

Refinement of Hexamethylenetetramine based on Diffractometer and Imaging-Plate Data

BY JACEK GROCHOWSKI AND PAWEŁ SERDA

*Regional Laboratory of Physicochemical Analysis and Structural Research, Jagiellonian University,
Ingardena 3, 30-060 Cracow, Poland*

AND KEITH S. WILSON AND ZBIGNIEW DAUTER

European Molecular Biology Laboratory, c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany

(Received 14 October 1993; accepted 19 February 1994)

Abstract

Two data sets were collected on single crystals of hexamethylenetetramine (urotropin) using a four-circle diffractometer with Cu $K\alpha$ radiation and an imaging-plate two-dimensional detector with Mo $K\alpha$ source using the rotation method. Both data sets extend to the same limit of $\sin \theta/\lambda = 0.62 \text{ \AA}^{-1}$, corresponding to a resolution of 0.81 \AA . Different processing protocols were employed for the two sets of data. Structure refinements carried out separately with each data set led to equivalent results of comparable accuracy. The imaging-plate scanner was able to provide X-ray data of high quality in a significantly shorter time than the diffractometer.

Introduction

1,3,5,7-tetraazaadamantane ($\text{C}_6\text{H}_{12}\text{N}_4$, hexamethylenetetramine or urotropin) crystallizes in the cubic space group $I\bar{4}3m$ with the cell parameter $a = 7.028(1) \text{ \AA}$. The structure of urotropin was first determined by Dickinson & Raymond (1923) and was later extensively studied by Becka & Cruickshank (1963), Duckworth, Willis & Pawley (1970), Stevens & Hope (1975) and recently Terpstra, Craven & Stewart (1993). As crystals are readily available and possess high but noncentrosymmetric symmetry, they are well suited to act as a standard test for accurate measurement of anomalous dispersion of light atoms such as nitrogen (Grochowski & Serda, 1992). Prior to such studies, a model of the structure, including the charge-density distribution, must be refined. It is also a convenient example for comparing a relatively novel technique of data collection [imaging plate (IP)] with a classic one (diffractometer). The former method of data collection and processing is routinely used in macromolecular crystallography (*e.g.* Dauter, Terry, Witzel & Wilson, 1990) but so far has not been commonly used for small-molecule structure analysis, especially not for so small a unit cell as that of urotropin.

Materials and methods

Commercial urotropin was resublimated under reduced pressure and gave well developed crystals of $\{110\}$ morphology having the shape of a rhombic dodecahedron. For the diffractometer experiment, a sample of 0.25 mm in diameter was mounted on a goniometer head with an additional external 1 mm thin-walled low-absorption capillary to slow down sublimation. The sample size was chosen to fit the homogeneous region of the fine-focus graphite-monochromatized beam on a CAD-4 Enraf–Nonius diffractometer. The copper tube was operated at 45 kV and 20 mA. A primary scan with a speed of $ca 5^\circ \text{ min}^{-1}$ was used to determine the required angular speed for the final scan such that $\sigma(I)/I \leq 0.01$. The slowest scan speed was limited by a preset maximum time of 120 s for measuring a single reflection. For very weak reflections with $\sigma(I)/I > 2$, the primary scan was accepted as final. During the total exposure time (38 h), one intensity control reflection was monitored every 1 h. For the strongest reflections, an attenuator filter was used (factor 23.5). The final scan was done in two opposite directions with double scan speed and the results were compared. Orientation stability was checked using two control reflections every 400 measurements and, if necessary, a reorientation procedure was applied based on a reference set of 25 reflections. Such procedures are routinely used in single-crystal diffractometry. The entire reciprocal sphere up to $150^\circ 2\theta$ was explored. All collected reflections were considered as observed and qualified for further processing with the local data-reduction program *DAREX*. Symmetry-equivalent reflections were merged without merging Friedel pairs. A summary of the data collection and merging is given in Table 1.

