

Table 4. Calculated triplet phases ($^{\circ}$) of GaAs 222 for different polarized incident beams; δ_T is the exact triplet phase

	$\bar{1}53$	$\bar{1}3\bar{1}$	$\bar{1}\bar{1}1$	$\bar{5}33$	$\bar{1}\bar{1}1$	$\bar{5}13$
	$3\bar{3}\bar{1}$	$3\bar{1}3$	331	$7\bar{1}\bar{1}$	131	$7\bar{1}\bar{1}$
δ_T	97	-72	97	-66	-74	98
$\delta_E(\sigma + \pi)$	98	-64	96	-69	-72	99
$\delta_E(\sigma)$	86	-65	82	-72	-76	102

chosen. Most surprisingly, the Th_{π} , not valid for calculating peak intensity (at $\Delta\varphi = 0$), gives reasonably good agreement with the experimental curves.

In conclusion, we have demonstrated experimentally how the beam polarizations affect multiple diffraction intensities and patterns. According to Juretschke (1986), the intensity asymmetry of a multiple diffraction profile may be reversed due to the influence of the dominant π -polarized waves under some special circumstances, for example the four-beam case, $\bar{1}3\bar{1}$ and 513 of Ge 222. In the present study, we have not encountered this situation for Ge and GaAs. It is, however, anticipated that with different crystals and wavelengths the predicted π -polarization effect could be encountered. Further experiments with well collimated synchrotron radiation are suggested for more precise measurement on multiply diffracted intensities of linearly polarized X-rays.

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Note added

Two papers (Alexandropoulos, McWhan, Juretschke & Kotsis, 1990; Schwegle, Hümmer & Weckert, 1990) on similar experiments with syn-

chrotron radiation were reported at the 15th IUCr Congress, Bordeaux, France, 1990. The anomalous asymmetries of N -beam diffraction profiles have been encountered.

References

- ALEXANDROPOULOS, N. G., MCWHAN, D., JURETSCHKE, H. J. & KOTSIS, K. (1990). *Acta Cryst.* **A46**, C416.
 CATICHA-ELLIS, S. (1969). *Acta Cryst.* **A25**, 666-673.
 CHANG, S. L. (1982). *Phys. Rev. Lett.* **48**, 163-166.
 CHANG, S. L., HUANG, M. T., TANG, M. T. & LEE, C. H. (1989). *Acta Cryst.* **A45**, 870-878.
 CHANG, S. L. & TANG, M. T. (1988). *Acta Cryst.* **A44**, 1065-1072.
 CHAPMAN, L. D., YODER, D. R. & COLELLA, R. (1981). *Phys. Rev. Lett.* **46**, 1578-1581.
 HØIER, R. & AANESTAD, A. (1981). *Acta Cryst.* **A37**, 787-794.
 HÜMMER, K. & BILLY, H. W. (1982). *Acta Cryst.* **A38**, 841-848.
 HÜMMER, K., WECKERT, E. & BONDZA, H. (1989). *Acta Cryst.* **A45**, 182-187.
 JURETSCHKE, H. J. (1982a). *Phys. Rev. Lett.* **48**, 1487-1489.
 JURETSCHKE, H. J. (1982b). *Phys. Lett.* **92A**, 183-185.
 JURETSCHKE, H. J. (1986). *Phys. Status Solidi B*, **135**, 455-466.
 LIPSCOMB, W. N. (1949). *Acta Cryst.* **2**, 193-194.
 MO, F., HAUBACK, B. C. & THORKILDSEN, G. (1988). *Acta Chem. Scand. Ser. A*, **42**, 130-138.
 MOON, R. M. & SHULL, C. G. (1964). *Acta Cryst.* **17**, 805-812.
 POST, B. (1977). *Phys. Rev. Lett.* **39**, 760-763.
 PRAGER, P. R. (1971). *Acta Cryst.* **A27**, 563-569.
 RENNINGER, M. (1937). *Z. Phys.* **106**, 141-176.
 SCHWEGLE, W., HÜMMER, K. & WECKERT, E. (1990). *Acta Cryst.* **A46**, C417.
 SHEN, Q. & COLELLA, R. (1988). *Acta Cryst.* **A44**, 17-21.
 TANG, M. T. & CHANG, S. L. (1988). *Acta Cryst.* **A44**, 1973-1078.
 ZACHARIASEN, W. H. (1965). *Acta Cryst.* **18**, 705-710.

