13] for hyaluronic acid are (37 4,—16 3°) and (54 5,—1 7°), respectively In their calcu-

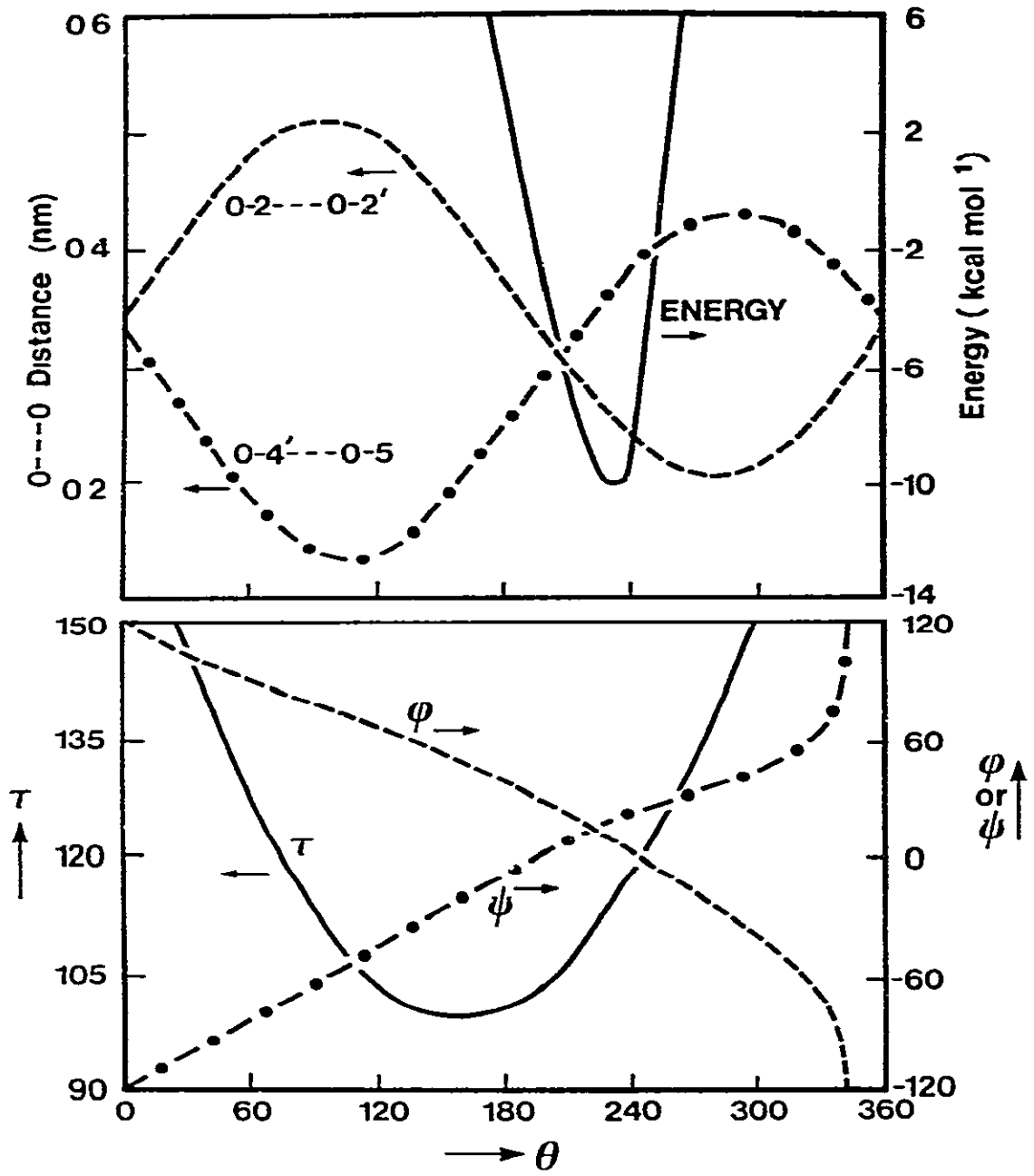


Fig 7 Same as Fig 5, but for the 3(-0 923) helix, with $(\phi_A,\psi_A) = (50,0°)$

