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## Disorder in Crystalline Tetraiodoethylene; Constrained Refinements of Neutron Powder Diffraction Data

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The 4 K results of Haywood & Shirley [*Acta Cryst.* (1977), **B33**, 1765–1773] have been reanalysed using the constrained-refinement program *EDINP*. The molecules are constrained to *mmm* symmetry and disorder is allowed on both molecular sites. One site is fully ordered, whereas the other is disordered to 21%. New room-temperature results indicate disorder on both sites, but as certain unexplained features appear in the diffraction scan at this temperature, scans at intermediate temperatures are required before a satisfactory explanation can be found. The significance of the low-temperature result is thought to be high and a possible significance test is suggested.

### Introduction

The crystal structure of tetraiodoethylene at room temperature has been studied by Khotsyanova, Kitaigorodsky & Struchkov (1952, 1953) and Kitaigorodsky, Khotsyanova & Struchkov (1953) using single-crystal X-ray diffraction. Later work by Kipps (1973)

did not give a definitive answer to the question posed by Kitaigorodsky's (1961) suggestion that the molecules in the structure are disordered between two orientations on each of the two independent sites in the crystal. The structure is monoclinic,  $P2_1/c$ , and although both molecules are sited on symmetry centres they are not related by symmetry.

The  $C_2I_4$  molecule can be considered as an arrangement of I atoms on a square with the ethylenic C=C bond lying in the plane of the square and parallel to one or other of its edges. Thus there are two orientations of this bond, and because the crystal potential would be little affected by the choice between these orientations, disorder between them is expected. Spectroscopic measurements by Thackeray, Shirley, Oralaratmanee, Kipps & Stace (1974) with crystals at 300 K suggest that more molecules are aligned with the ethylenic bond nearly parallel to  $a^*$ .

Although the X-ray studies should be the most reliable, the small scattering factor of C compared with that of I renders the technique insensitive, and as the neutron scattering lengths of the two elements are comparable it is clearly a problem for study with neutrons. Single-crystal neutron measurements require quite large crystals but the presence of twinning prevents the growth of suitable samples. Therefore, the most appropriate available technique is that of powder neutron diffraction.

The present work consists of an analysis of room-temperature powder neutron diffraction results and a reanalysis of the data already published by Haywood & Shirley (1977). A more positive result can be obtained

than in the earlier work, made possible by the use of constraints in the refinement procedure.

### Constrained refinements

Whenever there are data of any form which are known to an accuracy better than that which could possibly result from a refinement, it is always better to include these data in the refinement procedure at all stages. In the present example there is not sufficient structural information in the diffraction scans to merit the refinement of a large number of parameters, and therefore we can say with confidence that the data will not support a significant variation of the molecule shape from its expected  $mmm$  symmetry, or a significant difference between one molecule and another. It should be remembered, however, that any such assertion can be called into question at any stage and investigated by removing the constraints and applying a significance test. In this way, even 'facts' which are not definitely known can be included as constraints, but then the unconstrained result must be found and a comparison made. In the present case, however, no unconstrained

