Nuclear Magnetic Resonance Study of Molecules in Anisotropic Systems. Part 9.† Solution Structure and Preferred Orientation of *myo*-Inositol in a Lyotropic Liquid Crystal

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¹H N.m.r. spectra of *myo*-inositol have been measured in a lyotropic liquid crystal containing potassium laurate. Variable-temperature experiments revealed an increased participation of direct couplings in the spectra on lowering the temperature of a sample from an isotropic phase. Analysis of the ordered spectra obtained at 28 and 22 °C afforded values for the direct couplings between protons. The distance ratio $r_{2,6}/r_{3,5}$ was determined to be 0.92 from the relevant direct couplings. The direct coupling data have been interpreted in terms of a reasonable model of molecular structure; however, a full analysis of the direct couplings to give precise structural and order parameters was unsuccessful because of a relatively low degree of order in the mesophase. The principal axis system for the order tensor differs as much as 34° from that for the moment of inertia; it is therefore suggested that a type of solute–solvent interaction dominates the orientation of *myo*-inositol. The ¹H n.m.r. spectrum of *myo*-inositol in D_2O has also been analysed, to derive the indirect couplings used in the analysis of the ordered spectra.

We have previously studied the solution structure and orientation of several 1,2,3,4,5,6-hexachlorocyclohexane isomers [1-3] and some chloromethylstannanes [4] in thermotropic liquid crystals. To elucidate further the characteristics of molecules dissolved in an anisotropic medium, we have selected *myo*-inositol as a probe molecule for study in a lyotropic liquid crystal phase. The molecular orientation of various solutes in lyotropic liquid crystals has been studied.[5] Usually, the degree of order is very low for molecules with sufficient solubility in water,[6] but relatively few data are available for such systems.

Among the nine possible inositol isomers, *myo*-inositol is the most interesting because of its widespread occurrence in plants and animals:[7] it is found as a complex with vitamin B and possesses a beneficial effect on the repression of fatty liver.[8]

myo - Inositol

Experimental

¹H N.m.r. spectra were measured with a Varian XL-200 spectrometer at 200 MHz. Variable-temperature experiments were carried out in a conventional manner; the temperature fluctuation was within ±0.1 °C throughout the measurement. *myo*-Inositol (Tokyo Kasei Co. Ltd.) was used without further purification. The lyotropic liquid crystal used was composed of potassium chloride (4.0 wt %), potassium laurate (30.0 wt %), deuterium oxide (60.0 wt %), and decan-1-ol (6.0 wt %),[9] in which *myo*-inositol was dissolved (4.0 wt %). The ¹H n.m.r. spectrum of *myo*-inositol in an isotropic medium was obtained with a Hitachi R-90H spectrometer operating at 90 MHz and at 34 °C: a 10 wt % solution was used. The sample was spun for the measurement of ordered spectra.

For spectral analysis, the LAOCN5 program [10] was used for the isotropic sample, and the modified [11] LAOCN3 program for the oriented system. Structural and order parameters were derived from the direct coupling data with the aid of the SHAPE program.[12] These calculations were carried out with a NEAC S-1000 computer at the Computation Center, Osaka University.

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† Part 8, H. Fujiwara, J. Hirai, Y. Sasaki, and K. Takahashi, *Mol. Cryst. Liq. Cryst.*, in the press.
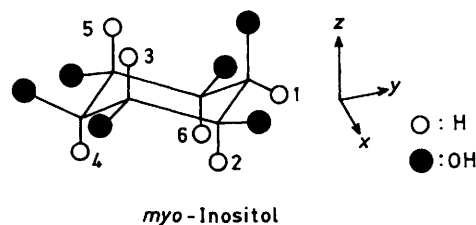
Results

¹H *N.m.r. Spectrum of* myo-*Inositol in an Isotropic Medium.*—The spectrum of *myo*-inositol in D_2O measured at 90 MHz was simulated starting from a set of indirect couplings observed at a higher magnetic field ($J_{1,2}$, $J_{2,3}$, and $J_{3,4}$) and reported [13] for 1,2,3,5/4,6-hexachlorocyclohexane, a chloro-analogue of *myo*-inositol (Figure 1). The resulting n.m.r. parameters are included in Table 1.

