# Detection of Radiation Damage in Organic Crystals*

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*Abstract*

In X-ray diffraction experiments with single crystals, the loss in intensity of Bragg reflections caused by radiation damage can become more severe at higher scattering angle, similar to the effect of increased thermal motion or disorder. Long exposure to X rays can also lead to an increase in the lattice dimensions, similar to the effect of thermal expansion. Both effects are illustrated by observations with 4, 5, 10, 11-tetraoxa-1, 2, 7, 8-tetraazatricyclo[6.4.1.1$^{2,7}$]tetradecane made in connection with a charge density study of this molecule. Recommendations are made for the choice of standard reflections and measurement strategies for accurate crystal structure analysis of radiation sensitive compounds.

## 1. Introduction

Ever since counter diffractometers began to come into general use for single-crystal intensity measurements, it has become normal practice to monitor the stability of the experimental system (X-ray source, crystal, counting device etc.) by remeasuring the intensity of one or a few chosen Bragg reflections at regular intervals throughout the duration of the experiment (see e.g. Stout and Jensen 1968; McCandlish *et al.* 1975). Corrections are then applied to compensate for any slow drift in the intensity of the standard reflections. In particular, slow decomposition of the crystal during the experiment can be detected by a gradual loss in intensity of the standard reflections and appropriate corrections made. To save counting time, crystallographers like to choose the standard reflections from among the most intense ones and, because of the general fall-off in intensity with increasing scattering angle, these are mostly low-order reflections. The confinement of standard reflections to the low-order region rests on the implicit assumption that low-order and high-order reflections will suffer the same proportional intensity loss due to radiation damage. The general validity of this assumption is by no means assured. In fact, protein crystallographers have been aware for years that the effects of radiation damage are more marked at higher scattering angles (Blake and Phillips 1962; Blundell and Johnson 1976).

* Dedicated to Dr A. McL. Mathieson on the occasion of his 65th birthday.

## 2. Preliminary Observations

We recently made low-temperature (96 K) X-ray intensity measurements for crystals of (1) in order to obtain electron density difference maps for the various types of bond in this molecule (Dunitz and Seiler 1983). The compound is obtained by gently warming a mixture of hydrazine, hydrogen peroxide and formaldehyde (von Girsewald and Silgens 1921); we had previously shown (Whittleton *et al.* 1981) that the generally accepted constitution (2) for the reaction product was incorrect and had to be revised to (1). The crystals are triclinic, space group P$\bar{1}$, and contain one centrosymmetric molecule per unit cell:
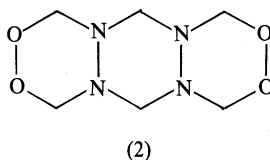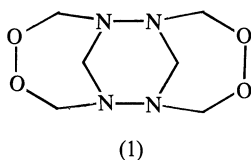


(1)                                          (2)

Table 1.  Changes in cell constants and standard reflections for crystal I during intensity measurements at 96 K extending over about four weeks

|  | Start | Finish | $hkl$ | $\sin\theta/\lambda$ (Å$^{-1}$) | $\Delta I$ (%) |
|---|---|---|---|---|---|
| $a$ (Å) | 6·0657(5) | 6·0718(4) | 120 | 0·25 | −6·6 |
| $b$ (Å) | 6·1349(5) | 6·1424(3) | 300 | 0·28 | −5·4 |
| $c$ (Å) | 6·5752(5) | 6·5818(6) | $\bar{3}80$ | 0·68 | −11·5 |
| $\alpha$ (deg.) | 105·991(6) | 105·977(6) | | | |
| $\beta$ (deg.) | 102·531(6) | 102·579(7) | | | |
| $\gamma$ (deg.) | 112·793(7) | 112·742(5) | | | |
| $V$ (Å$^3$) | 201·865 | 202·570 | | | |

During a first set of measurements (crystal I, with linear dimensions ~0·32 mm) the integrated intensities of three standard reflections were found to fall off by ≈ 1·5–3·0% per week. After about four weeks exposure time (Mo K$\bar{\alpha}$ radiation with graphite monochromator) the intensity decrease for the ($\bar{3}80$) reflection with $s = \sin\theta/\lambda = 0·68$ Å$^{-1}$ amounted to nearly 12%, roughly double the intensity decrease for the other two standard reflections with $s \sim 0·25$–$0·28$ Å$^{-1}$ (see Table 1). It was also noticed that over the four week exposure period the unit cell dimensions had increased slightly (by about 0·1% linearly). These cell dimensions were obtained by least-squares fitting of the setting angles of 22 reflections with $2\theta$ values in the range 56°–64°.