Table 1. *The final parameters in the profile refinements*

Model refined	Ordered	Disordered
Temperature	4 K	4 K
Wavelength (Å)	2.407	2.407
$2\theta$ range (°)	9.5 to 94.4	9.5 to 94.4
$2\theta$ step (°)	0.1	0.1
Scale factor	0.326 (5)	0.319 (5)
Zero of the scan (°)	0.354 (6)	0.356 (5)
Peak shape parameters, (°) <sup>2</sup> $\left\{ \begin{array}{l} u \\ v \\ w \end{array} \right.$	1.10 (12) -1.84 (15) 0.96 (04)	1.30 (12) -2.06 (15) 1.02 (04)
Background counts	260 (1)	259 (1)
Cell parameters $\left\{ \begin{array}{l} a \text{ (Å)} \\ b \text{ (Å)} \\ c \text{ (Å)} \\ \beta \text{ (°)} \end{array} \right.$	14.7710 (23) 4.2243 (04) 12.5468 (16) 108.36 (1)	14.7702 (20) 4.2243 (04) 12.5464 (16) 108.36 (1)
Cell volume (Å <sup>3</sup> )	743.0 (4)	743.0 (3)
Atom positions (Å) ( $I_z, C_y, C_z = 0$ ) $\left\{ \begin{array}{l} I_x \\ I_y \\ C_x \end{array} \right.$	1.819 (6) 1.807 (7) 0.688 (8) 1.375 (17) 2.132 (8)	1.828 (6) 1.777 (8) 0.704 (8) 1.408 (16) 2.103 (8)
C=C bond	2.2 (2)	1.3 (2)
C-I bond	2.527 (5)	2.539 (5)
Isotropic overall temperature factor $B$	2.027 (6)	2.026 (5)
Euler angles (radians) $\left\{ \begin{array}{l} \text{molecule 1} \\ \left\{ \begin{array}{l} \theta_1 \\ \psi_1 \end{array} \right. \\ \text{molecule 2} \\ \left\{ \begin{array}{l} \theta_2 \\ \psi_2 \end{array} \right. \end{array} \right.$	-3.296 (6) 3.031 (5) 1.190 (6) -2.902 (6)	-3.287 (5) 3.046 (5) 1.179 (5) -2.936 (6)
Occupation probabilities $\left\{ \begin{array}{l} \text{molecule 1, } p_1 \\ \text{molecule 2, } p_2 \end{array} \right.$	- -	1.02 (2) 0.79 (2)
Number of parameters	20	22
$R_w \times 10^{-4}$	67.1	59.4
$R$ factor (%)	6.1	5.7

refinement was attempted for reasons which will appear later.

The molecule can therefore be specified by three positional coordinates,  $C_x$ ,  $I_x$  and  $I_y$ , where we imagine the molecule to be in the plane  $Z = 0$  with the ethylenic bond along the  $X$  axis. The orientation of this molecule in the crystal can be specified by three Euler angles  $\phi$ ,  $\theta$ ,  $\psi$ . These are defined in the review article by Pawley (1971). The first Euler angle,  $\phi$ , is a rotation about the molecular  $Z$  axis, and therefore the difference between the disordered orientations at one site is a difference of  $\pi/2$  in  $\phi$ . Thus three parameters for each of the two molecular sites are required to orient all the molecules, and only two more parameters are necessary for the disordering on each site. The total number of positional, orientational and disorder parameters is thus 11, compared with 20 used by Haywood & Shirley (1977). Even this comparison is not accurate as the present model allows disorder of the whole molecule whereas Haywood & Shirley include it only on the C positions.

A very similar case was studied by Larsen, Little & Coppens (1975) with single-crystal measurements. The molecule of tetracyanoethylene (TCNE) in a complex with pyrene was found to be disordered by a rotation of  $\pi/2$  in its plane. The disorder parameter in this case was very well determined at 0.930 (3). The result of allowing the 7% disorder in the refinement was that the central C=C bond increased and the shape of the TCNE molecule approached that of the pure cubic TCNE. The single-crystal program used in that study was described by Pawley (1971) and a modification of this program (*EDINP*) has been produced for powder refinements (Pawley, Mackenzie & Dietrich, 1977).

The results of refinement will be presented in the next section, but there are some points of interest in the

method which are best discussed here. At no stage in the refinement was any restriction placed on the values of the occupation probabilities. These could therefore move to impossible values out of the range  $0 \leq p \leq 1$ . No result disobeyed this requirement by more than one standard deviation. A good example where three disordered sites exist in a crystal is described by Hazell, Hazell & Pawley (1977) where this necessary condition was again obeyed without outside interference.