TABLE III

PARAMETERS DEFINING THE BEST MODELS FOR LEFT-HANDED HELICES

	Across (1→4) linkage	*Across (1→3) linkage*
Model 3	$\phi_A,\psi_A = 30,-20°$	$\phi_B,\psi_B = 77,-11°$
	$\tau_A = 117\,5°$	$\theta = 137°$
	O-3′ O-5 0 274 nm	$\tau_B = 114\,8°$
	O-2 O-6′ · 0 24 nm(?)	O-4′ O-5 · 0 26 nm
Model 4	$\phi_A,\psi_A = 50,0°$	$\phi_B,\psi_B = 7,18°$
	$\tau_A = 117\,5°$	$\theta = 232°$
	O-3′ O-5 0 29 nm	$\tau_B = 114\,8°$
	O-2 O-6′ · 0 27 nm	O-2 O-2′ 0 26 nm

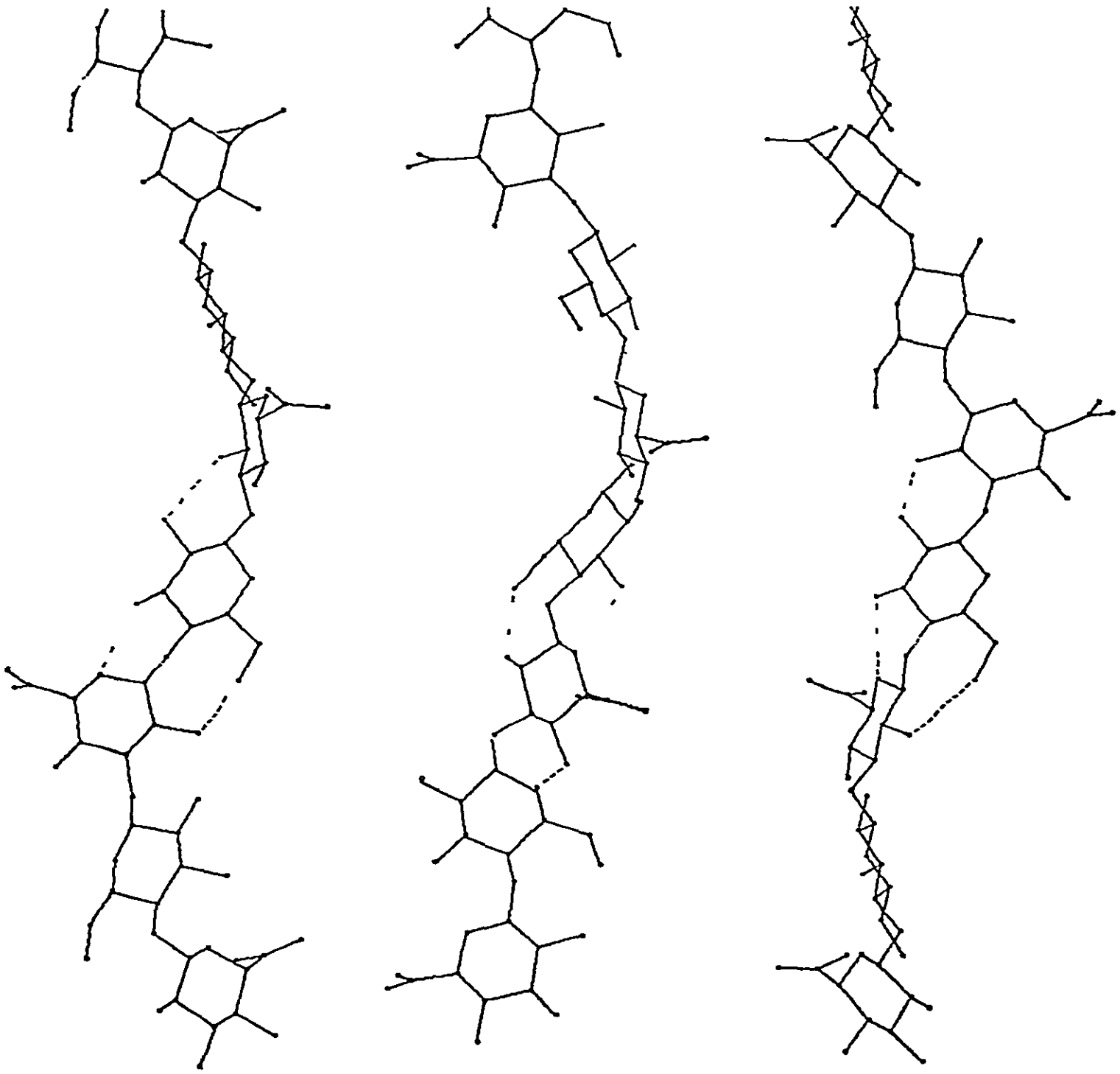


Fig 8 Projections of the stereochemically favored conformations of the Type III pneumococcal polysaccharide Model 2 (right), Model 3 (center), and Model 4 (left) are shown