Two other interesting examples of a change of cell dimensions on irradiation to X rays have been reported recently (Ohashi *et al.* 1981, 1982, 1983). These involve solid-state chemical reactions leading to racemization of chiral molecules and accompanied by changes in space group. The changes in cell dimensions observed in these examples are much larger (0·1–0·3 Å) than those observed here.

We could convince ourselves that the changes in intensity and in cell dimensions found for crystal I were not caused by instability of the X-ray equipment or the cooling system. It seems more likely that they were manifestations of crystal damage caused by exposure to X radiation.
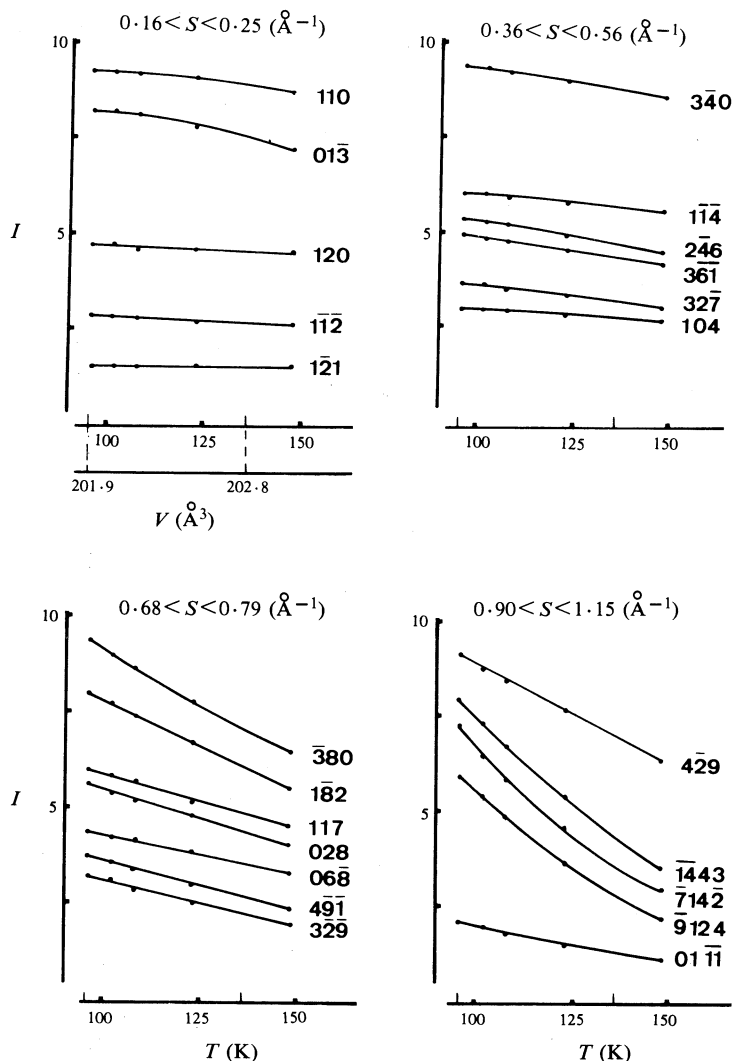
**Fig. 1.** Temperature dependence of intensities of 23 standard reflections measured at the start of the experiment with crystal II. For comparison with Fig. 3 the unit cell volumes at two temperatures (96 and 136 K) are indicated.