Often when a disordered arrangement is suggested for a structure some atoms of one molecule appear very close to atoms of the other orientation. In this situation it is impossible to resolve the close pairs of atoms, and therefore these atoms cannot be refined independently. In the work of Haywood & Shirley (1977) the I atoms occur in such close pairing that they did not attempt to separate them. Any such attempt would result in an ill-conditioned least-squares matrix, probably causing large and unphysical positional shifts. This is the reason why the fully unconstrained disordered model was not attempted. This problem does not arise in the constrained refinement of the disordered model. Indeed, in the successful low-temperature disordered refinement I atoms appear separated by 0.07 Å with no ill-conditioning. This is no new discovery as the work on azulene (Pawley, 1965) testifies. Instabilities arise when two parameters are trying to do the job of one, and it is quite clear that in the present case there is no such duplication of duty.

#### Low-temperature structure

Two refinements were performed with the 4 K data of Haywood & Shirley (1977), the first assuming total order as they reported, and the second allowing

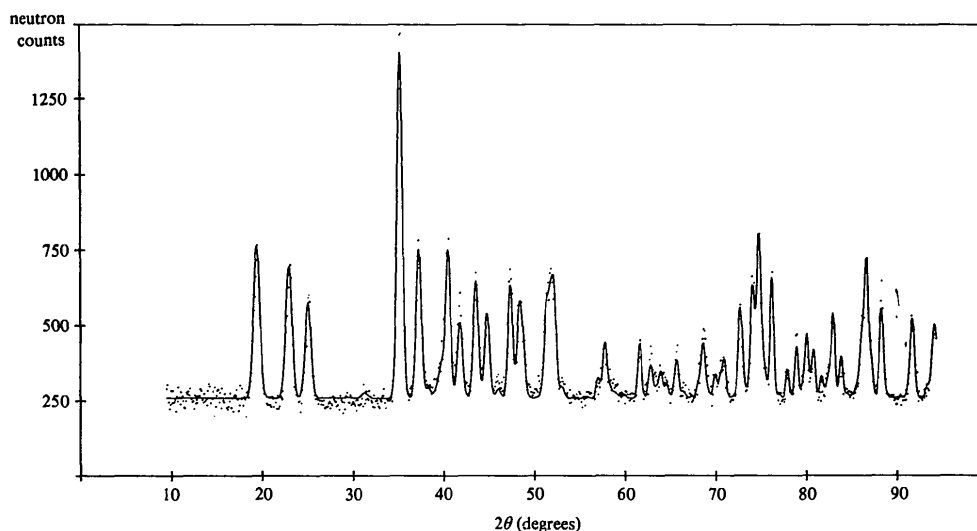


Fig. 1. The observed and calculated powder neutron diffraction scans for  $C_2I_4$  at 4 K. The observations are points, and the calculation is a continuous line.

disorder on both molecular sites. The results given in Table 1 indicate a better agreement with the disordered model, showing that one molecule is completely ordered whereas the other is disordered to about 21%. Of course we cannot accept this result simply by looking at these two probabilities, but all the evidence is that this result is highly significant. The ordered refinement has I atoms very nearly arranged on a square – complete disorder in the crystal would lead to a perfect square for the I atoms, distorted only by the correlation effect of half of the C atoms which would be misplaced in such a refinement. The fact that the I atoms move away from a square to a more rectangular arrangement supports the significance of the disordered refinement result.

Further support is given by the fact that both occupation probabilities lie (within error) within the range of physical reality. Furthermore, the values of the overall isotropic temperature factor in the two refinements strengthen the disordered result, considering that the measurements were made at 4 K. At this temperature the disordered refinement with  $B = 1.3$  (usual units) is more acceptable than the ordered refinement with  $B = 2.2$ . The reliability of temperature factors from powder refinements is not sufficient for these values to be interpreted as physical values, except perhaps at these very low temperatures, but it is well known that the effect of neglecting disorder when it is present is to make the temperature factors spuriously large, as in the ordered refinement.

The C=C bond extends when the disorder is incorporated in the model, and it is perhaps significant

that this is what happened in the TCNE refinement (Larsen *et al.*, 1975) already mentioned.

We therefore conclude that at 4 K one of the molecules [the second one as listed by Haywood & Shirley (1977)] is partially disordered whereas the other molecule is fully ordered.