lations, both τ_A and τ_B were restricted to 116 5° Allowing for this, as well as the slight difference in the observed fiber-repeat distance for the two polymers, these (ϕ_A,ψ_A) and (ϕ_B,ψ_B) values are in good agreement with those given in Table III for Model 3 On the basis of comparison with hyaluronic acid, the left-handed helix (Model 3) seems to be the probable conformation for Type III pneumococcal polysaccharide The presence of an O-4′ O-5 hydrogen-bond in both hyaluronic acid[13] and laminarabiose[16] supports this conclusion The O-2 O-2′ hydrogen-bond has been observed only in (1→3)-β-D-glucan, which has a flat-helical conformation with six-fold, screw symmetry[15,20] The three favored helices, Models 2, 3, and 4, are shown in Fig 8

PACKING ANALYSIS

A further comparison of the three most-favored conformational models was made by using stereochemical packing-analysis These models were each placed successively into the unit cell and refined against nonbonded, intermolecular atomic-contact criteria by using a computer program written by Zugenmaier and Sarko[17] With this program, molecular parameters may be so varied as to minimize the severity of short, intermolecular, nonbonded atom contact-distances The function minimized in this instance may be written as

$$\text{RPACK} = \sum_{i\ j} w_{ij}(d_{ij} - d_{0_{ij}})^2 \text{ for } d_{ij} < d_{0_{ij}}, \qquad (1)$$

where w_{ij} is a weight assigned to contacts between nonbonded atoms of the i^{th} and j^{th} type Here, d_{ij} is the distance between nonbonded atoms i and j, and $d_{0_{ij}}$ is the equilibrium nonbonded atomic-separation distance found for atoms of the i^{th} and j^{th} type in known crystal structures Only contact-distances less than the equilibrium contact-distance are included in the summation The values of w_{ij} and $d_{0_{ij}}$ were taken from Zugenmaier and Sarko[17]

Of the four possible space-groups, $P3_2$ and $P3_221$ accommodate left-handed helical conformations, whereas $P3_1$ and $P3_121$ accommodate right-handed helical conformations The measured density indicates that two polymer chains pass through the unit cell In trigonal space-groups, two helices of three-fold symmetry can only be placed on the three-fold axis parallel to c at $x = 1/3, y = 2/3$, and $x = 2/3, y = 1/3$ in the unit cell Under such conditions, the space groups $P3_2$ and $P3_1$ produce parallel-chain structures and the space groups $P3_221$ and $P3_121$ produce antiparallel-chain structures Stereochemical packing-analysis was initiated with the probable left-handed conformations in space groups $P3_2$ and $P3_221$, and the most probable right-handed conformation was refined in space groups $P3_1$ and $P3_121$ In space groups $P3_1$ and $P3_2$, only the rotation of the helix about the helix axis was allowed to vary. In space groups $P3_121$ and $P3_221$, translation along the helix axis and rotation about the helix axis were allowed

Results of stereochemical packing-refinement showed that the right-handed conformation either in space group $P3_1$ or $P3_121$ is highly unfavorable because of the presence of many severely short nonbonded-contact distances Packing of either of the left-handed conformations in space group $P3_2$ was likewise disallowed This leaves only the left-handed conformations in space group $P3_221$ as possibilities, with model 4 slightly favored over model 3 (see Table IV) As a final refinement of Models 3 and 4 in space group $P3_221$, the O-6′ hydroxymethyl and the uronic acid groups were allowed to rotate about the C-5′–C-6′ and C-5–C-6 bonds, respectively These groups did not move significantly away from the minimum-energy positions found during conformational analysis Without good quality, X-ray diffraction, intensity data, a definite selection of Model 3 or 4 in space group $P3_221$ is not possible, although Model 4 leads to a lower RPACK value Projections of Models 3 and 4 in the unit

TABLE IV

RESULTS OF THE PACKING CALCULATIONS FOR THE VARIOUS MODELS

Conformational model	*Space group*	*Minimum RPACK*
2	$P3_1$	784 0
	$P3_121$	74 6
3	$P3_2$	633 0
	$P3_221$	25 5
4	$P3_2$	683 0
	$P3_221$	19 9