At this stage we had measured about 5200 reflections within a limiting radius of $s = 0 \cdot 9 \, \text{Å}^{-1}$. Because of the radiation damage, crystal I seemed to have outlived its usefulness. As we still wished to measure reflections at higher scattering angle, we decided to continue with a fresh, slightly larger crystal II with linear dimensions of $\sim 0 \cdot 4$ mm, beginning the measurements at $s = 1 \cdot 22 \, \text{Å}^{-1}$ and working inwards, i.e. in shells of decreasing length of the scattering vector. With this new crystal we also had the opportunity to investigate the effects of radiation damage more closely.

The procedure of starting the measurements at high scattering angle and working inwards can sometimes have certain advantages. Insofar as the high-order reflections

suffer the greatest intensity loss through radiation damage, they should be measured first in order to obtain an optimal signal-to-noise ratio for the data set as a whole. Moreover, with increasing exposure to the X-ray beam, the reflection profile, as measured say by a $\omega$, $2\theta$ scan, broadens. Such an increase in the 'mosaic spread' of the crystal could just be detected at the end of our experiment and must be connected with an increase in the number of crystal defects. In any case, this broadening of the reflection profile is relatively harmless as far as the low-order reflections are concerned, but it can contribute to an increase in scan truncation error (Alexander and Smith 1962; Denne 1977) and hence to a systematic underestimation of the high-order reflections. To minimize this it is clearly beneficial to measure the high-order reflections first. Also, the loss in crystal perfection due to radiation damage will tend to reduce the extinction error and hence it may actually be advantageous to measure the strong low-order reflections last, as mentioned by McCandlish *et al.* (1975).
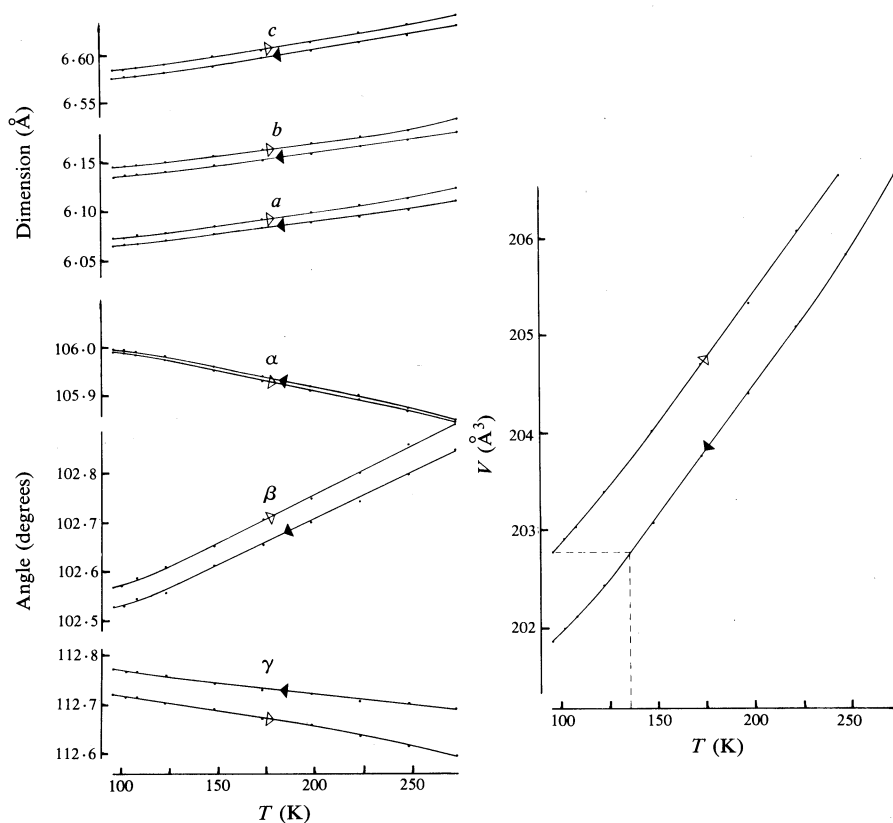


**Fig. 2.** Temperature dependence of unit cell dimensions measured at the start (solid arrowheads) and finish (open arrowheads) of the experiment with crystal II. Results obtained by warming or cooling the crystal are indistinguishable.

