By replacing $P_s(|\delta|)$ in (A11) with $P_s(|y|)$ from (A5) and (A6), one may verify the expressions for $R_{\alpha\beta}$ derived in equation (3) of the main text for the case of twinning by hemihedry.

An especially striking feature of these results is the deleterious effect of perfect twinning on the R factor for a correct structure model even at low values of N: for $N = 2$, the theoretical minimum values for R are 0·50 and 0·63 for non-centrosymmetric and centrosymmetric data, respectively.

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The Effect of Crystal Bending on Direct Phasing of Electron Diffraction Data from Cytosine

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Abstract

Limiting conditions for the crystal structure analysis of organics using electron diffraction intensity data from elastically bent microcrystals are shown for a representative aromatic structure, cytosine, $C_4H_5N_3O$. In a projection down the longest unit-cell axis, the normalized structure-factor magnitudes are greatly changed by slight bends, making the diffraction data useless for crystal structure analysis. This alteration of intensity is less severe for a projection down the shortest cell axis and allows a correct structure analysis for bends comparable to those measured experimentally. The correct crystal orientation, moreover, is only achieved by epitaxial growth and not solution growth.

Introduction

As effects due to crystal bending are routinely observed in experimental electron microscopy, their consequence for the crystal structure analysis from electron diffraction data should be considered. Direct phase determination has been successfully applied to electron diffraction intensity data from an orthorhombic paraffin and a cephalin (Dorset & Hauptman, 1976) to elucidate the aliphatic chain packing. The effect of n-beam dynamical scattering on the success of this phasing procedure has been recently reported (Dorset, Jap, Ho & Glaeser, 1979). Two organic structures, anhydrous cytosine and disodium 4-oxopyrimidine-2-sulphinate hexahydrate, were used to investigate the dependence of correct phasing on crystal thickness and electron beam energy. Dynamical structure factors from crystals up to about 75 Å thick, at 100 kV, gave correct kinematical phases and yielded correct crystal structures.

As shown by Cowley (1961), elastic bends also can appreciably affect diffraction patterns, altering both intensities and the apparent symmetry of the zone. This has been demonstrated with experimental data from several long-chain paraffinic materials (Dorset, 1979; Dorset, 1980). Since solvent-grown crystals of these materials have a long unit-cell edge parallel to the

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incident beam, the coherently scattering unit appears to be the repeat of the methylene 'subcell' packing of the zig-zag chains; contributions from other parts of the structure to the electron scattering are negligible.

The purpose of this paper is to extend the understanding of bending effects in simple organic materials; in particular their relation to the elucidation of the correct structure using direct methods. Anhydrous cytosine is used as a representative aromatic molecular crystal, considered here to find the effect of crystal bending on diffraction from structures with unit-cell dimensions smaller than the paraffinic materials already examined.

Crystal structure

The structure of anhydrous cytosine has been determined by X-ray crystallography. Cytosine crystallizes in space group $P2_12_12_1$ with lattice parameters a = 13·041 (2), b = 9·494 (1) and c = 3·815 (1) Å (Barker & Marsh, 1964). There are 32 nonhydrogen atoms in the unit cell. Solution-grown crystallites result in 0*kl* electron diffraction patterns. All zonal projections are centrosymmetric (*pgg*) so that the possible phases are limited to 0 and π.

Crystal bending

Cowley (1961) has proposed a model to describe the effects of crystal bending on kinematic diffraction intensities. For a crystal with the incident electron beam along the z axis, and equal bending in the xy plane [described by a Gaussian distribution $g_i(p)$], the resultant intensities are given by

$$I(\mathbf{s}) = \sum_i W_i(\mathbf{s}) \exp(2\pi i \mathbf{r}_i . \mathbf{s}) \exp(-\pi^2 s^2 z_i^2/\sigma^2). \quad (1)$$

$W_i(\mathbf{s})$ is the scattering factor corresponding to the Patterson peak of weight $w(\mathbf{r}_i)$, σ is the inverse of the crystal bending in radians and z_i is the z coordinate of the ith Patterson peak. The half-width of the Gaussian distribution $g_i(p)$ is proportional to the amount of bending.

$$g_i(p) = (\sigma/\pi^{1/2} r_i) \exp(-\sigma^2 p^2/r_i^2), \quad (2)$$

where p is the distance along the arc of the smeared-out Patterson peak. The contribution of a Patterson peak to the diffraction intensity for the *hk*0 zone depends upon its z coordinate, requiring a three-dimensional Patterson synthesis. Thus, for structures with large dimensions in the **c** direction, intensities of *hk*0 reflections may be significantly changed by crystal bending. Conversely, the modification of intensities due to bending will be smaller in zones where the projection is along a short unit-cell direction. (This consideration of crystal bending is valid only in the kinematic approximation and is independent of crystal thickness. The more accurate consideration of dynamical diffraction from bent deformed crystals is the subject of current work in our laboratory.)