Acta Cryst. (1991). **A47**, 510-515

Electron Diffraction Structure Analysis of Diketopiperazine – a Direct Phase Determination

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This paper is dedicated to Professor B. K. Vainshtein to celebrate his award of the Ewald Medal by the International Union of Crystallography

Abstract

Three-dimensional electron-diffraction intensity data from crystalline textures of diketopiperazine (2,5-

piperazinedione) published by B. K. Vainshtein in 1955 [*Zh. Fiz. Kim.* (1955), **29**, 327-344] are found to be suitable for *ab initio* structure analysis via conventional direct phase determination based on the

estimates of three- and four-phase structure invariants. Of the 289 non-zero reflections found in this data set, 196 with $|E| \geq 0.5$ were used to generate triples and quartets to phase 133 reflections correctly. The resultant [001] and [100] zonal electrostatic potential maps reveal an easily identifiable molecular structure and the atomic coordinates are close to the values found in an X-ray crystal structure.

Introduction

Of the three principal radiations used for scattering experiments intending to resolve interatomic distances, high-energy electrons are generally perceived to produce experimental data most often in need of complicated structure-based corrections. This is because matter scatters electrons very efficiently and, given their small wavelength, the simultaneous excitation of many beams must be reckoned with for any crystal orientation. Although these multiple-beam corrections are required as part of the interpretation of intensity data (Cowley, 1981), significant progress has been made in the visualization of inorganic structures in recent years (Spence, 1981). Similar claims can be made for thin macromolecular crystals, *e.g.* a two-dimensional array of an integral membrane protein such as bacterial porins (Jap, Downing & Walian, 1990; Sass *et al.*, 1989), for which structures can be resolved to *ca* 3.0 Å.

The application of electron-scattering techniques to small-molecule crystal-structure determination has perhaps not gained the same legitimacy for structural crystallographers as it has in the fields mentioned above, despite the fact that electron diffraction is widely used in polymer physics (Perez & Chanzy, 1989; Brisse, 1989), because of the sample crystallization difficulties. This is despite the fact that the quantitative interpretation of electron intensity data from organic microcrystals dates back to the early work of Rigamonti (1936) on paraffins and includes an intensive effort in the Soviet Union on a wide variety of molecular compounds.

Perhaps the suspicion that crystal-structure analyses based on electron-diffraction intensity data are not possible begins with well known comments in Heidenreich's (1964) book on electron microscopy where it was surmised that dynamical interactions may make the measurement of near kinematical structure-factor magnitudes very difficult and that the phasing of the measured amplitudes would thus be a more uncertain process than experienced in X-ray crystallography. Another difficulty lies in the fact that many of the early structure determinations carried out in the Soviet Union were on compounds for which the X-ray crystal structures were already known and, indeed, the atomic positions from these structures were often used to phase the electron-diffraction structure-factor magnitudes to allow the computation

Table 1. Distributions of $|E_h|$ for diketopiperazine

	Experimental	Theory	
		Centro-symmetric	Non-centro-symmetric
$\langle E_h ^2 \rangle$	1.000	1.000	1.000
$\langle E_h^2 - 1 \rangle$	0.995	0.968	0.736
$\langle E_h \rangle$	0.787	0.798	0.886
% $ E_h > 1.0$	29.9	32.2	36.8
% $ E_h > 2.0$	4.7	5.0	1.8
% $ E_h > 3.0$	0.3	0.3	0.01

of electrostatic potential maps. Since it is well known (Ramachandran & Srinivasan, 1970) that a correct set of crystallographic phases applied to permuted structure-factor amplitudes results in a Fourier map with a recognizable crystal structure, this early work unfortunately failed to answer the skeptical criticism of many other structural crystallographers that the measured electron diffraction intensity data *by themselves* may not be sufficient for an *ab initio* structure analysis.