## 3. Measurements with Crystal II

Over a period of about six weeks, the intensities of 23 standard reflections distributed between $s = 0 \cdot 16$ and $1 \cdot 15$ Å$^{-1}$ were measured periodically at 96 K. The

intensities of the standard reflections were also measured as a function of *temperature* at the beginning of the experiment (see Fig. 1). In addition, the temperature dependence of the cell dimensions was determined at the start of the experiment and also at its conclusion (Fig. 2), using the same procedure as described for crystal I.
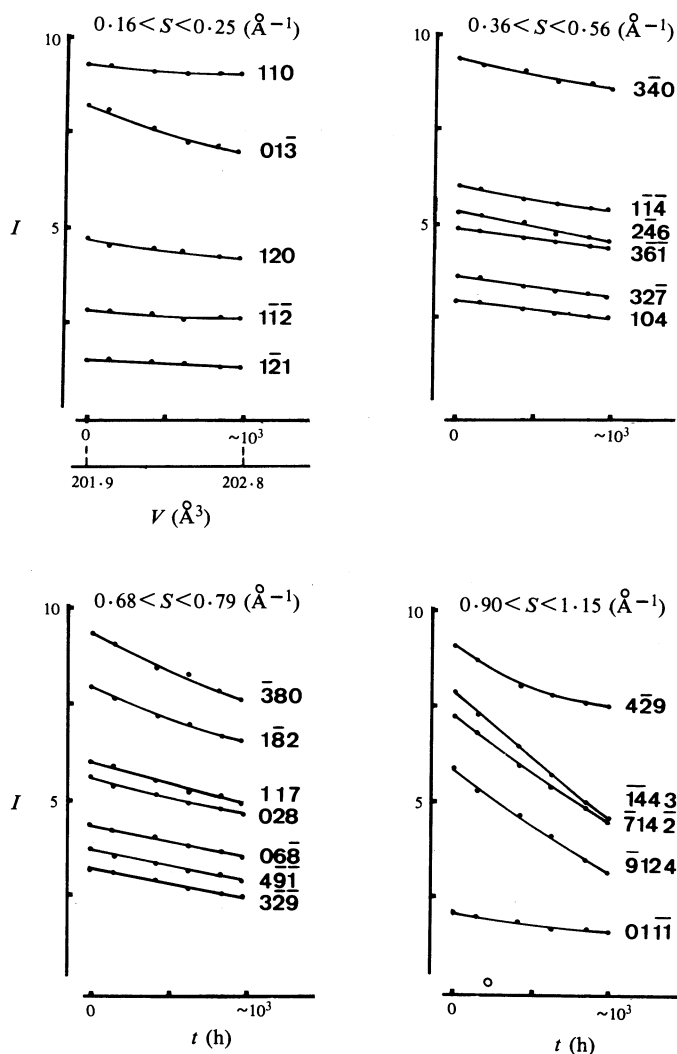


**Fig. 3.** Intensity changes of 23 standard reflections over a six-week period with crystal II at 96 K. The intervals along the abscissa are exposure times required to produce the same $\Delta V$ as produced by the temperatures indicated in Fig. 1.

Fig. 3 shows the behaviour of the 23 standard reflections as a function of radiation time during the measurement period. The differences between low-order and high-order reflections are unmistakable. Thus, for example, while the (110) reflection with $s = 0.16\ \text{Å}^{-1}$ decreases in intensity by only about 4%, the $(\bar{9}, 12, 4)$ reflection

with $s = 1 \cdot 15 \, \text{Å}^{-1}$ suffers an intensity loss of as much as 40%. In general, the proportional intensity fall-off increases with increasing scattering angle. Before we discuss this further, we need to say something about the meaning of the abscissa in Fig. 3.