TABLE V

CARTESIAN COORDINATES FOR ONE RESIDUE OF MODEL 3

Atom	X *(nm)*	Y *(nm)*	Z *(nm)*	*Atom*	X *(nm)*	Y *(nm)*	Z *(nm)*
*Glcp*A				*Glc*p			
C-1	0 4731	−0 1231	1 0664	C-1	0 3945	−0 0215	1 5784
C-2	0 5823	−0 1863	0 9812	C-2	0 2804	−0 0272	1 4777
C-3	0 5542	−0 1642	0 8334	C-3	0 3186	−0 1134	1 3584
C-4	0 4138	−0 2118	0 7986	C-4	0 4506	−0 0660	1 2991
C-5	0 3121	−0 1489	0 8932	C-5	0 5569	−0 0568	1 4081
C-6	0 1717	−0 2011	0 8711	C-6	0 6875	0 0007	1 3576
O-1	0 4958	−0 1571	1 1992	O-2	0 1638	−0 0781	1 5416
O-2	0 7083	−0 1310	1 0176	O-3	0 2160	−0 1063	1 2593
O-3	0 6501	−0 2357	0 7552	O-5	0 5118	0 0289	1 5141
O-4	0 3805	−0 1749	0 6650	O-6	0 6693	0 1290	1 2978
O-5	0 3465	−0 1782	1 0295	H-1	0 4127	−0 1167	1 6189
O-6′	0 1576	−0 3309	0 8963	H-2	0 2604	0 0702	1 4439
O-6″	0 0787	−0 1269	0 8325	H-3	0 3290	−0 2130	1 3900
H-1	0 4728	−0 0188	1 0546	H-4	0 4371	0 0287	1 2560
H-2	0 5843	−0 2896	1 0001	H-5	0 5745	−0 1528	1 4469
H-3	0 5621	−0 0617	0 8120	H-6A	0 7300	−0 0649	1 2875
H-4	0 4095	−0 3163	0 8077	H-6B	0 7535	0 0105	1 4387
H-5	0 3124	−0 0448	0 8795				

cell are shown in Fig 9 The intermolecular hydrogen-bonding schemes are not shown here, as neither the water molecules nor the sodium ion have been included in the calculations Their locations cannot be determined with the meagre X-ray data at present available The coordinates of the atoms in the asymmetric unit, corresponding to Models 3 and 4 in the unit cell, are given in Tables V and VI, respectively