One of the most complete three-dimensional electron-diffraction data sets ever collected from an organic crystal was the texture intensity pattern from diketopiperazine published by Vainshtein in 1955. In his electrostatic-potential maps, the atomic positions are very clearly defined but, again, the phases had been obtained from a previously determined X-ray crystal structure (Corey, 1938). It will be shown in this paper that the data recorded by Vainshtein are sufficient for a structure analysis which presumes no foreknowledge of the molecular packing or geometry.

Data and methods

Three-dimensional intensity data measured from texture electron-diffraction patterns of diketopiperazine (2,5-piperazinedione) by Vainshtein (1955) were used for this determination. Of the 318 reflections listed in his Tables 1 and 2, 289 are observed. The observed structure-factor magnitudes $|F_{hkl}^{\text{obs}}|$ were derived from the observed intensities I_{hkl}^{obs} by

$$|F_{hkl}^{\text{obs}}| = [I_{hkl}^{\text{obs}} / (d_{hkl} d_{h0l} p)]^{1/2}$$

where d_h are interplanar distances and p a multiplicity factor. Inclusion of d_{h0l} accounts for the platy texture of the azimuthally distributed crystallites. Among the 'observed' reflections are three I_{0k0} values not accessed by the sampling of reciprocal space (since they lie near the texture axis) which are estimated from the previous X-ray determination.

The monoclinic space group of diketopiperazine is $P2_1/a$ with $Z = 2$ (*i.e.* one-half molecule per asymmetric unit). Unit-cell constants determined by Vainshtein and converted to ångström units by Degeilh & Marsh (1959) are $a = 5.20$, $b = 11.45$, $c = 3.97$ Å, $\beta = 81.9^\circ$. It should be noted that only the extinction conditions for $h0l$ reflections could be observed in

Table 2. Phase values found from structure invariants generated for diketopiperazine