The number of reflections used in the model calculations, 65 for the 0*kl* zone and 239 for the *hk*0 zone, corresponds to a sin θ/λ cut-off at 0·77 Å^{-1}. The atoms were treated as point scatterers with no thermal motion, and hydrogen atoms were neglected. The scattering factors utilized were those of Doyle & Turner (1968). Calculations based on (1) were carried out for bending up to 10°.

The use of direct phasing procedures requires normalized structure factors E_{hkl} (Karle & Hauptman, 1956). These may be defined by

$$E^2_{hkl} = \frac{F^2_{hkl}}{\langle F^2_{hkl} \rangle}. \quad (3)$$

Evaluation of the expectation value of F^2_{hkl}, $\langle F^2_{hkl} \rangle$, is usually based on the assumption of randomly distributed atoms. This may be a poor approximation, and in fact if information about the structure is included $\langle F^2_{hkl} \rangle$ will change (Main, 1975). What we can obtain directly from our intensities are the quasi-normalized structure factors ξ_{hkl} corresponding to the point-atom structure. In space group $P1$ when all atoms are assumed to be randomly positioned $|\xi_{hkl}|^2 = |E_{hkl}|^2$ (Main, 1975). The $|E_{hkl}|$ values have been normalized using the theoretical property that the average value of $|E_{hkl}|^2$ = 1·000, and are then input to the direct phasing programs.

When crystal bending is considered, reflections which are normally extinct in space group $P2_12_12_1$ have non-zero intensity. These few reflections have been removed for the subsequent direct phasing. Table 1

Table 1. *Number of large* $|E_{hkl}|$ *values*

Bending	*hk*0 zone			0*kl* zone		
(°)	$\|E\| > 2$	$2 > \|E\| > 1·5$	$1·5 > \|E\| > 1·0$	$\|E\| > 2$	$2 > \|E\| > 1·5$	$1·5 > \|E\| > 1·0$
0	10	12	35	2	5	7
2·5	10	11	38	1	4	5
5·0	6	10	50	1	2	6
7·5	6	11	49	1	1	7
10·0	4	16	48	1	1	8

summarizes the number of large $|E_{hkl}|$ values at several bend angles and representative reflections are plotted in Figs. 1 and 2.

Several features may be readily seen. As the bending is increased the number of high $|E_{hkl}|$ values generally decreases, which will make direct phasing more difficult. Not all $|E_{hkl}|$ values decrease with increasing bend; some are very insensitive while others actually increase. This reordering of $|E_{hkl}|$'s will also hinder structure solution. There are very few large $|E_{hkl}|$ values for the 0*kl* zone, as in this projection many atoms overlap.

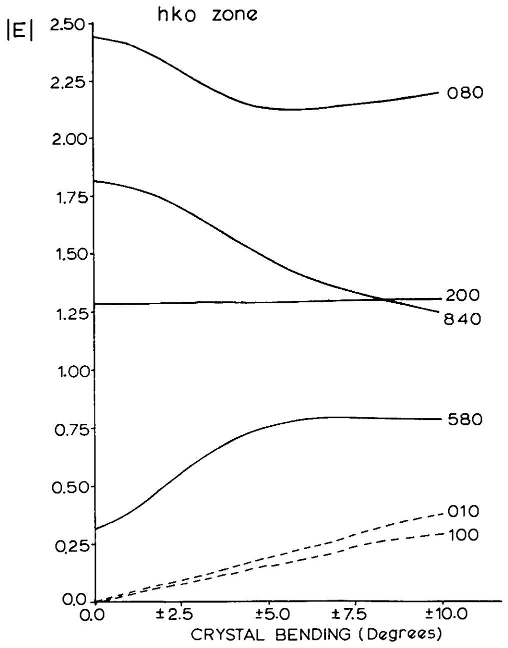

Fig. 1. Normalized structure factors for the *hk*0 zone of cytosine as a function of the degree of bending.