CONCLUSIONS

Conformational calculations and experimental data have shown that, whereas

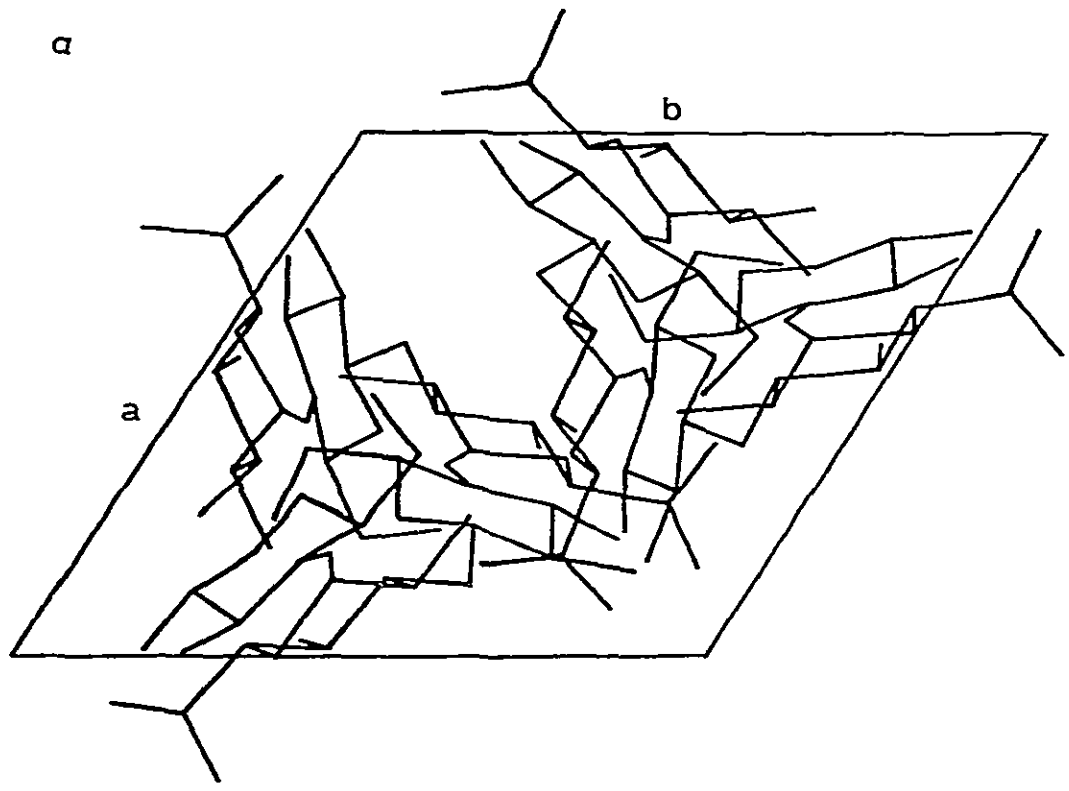
TABLE VI

CARTESIAN COORDINATES FOR ONE RESIDUE OF MODEL 4

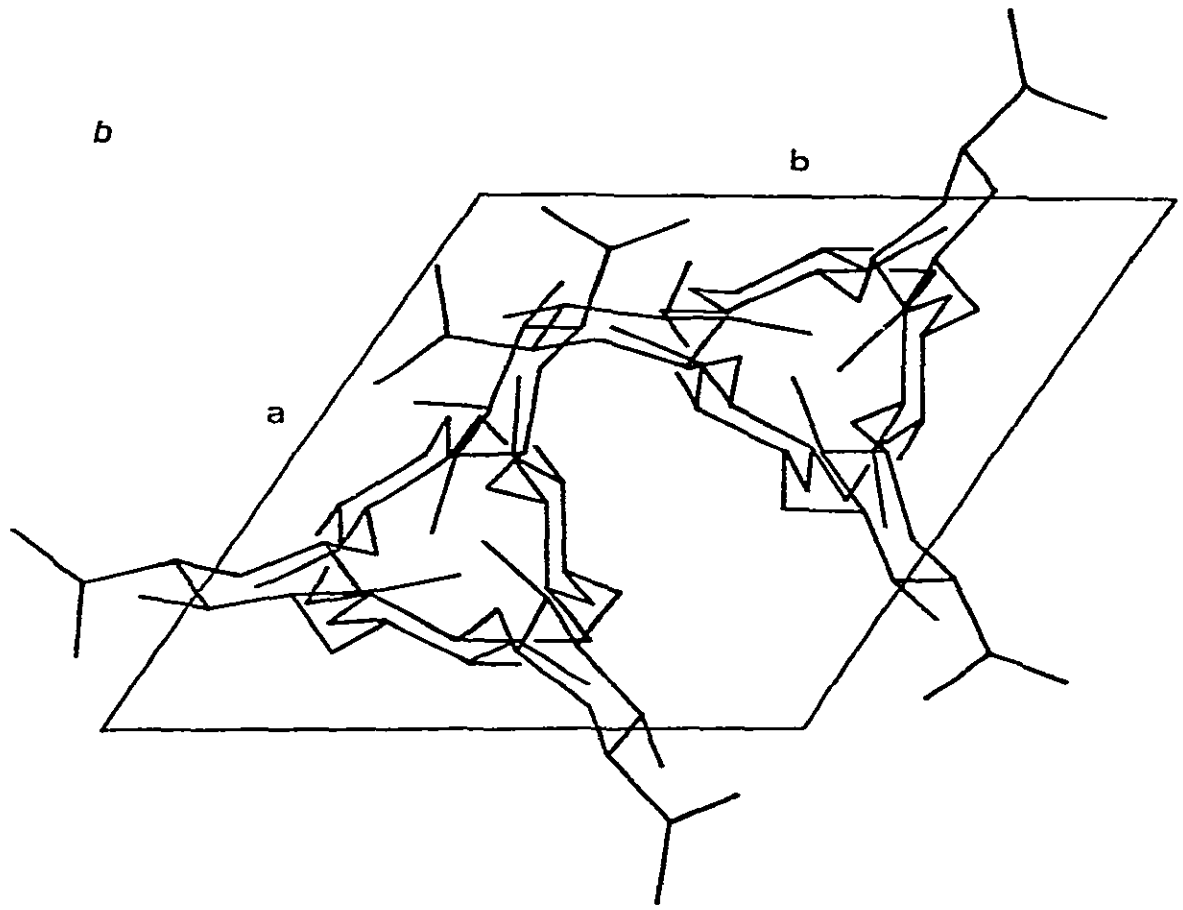
Atom	X *(nm)*	Y *(nm)*	Z *(nm)*	*Atom*	X *(nm)*	Y *(nm)*	Z *(nm)*
GlcpA				*Glcp*			
C-1	0 4301	0 0961	0 7028	C-1	0 6310	0 1549	1 1882
C-2	0 4337	−0 0065	0 5903	C-2	0 4795	0 1548	1 1724
C-3	0 3573	0 0446	0 4691	C-3	0 4387	0 0751	1 0495
C-4	0 2167	0 0871	0 5092	C-4	0 5134	0 1255	0 9267
C-5	0 2226	0 1841	0 6267	C-5	0 6634	0 1288	0 9540
C-6	0 0854	0 2213	0 6788	C-6	0 7423	0 1888	0 8396
O-1	0 4893	0 0399	0 8152	O-2	0 4200	0 1006	1 2898
O-2	0 5690	−0 0347	0 5566	O-3	0 2979	0 0876	1 0287
O-3	0 3502	−0 0582	0 3701	O-5	0 6908	0 2087	1 0701
O-4	0 1512	0 1516	0 4002	O-6	0 7336	0 1090	0 7216
O-5	0 2942	0 1249	0 7362	H-1	0 6662	0 0577	1 2065
O-6′	0 0192	0 1199	0 7339	H-2	0 4467	0.2539	1 1607
O-6″	0 0403	0 3375	0 6697	H-3	0 4622	−0 0261	1 0648
H-1	0 4805	0 1837	0 6745	H-4	0 4803	0 2225	0 9035
H-2	0 3882	−0 0950	0 6239	H-5	0 6971	0 0308	0 9711
H-3	0 4080	0 1274	0 4290	H-6A	0 8429	0 1987	0 8678
H-4	0 1617	0 0022	0 5373	H-6B	0 7037	0 2842	0 8186
H-5	0 2725	0 2714	0 5965				