<i>hkl</i>	$ E $	φ	φ_v^\dagger	<i>hkl</i>	$ E $	φ	φ_v^\ddagger
020	0.86	0	0	183	0.94	π	π
040	1.12	π	π	193	1.40	π	π
060	0.54	π	π	1,10,3	0.55	π	π
110	0.76	0	0	470	1.10	0	0
160	1.19	π	π	490	1.66	0	0
200	0.95	π	π	510	1.05	0	0
210	0.89	π	π	520	2.21	π	π
220	1.57	π	π	560	1.13	0	0
250	1.88	0	0	600	1.32	0	0
260	0.61	0	0				
280	1.53	0	0	001	0.63	π	π
290	2.20	π	π	011	0.41	0	0
2,10,0	1.78	0	0	021	0.31	π	π
310	0.13	π	0, π^\ddagger	051	0.85	π	π
330	1.45	π	π	071	0.93	π	0*
360	1.46	0	0	111	2.86	π	π
370	2.42	0	0	121	0.84	0	0
380	1.18	0	0	151	1.56	0	0
390	2.10	0	0	$\bar{1}11$	1.05	0	0
420	0.57	0	0	$\bar{1}31$	1.50	0	0
440	0.98	0	0	$\bar{1}71$	1.76	π	π
				$\bar{1}81$	1.35	π	0*
201	0.68	π	π	$\bar{1}91$	2.12	π	π
241	0.55	0	0	$\bar{1},10,1$	1.71	π	0*
271	0.67	0	0				
$\bar{2}01$	0.61	π	π	$\bar{4}31$	2.16	π	0*
$\bar{2}21$	1.34	0	0	$\bar{4}51$	0.97	0	π^*
$\bar{2}41$	1.89	0	0	$\bar{4}61$	1.09	0	0
$\bar{2}61$	1.31	π	π	$\bar{5}11$	0.76	π	π
$\bar{2}81$	2.76	π	π	611	1.05	0	0
$\bar{2}91$	0.76	0	π^*	631	0.90	0	0
311	1.19	0	0	002	0.57	π	π
321	0.67	0	0	022	0.87	π	π
331	0.58	0	0	042	1.05	π	π
341	0.63	π	π	062	0.54	0	0
361	1.30	π	π	072	0.53	0	0
391	1.31	π	π	082	1.90	0	0
3,10,1	2.89	0	0	092	1.22	0	0
$\bar{3}81$	1.52	0	π^*	0,10,2	0.82	0	0
$\bar{3},10,1$	1.46	0	π^*	172	0.61	π	π
441	1.15	0	0	$\bar{1}32$	1.59	π	π
451	1.29	π	π	$\bar{1}52$	0.98	π	π
471	1.31	π	π	$\bar{1}72$	2.38	0	0
481	1.47	π	0*	$\bar{1}82$	1.19	π	0*
401	1.68	π	π	$\bar{1}92$	1.52	0	0
411	1.78	π	0*	$\bar{1},10,2$	1.05	π	0*
421	1.61	π	π	202	1.99	0	0
				222	1.18	0	0
272	0.55	0	0	232	1.23	π	π
$\bar{2}72$	1.23	π	0*	242	1.00	π	π
$\bar{2}92$	1.59	π	0*				
312	0.72	0	0	262	1.14	π	π
$\bar{3}12$	2.79	0	0	183	0.64	0	π^*
$\bar{3}22$	2.38	0	π^*	$\bar{2}03$	2.08	π	π
$\bar{3}52$	3.80	π	π	$\bar{2}23$	1.65	π	π
$\bar{3}62$	1.84	π	0*	$\bar{2}33$	0.83	π	0*
402	1.02	π	π	$\bar{2}63$	1.18	0	0
452	1.20	0	0	313	1.42	π	π
492	1.12	π	π	323	1.51	0	0
402	1.33	0	0	343	0.76	0	0
442	1.65	π	π	$\bar{3}13$	1.15	π	π
562	1.71	0	0	423	1.20	0	0
$\bar{5}12$	1.03	π	π	513	1.44	0	0
023	0.69	0	0	$\bar{1}14$	1.97	0	0
043	1.64	0	0	$\bar{1}54$	0.89	π	π
063	0.38	π	π	284	1.20	0	0
083	2.06	π	π	$\bar{2}04$	0.73	0	0
093	1.27	0	0	005	0.91	π	π
153	1.01	0	0				
173	1.52	π	π				

$^\dagger \varphi_v$ phase values determined by Vainshtein from X-ray structure coordinates.

$^\ddagger 0, \pi$ depending on whether H-atom positions used.

the electron-diffraction data since the monoclinic *b* axis lies near the texture axis. To determine the space group unequivocally from electron-diffraction data, one would have to obtain another crystal orientation, e.g. by epitaxial growth, to visualize the absences for the $0k0$ reflections.

Normalized structure factors are calculated from F_{hkl}^{obs} in the usual way, i.e.

$$|E_h|^2 = |F_h|^2 / \varepsilon \sum_i f_i^2,$$

where ε is a multiplicity factor (with a value of 2 for $h0l$ and $0k0$ reflections) and f_i are Doyle-Turner (1968) electron scattering factors. A Wilson (1942) plot of the observed data indicates that the overall isotropic temperature factor is $B = 0.0 \text{ \AA}^2$; hence no thermal parameter was applied to the calculation of $|E_h|$'s.