The agreement between observation and calculation is shown in Fig. 1.

### Room-temperature structure

Only one refinement was attempted with the room-temperature results, on the assumption that ordering would not take place on increasing temperature. The refinement was considerably slower and poorer, but this behaviour is to be expected. Results are not given here as the refinement reached an unsatisfactory result with  $R = 15\%$ . There were a few weak features of the diffraction scan which could not be explained with the unit cell we used. There was an indication that the molecule which was fully ordered at low temperature may not be exactly 50% disordered at room temperature, but it is necessary to make measurements at intermediate temperatures to find the temperature variation of the site occupancies.

### The refinements

The results of the refinements with the low-temperature data are given in Table 1, with the correlation matrix in

Table 2. *The correlation matrix from the final refinement cycle using the disordered model*

	Back ground	Scan zero	Peak shape			Unit cell				Temp-erature factor $B$	Euler angles Molecule 1			Euler angles Molecule 2			Atom positions			Occupancies	
			$u$	$v$	$w$	$a$	$b$	$c$	$\beta$		$\phi_1$	$\theta_1$	$\psi_1$	$\phi_2$	$\theta_2$	$\psi_2$	$1_x$	$1_y$	$C_x$	$p_1$	$p_2$
Scale	-0.24	-0.01	-0.01	-0.06	0.17	0.07	-0.01	0.02	0.05	0.70	-0.14	0.25	-0.01	-0.18	0.14	0.04	0.16	0.42	0.28	0.06	0.17
Background	-0.02	-0.09	0.09	-0.15	0.02	0.02	-0.02	-0.07	0.14	-0.001	-0.002	-0.04	0.12	-0.01	0.04	0.17	0.05	-0.03	0.09		
Scan zero	0.01	-0.004	0.003	-0.49	-0.76	-0.51	0.10	-0.01	-0.001	-0.03	0.04	-0.01	-0.08	0.03	0.01	-0.01	0.01	0.06	-0.03		
Peak shape	$u$	-0.98	0.92	-0.002	0.002	0.03	0.05	-0.13	-0.02	0.04	-0.15	0.06	0.03	-0.09	-0.14	-0.20	0.03	-0.06	-0.16		
				-0.96	-0.002	-0.002	-0.03	-0.04	0.07	0.04	-0.05	0.16	-0.06	-0.04	0.09	0.14	0.19	-0.04	0.06	0.14	
Unit cell	$a$	0.28	0.14	0.01	0.03	0.04	-0.01	-0.05	0.04	-0.15	0.06	0.03	-0.09	0.12	0.18	0.03	-0.06	-0.13			
				0.09	-0.02	0.08	-0.02	0.03	0.09	-0.09	0.03	0.04	0.01	-0.03	0.01						
				0.17	-0.17	-0.01	-0.04	0.06	-0.04	0.11	-0.08	0.01	-0.02	-0.01	-0.10	-0.04					
				0.09	0.02	0.003	0.06	-0.14	-0.03	0.15	-0.01	-0.05	0.03	0.03	0.02	0.09					
Overall $B$	Molecule 1 Euler angles	$\phi_1$	0.04	0.03	0.03	0.09	-0.07	-0.04	0.05	0.06	0.21	0.08									
				0.04	0.03	0.09	-0.07	-0.04	0.05	0.06	0.21	0.08									
Molecule 2 Euler angles	$\phi_2$	-0.30	0.16	-0.17	-0.26	0.19	-0.09	0.02	0.54	-0.03	0.39	0.44									
				-0.02	0.56	0.16	-0.24	0.42	-0.01	-0.15	0.26	-0.10	-0.16								
Iodine positions	$1_x$	-0.06	0.01	0.43	0.15	0.15	-0.01	0.16	-0.16	0.01											
				-0.11	-0.31	0.41	0.04	0.06	0.19	-0.07	-0.08										
Carbon position $C_x$	Occupancy for molecule 1	$p_1$	0.06	-0.37	0.20	-0.32	-0.01	-0.14	-0.31												
				-0.27	0.09	0.15	0.13	-0.17	0.16												
Occupancy for molecule 2	$p_2$	0.01	0.03	0.21	-0.08	-0.05															
				0.01	0.03	0.21	-0.08	-0.05													
Carbon position $C_x$	Occupancy for molecule 1	$p_1$	0.03	0.21	0.39																
				0.03	0.21	0.39															
Carbon position $C_x$	Occupancy for molecule 1	$p_1$	0.03	0.21	0.39																
				0.03	0.21	0.39															
Carbon position $C_x$	Occupancy for molecule 1	$p_1$	0.03	0.21	0.39																
				0.03	0.21	0.39															