Temperature-dependent ¹H *N.m.r. Spectra of* myo-*Inositol in a Lyotropic Liquid Crystal.*—The spectrum of *myo*-inositol in the lyotropic liquid crystal changed with temperature as shown in Figure 2. Above 40 °C, the spectrum was almost the same as that in isotropic medium (D_2O), but the linewidth was to some extent broadened. On lowering the temperature below 35 °C, the spectrum became further split by direct couplings resulting from the molecular orientation in the mesophase. The changes in the spectrum detected on going from 34 to 28 °C (Figure 2) indicated the major contribution of the direct couplings involving protons 2, 3, 5, and 6. The splitting of the H-1 signal observed at 80 °C, due to the indirect coupling $J_{1,2}$, became masked by line broadening in the mesophase as a result of retarded molecular motion in this phase and small direct couplings with adjacent protons. At 22 °C the splitting of the H-1 signal reappeared, because direct couplings became relatively large as a result of the increased degree of orientation at the lower temperature.

The ordered spectra at 28 and 22 °C were analysed to obtain the direct coupling data (Table 2; Figure 3). A slight disagreement remained between observed and simulated spectra (*ca.* +5 Hz at 28 °C and *ca.* −40 Hz at 22 °C; Figures 3 and 4, respectively). This was hard to account for; it seemed to be due to overlapping of lines at the low degree of orientation involved. Attempts were made to study the spectrum of a sample in
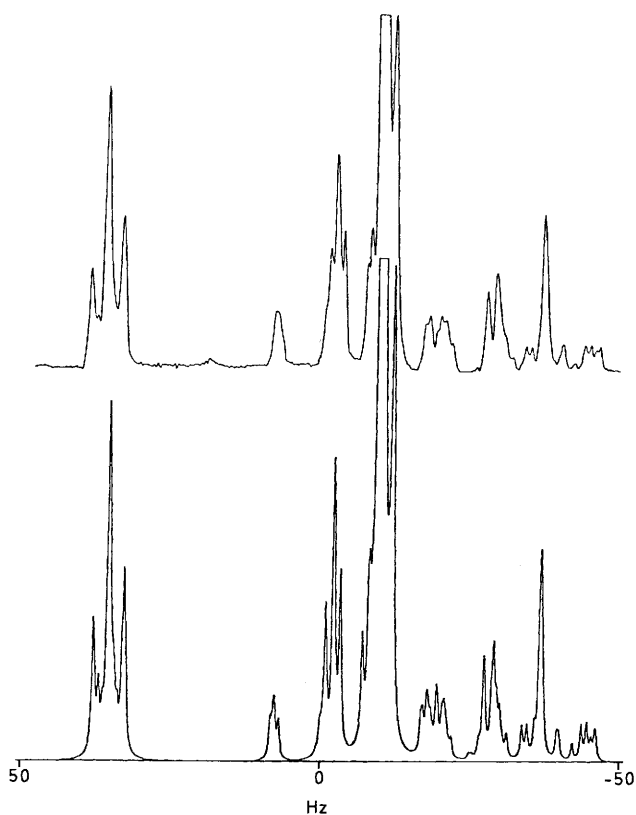
Figure 1. Experimental (upper) and simulated (lower) ^{1}H n.m.r. spectra of *myo*-inositol in D_2O (90 MHz). The experimental spectrum was obtained by accumulating 100 f.i.d.s with a 30° pulse and repetition time of 5 s; 16 k data points were used in the Fourier transformation against a spectral width of 900 Hz

Table 1. N.m.r. parameters of *myo*-inositol dissolved in D_2O

Indirect couplings (Hz)		$J_{3,4}$	9.53 ± 0.05
		$J_{3,5}$	-0.47 ± 0.06
$J_{1,2}$	2.91 ± 0.04		
$J_{1,3}$	-0.32 ± 0.04	Chemical shifts (Hz)[a]	
$J_{1,4}$	0.23 ± 0.04		
$J_{2,3}$	10.12 ± 0.05	ν_1	45.11 ± 0.03 (2.812 p.p.m.)
$J_{2,4}$	-0.26 ± 0.05	ν_2	-2.69 ± 0.05 (2.281 p.p.m.)
$J_{2,5}$	-0.14 ± 0.04	ν_3	5.66 ± 0.05 (2.374 p.p.m.)
$J_{2,6}$	0.33 ± 0.06	ν_4	-25.31 ± 0.03 (2.030 p.p.m.)