Direct phasing of the normalized electron-diffraction data was carried out using estimates of three- and four-phase structure invariants in space group $P\bar{1}$ (Hauptman, 1972; Hauptman & Green, 1976). In the former case, most triplet invariants used were of the Σ_2 type such that

$$\psi = \varphi_{h_1} + \varphi_{h_2} + \varphi_{h_3}$$

and $h_1 \neq h_2 \neq h_3$. One positive Σ_1 triple was also used. For the phase determination Σ_2 triples were listed in a hierarchy of *A* values where

$$A = k |E_{h_1} E_{h_2} E_{h_3}|$$

where $k = 2\sigma_3 / \sigma_2^{3/2}$ and $\sigma_n = \sum_{j=1}^N Z_j^n$ and Z_j^n is the scattering-factor value at $(\sin \theta) / \lambda = 0$. The larger values of *A* are usually associated with triple relationships with the highest probability of being correct. Quartet relationships were similarly arranged according to the magnitudes of

$$B = (2/N) |E_{h_1} E_{h_2} E_{h_3} E_{h_4}| \times (|E_{h_1+h_2}|^2 + |E_{h_2+h_3}|^2 + |E_{h_1+h_3}|^2 - 2).$$

Results

Using all the 318 *hkl* data listed in Vainshtein's paper it was first of interest to compare the distribution of computed $|E_h|$ values with the ones expected, respectively, for centrosymmetric or non-centrosymmetric structures in X-ray crystallography (Karle, Dragonette & Brenner, 1965). As is shown in Table 1, the experimental data are in close agreement with the centrosymmetric distribution expected for space group $P2_1/a$.

Next, three-dimensional Σ_2 triples were generated. After direct phase determination, it was found that, of the 146 invariants generated with $A \geq 2.0$, only two were incorrect. Similarly, of the 92 quartets generated with $B \geq 2.0$, two erroneous relationships were found. Three of the erroneous invariants were found to

involve the $\bar{1}11$ reflection and the other incorrect triple the $\bar{2}21$ reflection. These discrepancies could be resolved by the predominance of other relationships expressing the correct phase value. For the phase determination of some zonal reflections, 27 triples and 15 quartets were also generated as well as four three-dimensional negative quartets. One Σ_1 triple ($A=2\cdot 1$) was accepted to define $\varphi_{222}=0$.

Direct phasing was carried out manually after partially defining the origin with the phase values $\varphi_{312}=0$, $\varphi_{111}=\pi$. (A third value was not necessary.) After initially using symbolic values for some reflections, which were later defined during the progress of the phase determination, the phase values for 133 reflections were unambiguously assigned. These are listed in Table 2 and compared to the values given by Vainshtein. Note that there are 18 discrepancies between this direct phase determination and these values. There are no discrepancies if his atomic coordinates are used to calculate structure factors. The difference may be due to the electron scattering factors used in the earlier work (Vainshtein, 1964) which are slightly different from the values calculated by Doyle & Turner (1968). Of the 96 reflections with

$|E_h|\geq 1.0$, only seven are not assigned phase values by direct methods.

Although the first direct phasing was carried out with a data set incorporating the $0k0$ structure-factor values estimated by Vainshtein, one must, naturally, ask whether it is possible to attempt the structure analysis without these data. It would be preferable to do so, since they were not measured experimentally. This is particularly important in this case since φ_{040} is found to interact in many structure-invariant relationships. With the same origin-defining reflections it was found that an initial phase set of 93 values could be obtained and that the electrostatic potential maps are again similar to the ones calculated with the previously determined phase set, as shown below.

After completion of the phase determination, potential maps were calculated for two major unit-cell projections, containing the longest axis, with phased F_{hk0}^{obs} and F_{0kl}^{obs} structure-factor values from either of the two phase sets described above. As seen in Figs. 1 and 2, the molecular geometry is immediately recognizable. If the zonal maps are used to determine