Table 2. The behaviour of the isotropic temperature factor must be scrutinized as this forms part of the support for the significance of the result. Table 2 shows that the temperature factor is highly correlated with many other parameters. It may at first be surprising that the correlation with the background parameter is not the highest, but this is perhaps what we should expect as the background used in these refinements is a flat function. More work is being done to find the best form of background function as it is known that this increases with scattering angle. The procedure most often adopted is to take off an empirical background from the measurements before refinement (Rietveld, 1969), but as a next step, a function such as that used by Windsor & Sinclair (1976) should be considered.

The  $R$  factors given in Table 1 differ from those results with Rietveld's (1969) program in that the background is not subtracted and the differences over the whole of the scan are included:  $R_w = \sum_{\text{scan}} w(y^{\text{obs}} - y^{\text{calc}})^2$ ,  $R = \sum_{\text{scan}} |y^{\text{obs}} - y^{\text{calc}}| / \sum_{\text{scan}} y^{\text{obs}}$ ,  $w$  is the weight given to one point on the scan, the value of the intensity at that point being  $y^{\text{obs}}$ . Unit weights have been used throughout in this work – this is not as bad a choice as it is in single-crystal work as the powder method relies more on a small number of intense reflections. In both refinements scans of equal length, from  $2\theta = 9.5$  to  $94.4^\circ$ , were used.

One reason for giving all the parameter values and errors in Table 1 and the correlation matrix in Table 2 is to present a complete set of data at a time when we are not yet able to perform satisfactory significance tests. It would be best if we could simply apply Hamilton's (1965) test of significance on the  $R$  factors for the ordered and disordered result, but it is not at present possible to find the number of degrees of freedom for this test. A peak has a full width at half height of about 9 scan points and there are 850 points in the scan ( $0.1^\circ$  intervals of  $2\theta$ ); thus we may argue that the number of reflections contributing to our refinement is about  $M = 850/9 = 94$ .

Hamilton (1965) shows that

$$\mathcal{R} = R(\text{ordered})/R(\text{disordered})$$

is distributed as

$$\left( \frac{v_d - v_o}{M - v_d} F + 1 \right)^{1/2}$$

where  $v_d = 22$  and  $v_o = 20$  are the number of parameters in the disordered and ordered refinements respectively, and  $F$  is  $F$ -distributed with  $v_d - v_o$  and  $M - v_d$  degrees of freedom. The value of  $\mathcal{R}$  must be compared with  $6.1/5.7 = 1.07$  from the refinements. Calculated values for  $\mathcal{R}$  for different probability levels are given in Table 3, along with values using  $M/2$ ,  $2M$  and  $4M$  for comparison. The values for the suggested value of  $M$  indicate that the result we have obtained could be accidental with a chance of 1 in 1000. This

Table 3. *Points of the  $\mathcal{R}$  distribution*

$M$  = number of observations. The numbers of parameters used are  $v_o = 20$  and  $v_d = 22$ .

$M$	25%	10%	5%	1%	0.1%
376	1.0029	1.0046	1.0059	1.0089	1.0140
188	1.0062	1.0099	1.0126	1.0191	1.0303
94	1.0144	1.0231	1.0295	1.0451	1.0726
47	1.0422	1.0686	1.0888	1.1406	1.2382

would seem a very reasonable result, but it is clear that considerable experience must be gained before we can make such tests with confidence.