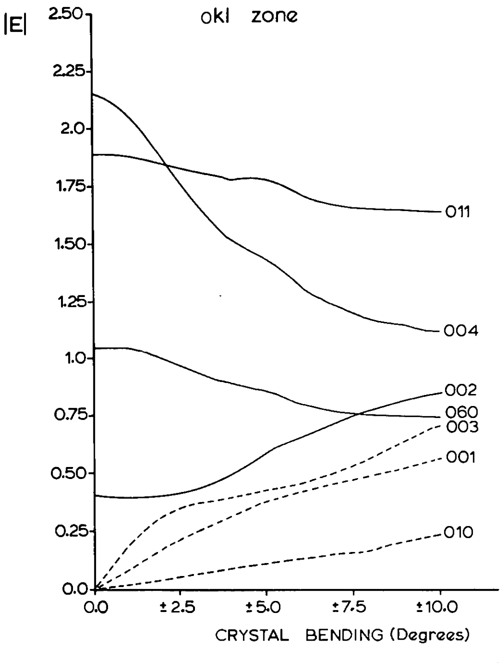

Fig. 2. Normalized structure factors for the 0*kl* zone of cytosine as a function of the degree of bending.

Direct phasing procedure

The direct phasing program *QTAN* (Langs & DeTitta, 1975) was used to estimate the cosine values of the three-phase invariants (Hauptman, 1972). Two reflections, which are not structure invariants or do not combine to form a structure invariant (Hauptman, 1972) are assigned arbitrary phases to define the unit-cell origin. The figure of merit NQEST (DeTitta, Edmonds, Langs & Hauptman, 1975) was used to predict the most probable solution. A large negative value of NQEST is likely to correspond with a correct phase solution but the calculated phases and E maps were also examined to ascertain the presence of a correct structure. In cases where a probable solution could not be found, the four- and five-phase relationships (Hauptman, 1980) were also used.

It proved impossible to obtain a consistent set of phases for the 0*kl* zone, even with no bending. This is a particularly unpropitious case as there is considerable non-eclipsing overlap of atoms in the (100) projection. The *hk*0 zone is more favorable, since the corresponding zone axis is nearly perpendicular to the aromatic rings and there are considerably more $|E_{hkl}|$ values which can be used for phasing. The results are summarized in Table 2. For the case of a flat crystal, and that bent by 5°, it was straightforward to obtain the correct solutions, although they did not have the lowest NQEST values of the sets of possible phases that were generated. The corresponding E maps showed the four cytosine molecules with no spurious peaks (Fig. 3). With the 7·5° bending data all the original sets of possible phases were incorrect. The most likely choice had 29 of the 70 phases wrong but the E map showed what appeared to be four cytosine molecules and a few extra peaks (Fig. 4). In this case the modification of E's due to bending has given rise to a plausible, but wrong, solution with the molecules incorrectly arranged within the unit cell. Inclusion of further phase ambiguities led to a solution that was essentially correct. It had the lowest NQEST value of all solutions generated and gave rise to an E map with no inexplicable peaks. However, changes in the starting set of E's, for example by reducing the sin θ/λ cut-off for reflections to 0·70 Å^{-1}, made elucidation of the correct structure more difficult. With the amount of bending further increased to 10° it was not possible to generate the correct phase set or a recognizable Fourier representation. Inclusion of four- and five-phase

Table 2. *Results of direct phasing for hk*0 *zone*

Bend (°)	Number of $\|E_{hkl}\|$ values used	Minimum value of $\|E_{hkl}\|$ used for calculating triples	Number of incorrect phases	NQEST figure of merit	Is Fourier map recognizable and correct?
0	70	0·93	0	−0·412*	yes
5·0	70	0·94	0	−0·064*	yes
7·5	70	0·97	29	−0·212	no†
7·5	70	0·97	1	−0·502	yes
10·0	70	0·98	16	−0·702	no

* Not lowest NQEST of phase sets generated.

† Cytosine molecules recognizable but incorrectly packed in the unit cell.

relationships did not improve the result, with the most likely solution generated containing sixteen incorrect phases.

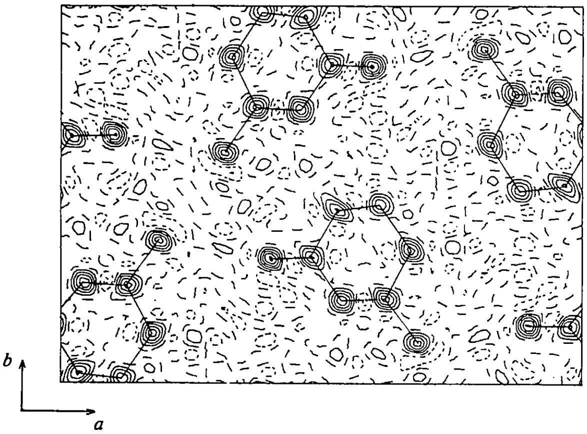

Fig. 3. *E* map calculated for the *hk*0 zone of cytosine from data including 5·0° bending. Correct molecular packing.