The imaging-plate data were collected on a larger crystal of 0.4 mm dimension, mounted in a sealed capillary, to fit the beam size of a normal-focus molybdenum tube powered at 60 kV and 50 mA. The

Table 1. *Details of data collection and refinement*

	Diffractometer	Imaging plate
Sample size (mm)	0.25	0.4
Radiation	Cu $K\alpha$, graphite monochromatized 45 kV, 20 mA	Mo $K\alpha$, graphite monochromatized 60 kV, 50 mA
Total exposure time (h)	38.5	6.5
Total experimental time (h)	43.5	7.5
Maximum resolution (Å)	0.81	0.81
hkl range	-8, 8	-8, 8
Scan direction	$\omega/2\theta$	ω - wide range
Scan range	1.0° + 0.15° $\tan \theta$ for $\theta < 45^\circ$ 1.1° + 0.15° $\tan \theta$ for $45^\circ < \theta < 67^\circ$ 1.3° + 0.25° $\tan \theta$ for $67^\circ < \theta < 70^\circ$ 1.5° + 0.25° $\tan \theta$ for $70^\circ < \theta < 75^\circ$	10° for 20 min exposures at 67 mm 15° for 2 min exposures at 130 mm
Variation of intensity standards	< 1.3%	
No. of reflections measured	1454	2429
No. of weak reflections	41 [$\sigma(I)/I > 2$]	None
Handling of strong reflections	Attenuator	Double distance/exposure time
Unique reflections	84 (52 with Friedels merged)	51 (Friedels merged) Reflection 011 rejected
$R(I)_{\text{symm}}$	0.034 (0.029 with Friedels merged)	0.034 (Friedels merged)
$R(\sigma)$	0.011	0.012
σ estimation	Counting statistics	Pixel-to-pixel statistics, t -plot corrected
Refinement	Full-matrix least squares against F^2 with <i>SHELXL93</i>	Full-matrix least squares against F^2 with <i>SHELXL93</i>
Weighting scheme	$1/[\sigma^2(F_o^2) + (aP)^2 + bP]$, $P = [\max(F_o^2, 0) + 2F_c^2]/3$ $a = 0.035, b = 0.07$	$1/[\sigma^2(F_o^2) + (aP)^2 + bP]$, $P = [\max(F_o^2, 0) + 2F_c^2]/3$ $a = 0.035, b = 0.04$
No. of parameters	15	15
Extinction parameter	0.030	0.075
Discrepancy factors	For all 84 data	With 011 rejected
$wR2$	0.073	0.062
$R1$	0.029	0.034
Goodness of fit	1.37	1.54

radiation was monochromatized by pyrolytic graphite. The instrumental set-up consisted of a Mar Research imaging-plate scanner equipped with a single-axis φ -rotation motor. The scanner was controlled by a VAX 3100 workstation. The rotation method, standard for macromolecular X-ray data collection, was employed (Arndt & Wonacott, 1977).

The flat circular imaging plate of 180 mm diameter placed in front of the crystal, even at the shortest possible crystal-to-plate distance of 67 mm, limited the maximum 2θ angle to 53° ; hence, the shorter wavelength of the molybdenum tube was used to achieve a resolution comparable with that of the diffractometer experiment. The imaging-plate scanner has an intrinsic limitation in dynamic range, resulting from the 16-bit electronic hardware. To overcome this problem, two sets of data were collected with different exposure times to adequately cover the weak and strong intensities. The first was collected at a distance of 67 mm and 10° oscillation with 20 min exposure, the second at a distance of 130 mm with 15° oscillation and 2 min exposure. A total of 180° of rotation was recorded for each set.

The images were processed with the program *DENZO* (Otwinowski, 1992). Only fully recorded reflections were used with no attempt made to utilize the summed intensities of partially recorded reflec-

tions on two successive images. To obtain a sufficient number of reflections recorded on a single image, required to refine the crystal orientation matrix as well as other relevant parameters, a rather wide oscillation range was chosen for this crystal with very small unit-cell dimensions in comparison with protein crystals. This also increased the ratio of fully recorded reflections to partials. All measured reflections were used for subsequent merging, including Friedel pairs, as for Mo radiation the anomalous effect of nitrogen is negligible. The estimation of $\sigma(F^2)$ values for IP data is based on pixel-to-pixel statistics of background area for individual intensity integration. This, however, is not completely equivalent to the counting statistics, as the response of the imaging plate is not necessarily adjusted to 1 analogue-to-digital-converter unit per X-ray quantum. The σ values are corrected *a posteriori* using a t plot and are set to the statistically expected level. The details of the imaging plate data collection are included in Table 1.

Results

(a) Crystal data

Formula $C_6H_{12}N_4$, cubic, space group $I\bar{4}3m$, $Z = 2$, lattice parameter $a = 7.028(1) \text{ \AA}$, $V = 347.13 \text{ \AA}^3$,

$F(000) = 152$, calculated density $D_x = 1.343 \text{ Mg m}^{-3}$, linear absorption coefficient $\mu = 0.71 \text{ mm}^{-1}$ for Cu and $\mu = 0.09 \text{ mm}^{-1}$ for Mo radiation.