[a] In Hz at 90 MHz from an arbitrary frequency. In parentheses are listed values in p.p.m. from internal t-butyl alcohol.

thermotropic liquid crystals at variable temperatures, with a negative result. The inositol was insoluble in ZLI1132 (Merck), and in ZLI1167 (Merck), in which it was slightly soluble, it gave only seventeen lines with a half-width of *ca.* 15 Hz at a spectral width of 1 600 Hz.

Discussion

The ^{1}H N.m.r. Spectrum in an Isotropic Medium.—The assignment of ^{1}H chemical shifts in the present study coincides with those in refs. 14 ($\delta_1 = 4.06, \delta_2 = 3.54, \delta_3 = 3.63$, and $\delta_4 = 3.28$ p.p.m. from sodium 3-trimethylsilylpropane-1-sulphonate) and 15 ($\delta_1 = 4.069, \delta_2 = 3.554, \delta_3 = 3.619$, and $\delta_4 = 3.209$ p.p.m. from sodium 3-trimethylsilyltetradeuteriopropionate). However, the assignment in ref. 16 is inconsistent with these results: $\delta_1 = -0.29, \delta_2 = 0.14, \delta_3 = 0.24$, and $\delta_4 = 0.49$ p.p.m. from dioxane are given in heteronuclear decoupling experiments,
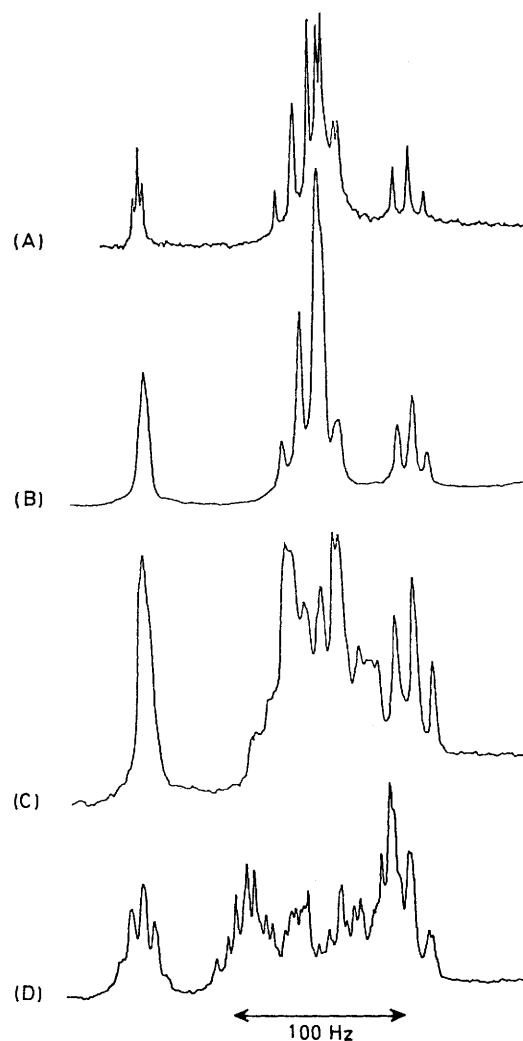

Figure 2. Temperature-dependent ^{1}H n.m.r. spectra of *myo*-inositol dissolved in a lyotropic liquid crystal (200 MHz); temperatures of measurement: (A) 80.3 °C (isotropic), (B) 34.0 °C, (C) 28.0 °C, (D) 22.0 °C

relative chemical shifts of H-2 and H-3 being interchanged. The correctness of our assignment is obvious from the first-order splitting in the ^{1}H spectrum.

Interpretation of the Direct Couplings observed in the Lyotropic Liquid Crystal.—The direct couplings (Table 2) include information on the molecular structure and order parameters of the solute in the mesophase. However, a full analysis of the direct couplings was not achieved, because of the relatively low accuracy of the experimental data. Calculation resulted in estimated errors as large as 50% in the values of the distance ratios and the order parameters, except for $S_{x,x}$, $r_{1,3}/r_{3,4}$, and $r_{1,4}/r_{3,4}$. Therefore, a reasonable model was sought which would explain well the observed direct couplings, since the geometry of solute was expected not to be greatly deformed, as indicated by the estimated errors in the mesophase. First, a standard geometry was fixed and only order parameters were simulated (three-parameter calculation, model 1 in Table 2). For the standard geometry, literature values[17] of $r_{CC} = 1.521$ Å and $r_{CO} = 1.429$ Å were taken, and $r_{CH} = 1.07$ Å, $r_{OH} = 0.96$ Å, and bonding angles of 109.47° for C atoms were assumed in accord with the C_s symmetry. This model did not give a satisfactory fit for $D_{2,6}$ and $D_{3,5}$ (Table 2), which give different