The standard deviations that are given in Table 1 are probably over-optimistic as they are calculated with the total number of reflections used in the calculation, namely 266. If the number used were 96, all standard deviations would be larger by  $[(266 - 22)/(94 - 22)]^{1/2} = 1.85$ , using  $v_d = 22$ .

## Conclusion

This work shows that the neutron powder-profile refinement procedure with appropriate constraints is capable of discovering the amount of disorder in the  $\text{C}_2\text{I}_4$  system at 4 K. At this temperature one molecule is ordered and the other 21% disordered, whereas at room temperature the disorder is probably 50% on both molecular sites. The significance of this result is discussed and is considered to be beyond doubt. This result is in accordance with a suggested  $R$ -factor ratio test.

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## The Crystal Structure and Conformation of the Cyclic Dipeptide *cyclo*(-L-Seryl-L-histidyl-) Monohydrate

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$C_9H_{12}N_4O_3 \cdot H_2O$  is orthorhombic, space group  $P2_12_12_1$  with  $a = 8.162$  (3),  $b = 23.191$  (7),  $c = 5.756$  (4) Å,  $Z = 4$ . The final  $R = 0.036$ . The diketopiperazine ring is planar. The two side chains are folded above the central ring:  $\chi_1^1 = 69.8^\circ$  for the seryl and  $\chi_2^1 = 62.2^\circ$  for the histidyl residues. The conformation is different from that of *cyclo*(-L-threonyl-L-histidyl-) but looks more like the conformation of *cyclo*(-L-seryl-L-tyrosyl-). Such a doubly folded conformation was found to be the most stable from empirical calculations and, from NMR studies, is partly populated in aqueous solutions.

### Introduction

As part of a study of short peptides with polar side chains, we present the structure of the cyclic dipeptide *cyclo*(-L-Ser-L-His-) monohydrate (Ser = seryl, His = histidyl). Recently, we determined the structure of *cyclo*(-L-Thr-L-His-) dihydrate (Thr = threonyl) (Cotrait, Ptak, Busetta & Heitz, 1976), which allows comparison of their conformations.

The seryl and histidyl residues associated with an aspartyl residue are functional in the active site of many serine proteases, e.g.  $\alpha$ -chymotrypsin (Birktoft & Blow, 1972). The catalytic properties of some synthetic peptides (Kapoor, 1972) considered as model enzymes might also involve interactions between such residues. Cyclic dipeptides are used here as models to investigate the side-chain–side-chain interactions between two neighbouring residues. Our crystallographic studies were made in parallel with NMR studies in solution (Ptak, Heitz & Dreux, 1977) and empirical calculations of intramolecular energy (Genest & Ptak, 1976), in order to give a complete view of the conformational properties of short peptides containing functional polar residues.

### Experimental

*cyclo*(-L-Ser-L-His-) was prepared by cyclization of *Z*-dipeptide methyl esters by catalytic hydrogenation. It crystallized in the neutral form from water–acetone solutions as thin colourless needles.

The cell constants were deduced from Eulerian angle measurements for 15 reflections made with a Siemens four-circle diffractometer. The calculated density is  $1.476 \text{ g cm}^{-3}$  if one water molecule per dipeptide molecule is assumed (formula  $C_9H_{12}N_4O_3 \cdot H_2O$ ). This is appreciably higher than that for *cyclo*(-L-Thr-L-His-) dihydrate ( $1.32 \text{ g cm}^{-3}$ ). The intensities of 1150 reflections ( $\theta < 70^\circ$ ) were collected by  $\theta$ – $2\theta$  scans and the five-point measurement technique with Cu  $K\alpha$  radiation ( $\lambda = 1.5418 \text{ \AA}$ ). No absorption corrections were applied.

The structure was solved by direct methods with a program similar to that described by Germain, Main & Woolfson (1971). An  $E$  map with the set of phases having the highest figure of merit displayed most of the atoms. Missing atoms and the O atom of the water molecule were located by a Fourier synthesis. The structure was refined first with isotropic, then aniso-