(b) Diffractometer measurement

1454 collected intensities were merged to yield 84 unique reflections with $R(I)_{\text{symm}} = 0.034$ and $R(\sigma) = 0.011$, defined as $R(I)_{\text{symm}} = \sum |F_o^2 - \langle F_o^2 \rangle| / \sum \langle F_o^2 \rangle$, summed over all input reflections for which more than one symmetry equivalent is averaged, and $R(\sigma) = \sum \sigma(F_o^2) / \sum F_o^2$, summed over all reflections in the merged list.

The model was refined on F^2 using *SHELXL93* (Sheldrick, 1993), with 15 least-squares parameters (including all independent positional and anisotropic displacement parameters of C, N and H atoms and extinction) against 84 observations (all unique observations). The weighting scheme applied – as suggested by *SHELXL93* to obtain a flat analysis of variance in terms of F_c^2 – was $1/[\sigma^2(F_o^2) + (0.035P)^2 + 0.07P]$, where $P = [\max(F_o^2, 0) + 2F_c^2]/3$. The final discrepancy factor $wR2$ was 0.073 for 84 data and the ‘traditional’ discrepancy factor, calculated using F 's, was $R1 = 0.029$; goodness of fit $S = 1.366$. The Flack x parameter (Flack, 1983) converged to 0.99 with e.s.d. 1.7 and to -0.03 with e.s.d. 1.7 for the inverted structure. This would suggest the opposite polarity for the structure but the result cannot be considered conclusive because of the high standard deviation.

(c) Imaging-plate experiment

2429 observed intensities were merged including Friedel pairs to yield 52 unique reflections with $R(I)_{\text{symm}} = 0.034$ and $R(\sigma) = 0.012$. The model was refined against F^2 using the same program, *SHELXL93*, with 15 least-squares parameters (including extinction), against 51 data – reflection 011 was discarded because the area of the imaging plate where this lowest-resolution reflection occurred was affected by the shadow of the beam stop. The weighting scheme applied was $1/[\sigma^2(F_o^2) + (0.035P)^2 + 0.04P]$. The final discrepancy factor $wR2$ was 0.062 for 51 data and $R1$ was 0.034 for all data and 0.021 for the 49 reflections for which $F_o > 4\sigma(F_o)$; goodness of fit $S = 1.543$.

Discussion

(a) Comparison of intensity measurements

The most important difference between the two methods results from the fact that on the IP a two-dimensional mapping of the reciprocal lattice is recorded. During data processing, integration is carried out on a rectangle of pixels, within which an elliptical contour separates the peak and the background areas. Visualization of a diffraction image

on the IP provides the opportunity to see the reflection shape, satellites and background anisotropy. In single-crystal diffractometry, a one-dimensional profile is recorded and integrated along a chosen scan direction. Consequently, the wider two-dimensional vicinity of a reflection cannot be directly explored.

In the imaging-plate technique, dealing with weak reflections and reflections that are too strong (outside the dynamic range of the IP scanner) requires the use of multiple exposures with different crystal-to-plate distances and recording times. In the longer-exposure data set, there were 122 overloaded intensities, which were all discarded and recorded adequately in the short-exposure series.

With a diffractometer, time is a limiting factor for measurements of very weak reflections and attenuator-filter and dead-time corrections are used to deal with very high intensities. In the diffractometer experiment, no weak reflections were rejected (see Table 1). The 49 strongest reflections were measured with the attenuator filter. A comparison of the weighting schemes indicates that the most intense reflections have smaller weights in the diffractometer experiment than in the IP one.

(b) Blind region

At high resolution in the single-axis rotation method, the so-called ‘blind region’ of the reciprocal lattice, containing reflections (near the rotation axis) that cannot cross the Ewald sphere, is relatively large (Arndt & Wonacott, 1977). In the case of the highly symmetric crystal of urotropin, this effect plays a minor role, owing to the high multiplicity of reflections. It nevertheless decreases the total number of observations in comparison with those recorded on a diffractometer with κ geometry, where the accessibility of reflections is only limited by the collision positions.

(c) Exposure time

Owing to simultaneous recording of reflections, an IP offers a significant decrease in data-collection time in comparison with a single-crystal diffractometer. The larger the unit-cell volume, the greater this time gain. In the present case, the IP exposure was six times shorter than the diffractometer one. The magnitude of this ratio is, however, influenced by such obvious factors as the different crystal sizes, different primary-beam intensities and different radiations used in the two experiments.