Table 2. Direct couplings observed and simulated at 22 °C

	Observed	Simulated		
		Model 1	Model 2	Model 3
Direct couplings (Hz)				
$D_{1,2}$	-5.28 ± 0.09	-6.30	-6.33	-5.23
$D_{1,3}$	3.11 ± 0.15	3.19	2.97	2.83
$D_{1,4}$	0.69 ± 0.15	0.28	0.08	0.50
$D_{2,3}$	10.29 ± 0.10	7.35	7.06	10.26
$D_{2,4}$	1.12 ± 0.13	0.92	1.03	0.91
$D_{2,5}$	0.73 ± 0.10	1.30	1.46	0.90
$D_{2,6}$	-20.36 ± 0.10	-17.94	-20.34	-20.37
$D_{3,4}$	1.32 ± 0.08	1.58	1.59	1.08
$D_{3,5}$	-15.88 ± 0.13	-17.94	-16.52	-15.96
R.m.s. error (Hz)[a]		1.54	1.21	0.16
Order parameters[b]				
$S_{x,x}$		0.0023	0.0021	0.0020
		(0.0002)	(0.0003)	(0.0003)
$S_{z,z}$		-0.0014	-0.0013	-0.0013
		(0.0005)	(0.0007)	(0.0006)
$S_{y,z}$		-0.0014	-0.0014	-0.0010
		(0.0004)	(0.0004)	(0.0005)

[a] R.m.s. error between observed and the simulated direct couplings. [b] $r_{3,4} = 3.00$ Å assumed.[17] Standard deviations are given in parentheses.

experimental values, indicating different distances between the relevant protons. To take this into account, the x-co-ordinates of H-2 and H-6 were varied (four-parameter calculation, model 2 in Table 2), since the y- and z-co-ordinates could not discriminate between the two direct couplings. This model improved the fit for $D_{2,6}$ and $D_{3,5}$. However the root mean square error between observed and the recalculated direct couplings was still high because of the discrepancy in $D_{2,3}$. This could be removed effectively by varying the z-co-ordinates of H-2 and H-3 (five-parameter calculation, model 3 in Table 2), where $z_2 = z_4 = z_6 = -z_3 = -z_5$ is assumed). According to this calculation, all the direct couplings can be reproduced within an error of 0.25 Hz. Distance ratios are listed in Table 3 for these calculations together with the crystal data. The model 3 is seen to give results consistent with the crystal data, if one allows the inclusion of errors of the order of 0.05 for the distance ratios in the crystal. The distance ratio $r_{2,6}/r_{3,5}$ can be determined directly from the relevant direct couplings observed, since only $S_{x,x}$ contributes to $D_{2,6}$ and $D_{3,5}$ in accord with the C_s symmetry [equation (1)]. This equation gives $r_{2,6}/r_{3,5} =$

$$r_{2,6}/r_{3,5} = (D_{2,6}/D_{3,5})^{-1/3} \qquad (1)$$

0.921 ± 0.004, consistent with the value of 0.94 ± 0.03 derived from the crystal data. This result does not depend on the model; hence the shortening of $r_{2,6}$ relative to $r_{3,5}$ is proved also for the mesophase treated here.

Similar results have been obtained from the direct couplings measured at 28 °C. However, these are subject to larger errors because of signal broadening in the original spectrum: $r_{1,2}/r_{3,4} = 0.78 \pm 0.03$, $r_{1,3}/r_{3,4} = 1.26 \pm 0.08$, $r_{1,4}/r_{3,4} = 1.35 \pm 0.09$, $r_{2,3}/r_{3,4} = 1.00 \pm 0.00$, $r_{2,4}/r_{3,4} = 0.83 \pm 0.05$, $r_{2,5}/r_{3,4} = 1.29 \pm 0.02$, $r_{2,6}/r_{3,4} = 0.79 \pm 0.04$, $r_{3,5}/r_{3,4} = 0.84 \pm 0.06$, $S_{x,x} = 0.00177 \pm 0.0008$, $S_{z,z} = -0.00117 \pm 0.00003$, and $S_{y,z} = -0.00086 \pm 0.00001$ (root mean square error of fitting 0.29 Hz).