the (1→4)-linked β-D-glucans crystallize almost exclusively in extended conformations[5−8 18], both extended and flat-helical conformations are theoretically favorable for the (1→3)-linked β-D-glucans[18], although only flat-helical conformations have been observed experimentally thus far[6−8,15,20]. Both polysaccharides, hyaluronic acid and the Type III polysaccharide, having alternating (1→4) and (1→3) linkages, crystallize with a slowly winding, almost extended helix Comparison of the (ϕ_B,ψ_B) values of Model 3 with the calculations of Sathyanarayana and Rao[14] shows that these values correspond to $n \sim 6$ and $h \sim 0.4$ nm for (1→3)-β-D-glucan Thus, it is the residues in the 1→3 linkage that provide the serpentine twist to the helix, when this type of linkage alternates with the β-D-(1→4) linkage A similar feature was observed for mycodextran, which is poly[(1→4)-α-D-Glc*p*-(1→3)-α-D-Glc*p*] It is known that (1→4)-linked α-D-Glc*p* residues (amylose) crystallize in a flat-helical conformation, whereas the (1→3)-linked α-D-Glc*p* residues favor extended helical structures[18] Alternation of these linkages, in mycodextran, leads to an extended conformation, which resembles a corrugated ribbon[19] Thus, from the X-ray data available thus far on polysaccharides having alternating (1→4) and (1→3) linkages, it would seem that the function of the linkage which would promote a flat-helical structure in the homopolymer is to provide a "worm-like" character to the copolysaccharide

In view of the biologists' description of bacterial polysaccharides as "slimes", the extended conformation proposed suggests that a brusheap gel-structure is present



Model 3



Model 4

Fig 9 Projections of the packing of (*a*) Model 3 and (*b*) Model 4 helices in the unit cell

in water Weak, interchain forces of the kind responsible for cellulosic or pectin gels may be expected The underlying mechanism whereby this gel structure would provide protection against bacteriophages would then revert to a simple, mechanical exclusion, as in gel-permeation chromatography Only a phage able to bore a wide enough hole by chain scission would pass through the gel to the host surface

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