(d) Lattice-parameter determination

Because on an imaging plate the scattering angle is not measured directly, the use of an IP for unit-cell-parameter determination requires prior calibration of

Table 2. Comparison of structural parameters with those obtained by other authors

The results of the refinements based on Cu K α diffractometer data and Mo K α diffractometer data are marked as Cu-Dif and Mo-IP, respectively. Cu-BC and Mo-BC denote the results of Becka & Cruickshank (1963) (298 K Cu and Mo); N-DWP refers to the neutron diffraction data of Duckworth, Willis & Pawley (1970); Mo-SH refers to the results of Stevens & Hope (1975) for 0.77 Å resolution; the Terpstra, Craven & Stewart (1993) refinement based on DWP neutron data is identified by DWP-TCS and that based on SH by SH-TCS. A lack of e.s.d.'s means that the corresponding values were assumed.

(a) Fractional atomic coordinates for atoms in the asymmetric unit: C (<i>u</i> , 0, 0); N (<i>v</i> , <i>v</i> , <i>v</i>); H (<i>x</i> , <i>x</i> , <i>z</i>)											
	<i>u</i>	<i>v</i>	<i>x</i>	<i>z</i>							
	0.23853 (33)	0.12225 (17)	0.09088 (157)	-0.31617 (205)	Cu-Dif						
	0.23841 (32)	0.12213 (18)	0.08789 (215)	-0.31721 (215)	Mo-IP						
	0.23783 (97)	0.12322 (77)	0.08855	-0.32751	Cu-BC						
	0.23774 (46)	0.12354 (31)	0.08855	-0.32637	Mo-BC						
	0.2370 (5)	0.1222 (2)	0.0909 (6)	-0.3266 (7)	N-DWP						
	0.2369 (2)	0.1221 (1)	0.0874 (8)	-0.3155 (8)	Mo-SH						
	0.2379 (4)	0.1222 (6)	0.0894 (9)	-0.3229 (11)	DWP-TCS						
	0.23813 (18)	0.12251 (14)			SH-TCS						

(b) Independent components of the thermal displacement tensor (Å^2)											
	$U_{11}(C)$	$U_{22}(C)$	$U_{33}(C)$	$U_{11}(N)$	$U_{12}(N)$	$U_{11}(H)$	$U_{33}(H)$	$U_{12}(H)$	$U_{23}(H)$		
	0.0322 (10)	0.0693 (11)	0.0011 (16)	0.0525 (9)	-0.0091 (7)	0.1043 (113)	0.0458 (88)	-0.0093 (120)	0.0504 (81)		Cu-Dif
	0.0284 (10)	0.0665 (12)	-0.0019 (18)	0.0490 (10)	-0.0093 (6)	0.1073 (130)	0.0529 (95)	-0.0031 (130)	0.0513 (90)		Mo-IP
	0.0298 (32)	0.0648 (40)	0.0	0.0490 (33)	-0.0105 (17)	0.1265	0.0359	0.0069	0.0105		Cu-BC
	0.0324 (14)	0.0646 (18)	0.0	0.0492 (13)	-0.0117 (8)	0.1265	0.0359	0.0069	0.0105		Mo-BC
	0.0258 (15)	0.0648 (15)	0.0003 (25)	0.0491 (8)	-0.0103 (8)	0.1111 (35)	0.0516 (27)	-0.0053 (40)	0.0238 (20)		N-DWP
	0.0304 (3)	0.0690 (1)	-0.0028 (3)	0.0514 (1)	-0.0103 (1)	0.1025 (25)	0.0399 (22)	0.0058 (25)	0.0403 (22)		Mo-SH
	0.0262 (12)	0.0648 (13)	0.0004 (19)	0.0497 (8)	0.0101 (6)	0.0501 (29)	0.1109 (23)	-0.0246 (15)	0.0049 (26)		DWP-TCS
	0.0263 (4)	0.0646 (7)	0.0010 (9)	0.0475 (4)	-0.0110 (4)						SH-TCS