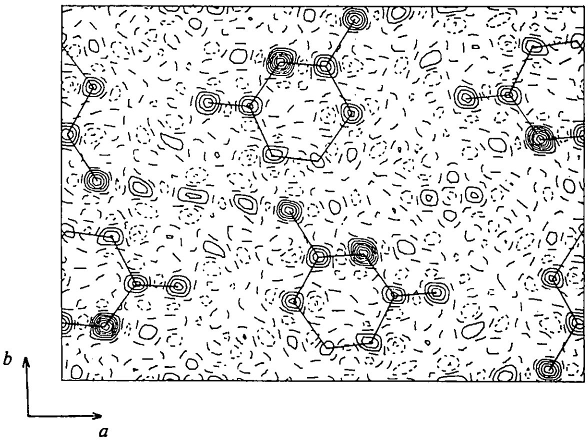

Fig. 4. *E* map calculated for the *hk*0 zone of cytosine from data including 7·5° bending. The molecular packing is incorrect.

Discussion

It is again shown that the direct phasing of electron diffraction data can be seriously affected by crystal bending, leading one to consider the optimum conditions for obtaining usable diffraction data. Zones with the smallest unit-cell dimension in the beam direction will be less perturbed by bending than other projections. Practically speaking, this demands an epitaxial crystal growth for specimen preparation, since normal growth from solution generally ensures that the longest unit-cell axes will be perpendicular to the largest crystal faces (Jensen, 1970).

Fortunately, the appropriate epitaxial growth conditions have been realized for various aromatic systems including phthalocyanines (Uyeda, Kobayashi, Suito, Harada & Watanabe, 1972; Murata, Fryer & Baird, 1976), fused ring compounds (Fryer, 1978, 1979), and linear polymers (Wittman & Manley, 1978; Wittman & Lotz, 1981). High-resolution, low-dose electron microscopy on these materials has already shown the outline of the molecular images. Since the zonal projection onto the aromatic rings will give diffraction patterns with nearly radial intensity distribution, ensuring a suitable number of large $|E|$ values per atom in the asymmetric unit, the electron diffraction data from these materials should be quite usable for crystal structure analysis, for example by direct phasing, using conditions which are optimal from standpoints of least perturbation and adequate number of data. This would enable the structural determinations to be extended to atomic, rather than molecular, resolution.

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Combination of Convergent-Beam Electron Diffraction and 1 MeV Structure Imaging in a Structure Determination of $Na_2Ti_9O_{19}$

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Abstract

The space group and atom configuration of monoclinic $Na_2Ti_9O_{19}$ have been studied by combining 1 MeV high-resolution electron microscope imaging with the convergent-beam electron diffraction (CBED) technique. The crystal has monoclinic symmetry with lattice parameters $a = 12{\cdot}2$, $b = 3{\cdot}78$, $c = 15{\cdot}6$ Å and $\beta = 105°$. The space group was determined uniquely to be centrosymmetric $C2/m$ by selecting the point group from the symmetry of the CBED pattern. On the basis of the 1 MeV structure-image observation reported previously and the space-group determination, the crystal structure of monoclinic $Na_2Ti_9O_{19}$ is proposed and atom coordinates are given.

The high-voltage high-resolution electron microscope working at NIRM, Japan, provides a very powerful method to determine a crystal structure on the basis of structure images taken along two or more principle directions, in which each cation site is resolved (Horiuchi, Kikuchi & Goto, 1977; Bando, Watanabe, Sekikawa, Goto & Horiuchi, 1979; Bando, Watanabe & Sekikawa, 1979, 1980; Matsui, Kato, Kimizuka & Horiuchi, 1979). The possible space groups have been selected by extinction rules obtained from normal electron diffraction. However, the question still remains whether the crystal is non-centrosymmetric or centrosymmetric, since both space groups have the same Bragg conditions.

To distinguish between centrosymmetric and non-centrosymmetric space groups, the convergent-beam electron diffraction (CBED) method is very useful (Goodman, 1975; Buxton, Eades, Steeds & Rackham, 1976; Tanaka, Saito & Watanabe, 1980). A combination of structure imaging and CBED should then be very successful for determining unknown crystal structures. Bando, Sekikawa, Yamamura & Matsui (1981) have tried to combine 1 MeV high-resolution electron micrographs with CBED patterns to determine the crystal structure and space groups of $Ca_4YFe_5O_{13}$.

In a previous paper (Bando, Watanabe & Sekikawa, 1979), the crystal structure of monoclinic $Na_2Ti_9O_{19}$ was studied on the basis of 1 MeV high-resolution electron micrographs coupled with normal electron

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