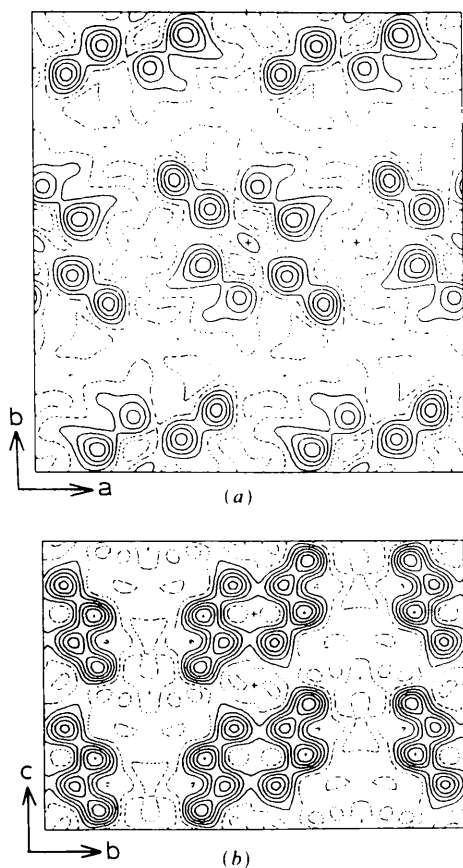


Fig. 1. Electrostatic potential maps of diketopiperazine calculated from $|F_{hk0}^{\text{obs}}|$ zonal data phased by direct methods. (a) [001] projection, (b) [100] projection.

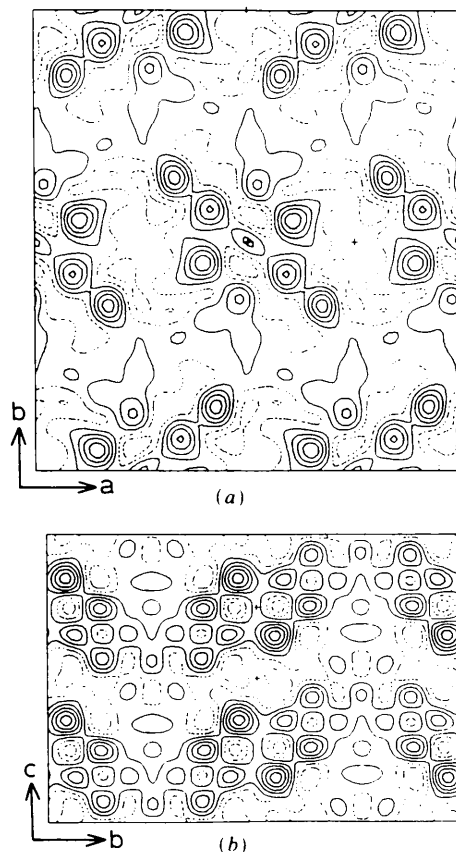


Fig. 2. Electrostatic potential maps calculated from zonal data with phase values determined after unmeasured $0k0$ reflections were removed from the data set. (a) [001] projection, (b) [100] projection.

Table 3. Fractional coordinates of unique atoms in diketopiperazine

Atom	This determination			Previous electron diffraction*			X-ray structure†		
	x	y	z	x	y	z	x	y	z
C1	-0.175	0.072	0.708	-0.181	0.072	0.708	-0.1820	0.0697	0.7170
C2	0.051	0.120	0.502	0.045	0.120	0.505	0.0515	0.1233	0.5150
N	0.212	0.049	0.308	0.221	0.047	0.310	0.2198	0.0432	0.3098
O	-0.345	0.130	-0.138	-0.338	0.120	-0.113	-0.3311	0.1328	-0.0956

* Vainshtein (1955).

† Degeilh & Marsh (1959).

atomic fractional positions, one finds a reasonably close match to the X-ray crystal structure of Degeilh & Marsh (1959) as shown in Table 3. Bond distances and angles calculated from these atomic coordinates are also chemically reasonable values (Fig. 3).

If the coordinates from this determination are used to calculate structure factors, then the match to the observed data is $R = 0.34$ or $R = 0.32$ if non-zero $|F_{\text{obs}}|$ are used. The coordinates listed by Vainshtein, which are based on the potential map using F_{hkl} phased from the X-ray crystal structure, generate another F_{hkl}^{calc} set with a slightly better fit to the observed data ($R = 0.30$). If H-atom positions suggested by Vainshtein are added, $R = 0.26$ for all data and $R = 0.25$ for non-zero data.