Molecular Orientation of myo-*Inositol in the Lyotropic Liquid Crystal.*—The principal axes (x',y',z') of the order matrix can be
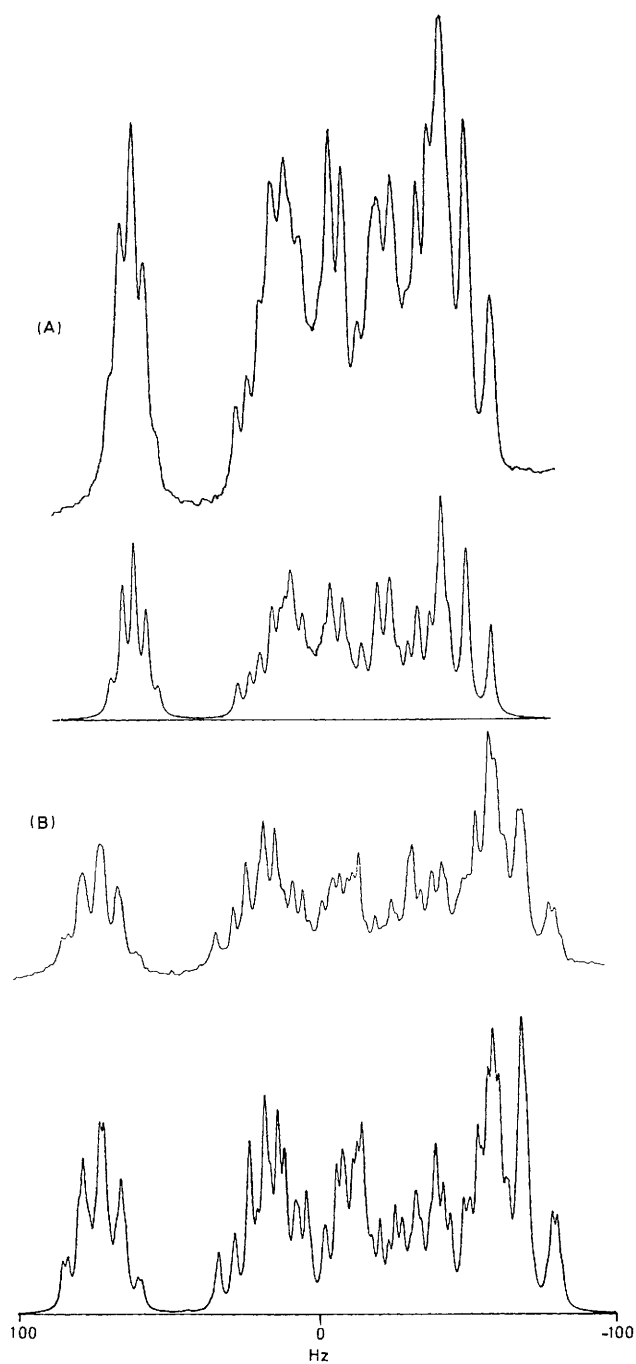

Figure 3. Experimental (upper) and simulated (lower) ^{1}H n.m.r. spectra of *myo*-inositol in the lyotopic liquid crystal at (A) 28 °C, (B) 22 °C. A hundred f.i.d.s were accumulated by 35° pulses with a repetition of 2 s; 8 k data points were used in the Fourier transformation against a spectral width of 1 000 Hz. The r.m.s. errors between observed and simulated spectra were 0.31 Hz for 21 lines at 28 °C and 0.59 Hz for 42 lines at 22 °C. The chemical shifts simulated at 22 °C were $v_1 = 79.0 \pm 0.1$, $v_2 = -25.4 \pm 0.1$, $v_3 = -11.2 \pm 0.1$, and $v_4 = -74.3 \pm 0.3$ Hz at 200 MHz from an arbitrary reference

determined by diagonalising the matrix obtained experimentally. For this purpose, y- and z-axes are assumed to be rotated anticlockwise around the x axis by an angle ω to be superposed on the y' and z'-axes (Figure 4). Under these conditions $S_{y',z'}$ is expressed as equation (2), where $\theta_p^{y'}$ means an angle between

$$S_{y',z'} = \sum_{p,q} \cos\theta_p^{y'} \cos\theta_q^{z'} S_{p,q} \qquad p,q = x, y, z \qquad (2)$$

Table 3. Distance ratios estimated from direct couplings at 22 °C according to the various models