(c) Comparison of determined cell parameters (Å), bond lengths (Å) and angles ($^\circ$)											
	<i>a</i>	C-N	C-H	N-C-N	C-N-C						
	7.028 (1)	1.464 (2)	1.055 (14)	112.2 (2)	108.1 (1)						Cu-Dif
	7.028 (1)*	1.464 (4)	1.034 (15)	112.1 (3)	108.1 (2)						Mo-IP
	7.021 (9)	1.478 (4)	-	113.3 (9)	107.5 (5)						Cu-BC
	7.021 (9)	1.479 (2)	-	113.7 (4)	107.3 (2)						Mo-BC
	7.019	1.474	1.104	113.7	107.3						N-DWP
	7.028 (2)	1.462 (5)	1.071 (7)	112.4 (3)	108.0 (3)						SH-TCS

* The cell parameter for IP refinement was taken from diffractometer measurements.

the crystal-to-plate distance. Moreover, the program *DENZO* used for integration of the intensities refines all parameters (orientation matrix, lattice parameters, distortion corrections *etc.*) necessary to calculate the predicted pattern of reflections and match it with the observed one, on the basis of a single individual oscillation image; in this situation, the resulting values are not always highly accurate. A diffractometer measurement provides values based on direct and more precise measurement of scattering angles. For both structural models, the lattice parameter as refined from diffractometer measurements carried out on several crystals was used.

(e) Refinement

A comparison of the atomic positional parameters (Table 2) indicates that the values refined from the diffractometer and the IP data are in good agreement. Their differences are within one standard deviation. Neither are there any big differences in thermal parameters. The positional parameters reported by Becka & Cruickshank (1963), refined from photographic Cu- and Mo-radiation experiments, differ slightly more from the presently obtained values but are nevertheless internally consistent for Cu and Mo radiations. This is quite remarkable in view of the fact that their experiment was carried out using photographic film and a Weissenberg goniometer 30 years ago. A comparison with structural parameters obtained by other authors employing various methods is made in Table 2.

An extinction correction was introduced (in *SHELXL93*) so that F_c 's were multiplied by $k[1 + 0.001x F_c^2 \lambda^3 / \sin 2\theta]^{-1/4}$, where k is the overall scale factor. The extinction parameter x was refined and converged to 0.03 for the diffractometer data and to 0.075 for the IP data.

A summary of the molecular geometry is given in Table 2(c). Except for the C–H distance, the bond lengths and angles resulting from the two refinements are virtually the same, within half a standard deviation.

Concluding remarks

Equal-resolution diffraction experiments carried out using a four-circle diffractometer and a single-axis imaging-plate scanner resulted in comparable accuracy and precision of refined structural parameters. This proves that an IP scanner could be used successfully for small-molecular crystal data collection. The main advantage of the IP technique is shortening of the exposure time and two-dimensional recording of reflections. The diffractometer offers, on the other hand, a more flexible, albeit slower, exploration of the reciprocal lattice. Based on the proven measuring method, a higher-resolution experiment is being prepared for an imaging plate with larger diameter using Ag radiation.

This work was supported in part by the Polish State Committee of Scientific Research under grant no. 1297/P4/04/94.

References

- ARNDT, U. W. & WONACOTT, A. J. (1977). Editors. *The Rotation Method in Crystallography*. Amsterdam: North Holland.
- BECKA, L. N. & CRUICKSHANK, D. W. J. (1963). *Proc. R. Soc. London Ser. A*, **273**, 435–454.
- DAUTER, Z., TERRY, H., WITZEL, H. & WILSON, K. S. (1990). *Acta Cryst.* **B46**, 833–841.
- DICKINSON, R. G. & RAYMOND, A. L. (1923). *J. Am. Chem. Soc.* **45**, 22–29.
- DUCKWORTH, J. A. K., WILLIS, B. T. M. & PAWLEY, G. S. (1970). *Acta Cryst.* **A26**, 263–271.
- FLACK, H. D. (1983). *Acta Cryst.* **A39**, 876–881.
- GROCHOWSKI, J. & SERDA, P. (1992). *Acta Phys. Polon.* **A82**, 147–156.
- OTWINOWSKI, Z. (1992). *DENZO: a Film Processing Program for Macromolecular Crystallography*. Yale Univ., New Haven, Connecticut, USA.
- SHELDRIK, G. M. (1993). *SHELXL93. Program for Crystal Structure Refinement*. Univ. of Göttingen, Germany.
- STEVENS, E. D. & HOPE, H. (1975). *Acta Cryst.* **A31**, 494–498.
- TERPSTRA, M., CRAVEN, B. M. & STEWART, R. F. (1993). *Acta Cryst.* **A49**, 685–692.