Discussion

From the results described above, it is apparent that electron-diffraction data from organic microcrystals can be suitable for *ab initio* crystal-structure analysis, yielding results similar to those obtained by X-ray crystallography, although, certainly, not quite as accurate as those when the diffraction data are free of perturbations due to multiple scattering. The direct phasing progressed in a fashion similar to an equivalent analysis of X-ray diffraction data and, as will be shown in a number of other publications from this laboratory, this phasing procedure appears to have a universal application to electron crystallographic determinations. Sometimes it can also be supplemented by high-resolution electron-microscope images. It is apparent, therefore, that many negative criticisms of pioneering electron-diffraction determinations have been unwarranted; it was not

correct to question the validity of these data just because they were phased from an existing X-ray crystal structure.

Although these data conform well enough to the kinematical scattering approximation to allow a correct structure determination, there are still problems which need to be resolved. For example, the isotropic temperature factor used for the atoms, $B = 0.0 \text{ \AA}^2$, is not physically meaningful for such a structure, especially when thermal motion was a refined parameter in the X-ray crystal structure (Degeilh & Marsh, 1959). From our experience, such an unrealistically low temperature factor is a sign of multiple-scattering effects (Dorset, 1976) which, on average, tend to suppress the relative scattering amplitudes of low-angle reflections. This multiple scattering can be coherent in the form of n -beam dynamical scattering (Cowley & Moodie, 1959) or incoherent in the form of so-called 'secondary' scattering (Cowley, Rees & Spink, 1951). Multiple-scattering effects can often be corrected for in data from single crystals. However, compensation for such perturbations of the intensity data relies on a preliminary knowledge of the crystal structure and crystal habit. It is, therefore, even more difficult to make this correction if a number of crystal orientations is included in the sampled specimen area (Turner & Cowley, 1969).

The challenge to electron crystallographic analyses, therefore, lies not in the determination of a nearly correct structure (presuming that care is taken to optimize experimental parameters) but in the refinement of such structures. In this example, refinement of the temperature factor was unsuccessful, as implied above. Calculation of Fourier maps based on the complete three-dimensional data did not improve the structure by yielding better atomic coordinates. Conventional unconstrained least-squares refinement was not tried, since previous experience indicates that an actual compensation for the multiple-scattering effects can lead to unreasonable bond lengths and angles.

To summarize this paper, it is apparent that the pioneering work of Vainshtein and his co-workers on the electron-diffraction structure analysis of organic compounds often led to chemically realistic results. This work must not be ignored and it should serve as an impetus to improve the procedures for using polycrystalline data for *ab initio* structure determina-

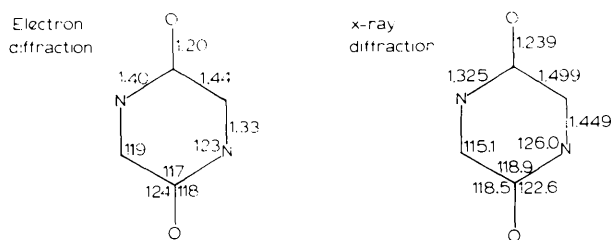


Fig. 3. Comparison of bond distances and angles determined by electron-diffraction structure analysis of diketopiperazine with the previous X-ray structure of Degeilh & Marsh (1959).

tion, especially given the greater possibilities proffered by modern electron microscopes which operate at higher accelerating voltages and have well designed goniometer stages for sample orientation. Although the determination of crystal structures already solved by X-ray methods may seem pointless, one should be reminded that many interesting materials cannot be crystallized sufficiently for X-ray data collection. Hence this 'benchmark' comparison with a known structure emphasizes the utility of electron crystallography for yielding reasonably accurate structural geometries.