	Model 1	Model 2	Model 3	Crystal[a]	At 28 °C[b]
$r_{1,2}/r_{3,4}$	0.75	0.74	0.79 ± 0.01	0.75	0.78 ± 0.03
$r_{1,3}/r_{3,4}$	1.23	1.23	1.29 ± 0.04	1.24	1.26 ± 0.08
$r_{1,4}/r_{3,4}$	1.24	1.24	1.39 ± 0.05	1.36	1.35 ± 0.09
$r_{2,3}/r_{3,4}$	1.05	1.05	1.00 ± 0.00	0.99	1.00 ± 0.00
$r_{2,4}/r_{3,4}$	0.76	0.75	0.85 ± 0.03	0.86	0.83 ± 0.05
$r_{2,5}/r_{3,4}$	1.29	1.28	1.30 ± 0.01	1.31	1.29 ± 0.02
$r_{2,6}/r_{3,4}$	0.76	0.71	0.79 ± 0.02	0.83	0.79 ± 0.04
$r_{3,5}/r_{3,4}$	0.76	0.76	0.86 ± 0.04	0.88	0.84 ± 0.06

[a] Calculated from the data in ref. 17. Averages were taken for chemically equivalent bonds with respect to the two inequivalent molecules in the crystal. [b] Estimated from direct couplings at 28 °C according to model 3.
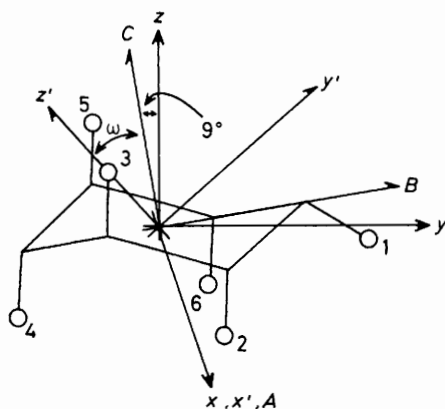

Figure 4. The molecule of *myo*-inositol and its cartesian co-ordinates (x, y, z), the principal axes of the order parameter (x', y', z'), and the principal axes of the moment of inertia (A, B, C). The axes x, x', and A are the same from C_s symmetry. The value of $\omega = 43°$ is obtained from the order parameters measured at 22 °C. The OH groups are not shown for simplicity

the *p*- and *y'*-axes. Since $S_{x,y} = S_{x,z} = 0$ in C_s symmetry and $S_{x,x} + S_{y,y} + S_{z,z} = 0$, the angle ω which causes $S_{y',z'}$ to vanish is given by equation (3), and the principal values are expressed

$$\tan(2\omega) = 2S_{y,z}/(S_{y,y} - S_{z,z}) = -2S_{y,z}/(S_{x,x} + 2S_{z,z}) \quad (3)$$

as $S_{x',x'} = S_{x,x}$ and equation (4). From the order parameters

$$S_{z',z'} = \sin^2\omega S_{y,y} + \cos^2\omega S_{z,z} - \sin(2\omega)S_{y,z}. \quad (4)$$

determined at 22 °C, $\omega = 43°$, values of $S_{x',x'} = 0.0204$, and $S_{z',z'} = -0.00334$ are obtained. Therefore, the most favoured orientation of *myo*-inositol is found to be that with its z'-axis perpendicular to the external magnetic field. This orientation is equivalent to that with the z'-axis parallel to the cylindrical

superstructure of the alkyl chain, and to that with the z'-axis perpendicular to the alkyl chain of the mesophase.[6] This principal axis system may be compared with that for the moment of inertia (axes A, B, and C in Figure 4), which is obtained by assuming a zig-zag conformation for C–C–O–H and a binding angle of 109.47° for the O atoms. The principal values of the moment of inertia are $I_A = 453.6$, $I_B = 540.6$, and $I_C = 885.1$ uÅ^2, and the C-axis deviates from the z-axis anticlockwise by 9°. Therefore, the C and z' axes deviate 34° from each other. In the case of 1,2,3,5/4,6-hexachlorocyclohexane, a chloro analogue of *myo*-inositol, dissolved in a thermotropic liquid crystal (EBBA), the two principal axis systems virtually coincide.[1] In a lyotropic liquid crystal, solutes may be subject to various molecular interactions with the medium, such as acid–base, hydrogen bonding, electrostatic, *etc.* This is the case especially for solutes very soluble in water. Therefore, molecular interactions are considered to dominate the orientation of *myo*-inositol found here. Further discussion, however, may not be justified because of the very low degree of order observed.

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