References

- BRISSE, F. (1989). *J. Electron Microsc. Tech.* **11**, 272-279.
 COREY, R. B. (1938). *J. Am. Chem. Soc.* **60**, 1598-1604.
 COWLEY, J. M. (1981). *Diffraction Physics*, 2nd ed. Amsterdam: North-Holland.
 COWLEY, J. M. & MOODIE, A. F. (1959). *Acta Cryst.* **12**, 360-367.
 COWLEY, J. M., REES, A. L. G. & SPINK, J. A. (1951). *Proc. Phys. Soc. London Sect. A*, **64**, 609-619.
 DEGEILH, R. & MARSH, R. E. (1959). *Acta Cryst.* **12**, 1007-1014.
 DORSET, D. L. (1976). *Acta Cryst.* **A32**, 207-215.
 DOYLE, P. A. & TURNER, P. S. (1968). *Acta Cryst.* **A24**, 390-397.
 HAUPTMAN, H. A. (1972). *Crystal Structure Determination, the Role of the Cosine Seminvariants*. New York: Plenum.
 HAUPTMAN, H. & GREEN, E. A. (1976). *Acta Cryst.* **A32**, 45-49.
 HEIDENREICH, R. D. (1964). *Fundamentals of Transmission Electron Microscopy*, pp. 202-203. New York: Interscience.
 JAP, B. K., DOWNING, K. H. & WALIAN, P. J. (1990). *J. Struct. Biol.* **103**, 57-63.
 KARLE, I. L., DRAGONETTE, K. S. & BRENNER, S. A. (1965). *Acta Cryst.* **19**, 713-716.
 PEREZ, S. & CHANZY, H. (1989). *J. Electron Microsc. Tech.* **11**, 280-285.
 RAMACHANDRAN, G. N. & SRINIVASAN, R. (1970). *Fourier Methods in Crystallography*, pp. 62-66. New York: Interscience.
 RIGAMONTI, R. (1936). *Gazz. Chim. Ital.* **66**, 174-182.
 SASS, H. J., BÜLDT, G., BECKMANN, E., ZEMLIN, F., VAN HEEL, M., ZEITLER, E., ROSENBUSCH, J. P., DORSET, D. L. & MASSALSKI, A. M. (1989). *J. Mol. Biol.* **209**, 171-175.
 SPENCE, J. C. H. (1981). *Experimental High-Resolution Electron Microscopy*. Oxford: Clarendon Press.
 TURNER, P. S. & COWLEY, J. M. (1969). *Acta Cryst.* **A25**, 475-481.
 VAINSHTEIN, B. K. (1955). *Zh. Fiz. Khim.* **29**, 327-344.
 VAINSHTEIN, B. K. (1964). *Structure Analysis by Electron Diffraction*, pp. 124, 402. Oxford: Pergamon Press.
 WILSON, A. J. C. (1942). *Nature (London)*, **150**, 151-152.

Acta Cryst. (1991). **A47**, 515-521

The Direct Determination of Phase Invariants Provided by Diffraction Data Measured at Two Different Temperatures

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In memory of David Harker

'He had bought a large map representing the sea,
 Without the least vestige of land:
 And the crew were much pleased when they found it to be
 A map they could all understand.

"What's the good of Mercator's North Poles and Equators,
 Tropics, Zones, and Meridian Lines?"
 So the Bellman would cry: and the crew would reply
 "They are merely conventional signs!"

(Louis Carrol, 'The Hunting of the Snark')

Abstract

A procedure is described for the determination of the crystal structure phase invariants of a compound based on diffraction data measured at two different temperatures. This temperature difference replacement (TDR) technique is shown to provide phase-invariant information from experimentally measured X-ray diffraction data for two different test structures. Although the new method does not appear to be as powerful as single-derivative isomorphous replacement (SIR) phasing, it does appear to be capable of reliably determining a limited number of negative as well as positive phase-restricted invariants for structures containing as many as 300 non-H atoms in the asymmetric unit.

Introduction

Common solutions to the crystallographic phase problem are often based on trigonometric diffraction magnitude relationships among derivative groups of data measured for the same or Friedel-related reflecting planes. These derivative groups can include a native and various isomorphous and anomalous-scattering crystal specimens as well as structures for which a partial molecular-replacement solution may be determined. The determination of non-centrosymmetric phases requires, in principle, a minimum of a native and two derivative data sets for which the underlying derivative substructure can be determined (Bokhoven, Schoone & Bijvoet, 1951; Harker, 1956). In practice the phases of certain reflections may be