From Fig. 2 it is clear that radiation damage has led to quite perceptible changes in the cell dimensions. The $a$, $b$ and $c$ axes lengthen by $\sim 0 \cdot 01 \, \text{Å}$ ($\sim 15\sigma$), and the interaxial angles change by up to $0 \cdot 05°$ ($\sim 9\sigma$). The increase in the unit cell volume after six weeks radiation amounts to about 0·6%. As indicated by the dashed line in Fig. 2, this is about the same change in volume $\Delta V$ as would have been produced by warming the fresh undamaged crystal from 96 K to about 136 K, a temperature increment of 40 K. We may therefore regard $\Delta V$ as a function of radiation time $\Delta V = f(t)$ or of temperature $\Delta V = g(T)$. The abscissa of Fig. 3 is scaled so that the time intervals correspond to the effective increases in temperature that would have produced the same $\Delta V$. The comparison with Fig. 1 now shows that for all 23 standard reflections there is a striking similarity between the intensity fall-off with increasing exposure time and with increasing temperature. These observations suggest that the main initial effect of radiation damage is to increase the disorder in the crystal. The resulting change in the X-ray diffraction intensities can be simulated by introducing an additional overall temperature factor which, to a first approximation for this crystal at least, is not too far from isotropic. The effective increase after the six weeks radiation time amounted to about $\Delta B \sim 0 \cdot 4 \, \text{Å}^2$ or $\Delta U \sim 0 \cdot 0050 \, \text{Å}^2$ for the crystal studied.
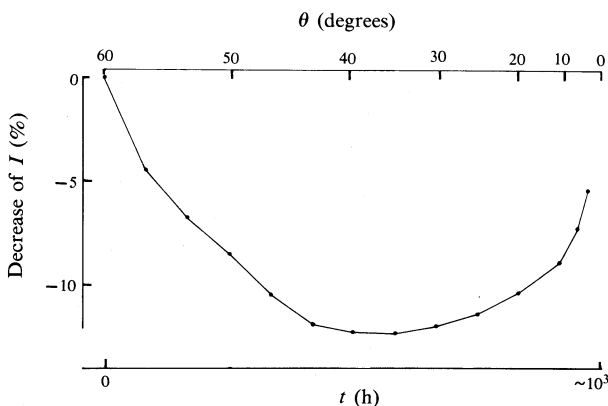


**Fig. 4.** Proportional intensity decrease of crystal II reflections as a function of scattering angle (or exposure time). Scaling of reflection intensities based on this curve gave satisfactory results.

## 4. Conclusions

It is clear that a correction factor for radiation damage which is based only on the intensity fall-off of low-order reflections can lead to serious errors. The main effect on a crystal-structure determination will be to produce artificially large atomic displacement parameters. The correction required in any given case will depend on the measurement strategy. For our crystal, satisfactory results were obtained with

a nonlinear scaling correction where the dependence on scattering angle is explicitly recognized (Fig. 4). In fact, the scale factor and overall temperature factor estimated from three high-order least-squares refinements ($s > 0 \cdot 8$ Å$^{-1}$, $s > 0 \cdot 9$ Å$^{-1}$ and $s > 1 \cdot 0$ Å$^{-1}$) including all reflections with $I > 3\sigma(I)$ agree to within about a standard deviation ($R = 0 \cdot 013$–$0 \cdot 014$). A similar correction, based on the intensity fall-off of the three standard reflections for crystal I, was also applied for that crystal.

Least-squares refinements for crystals I and II using the common reflections out to $s = 0 \cdot 9$ Å$^{-1}$ then gave practically identical results as far as the atomic positional and displacement parameters are concerned ($R_{\text{I}} = 0 \cdot 026$ and $R_{\text{II}} = 0 \cdot 029$). The extinction factor $y$, which is the ratio of the diffracted intensity $P$ to its kinematic value $P_k$, for the three strongest reflections was indeed, as expected, slightly larger (2–3%) for the crystal II measurements in spite of the larger crystal size. It is recommended that where extensive intensity measurements for a radiation sensitive crystal are required, the reflection sphere should be covered beginning at the outside limit and working inwards in shells of diminishing radius.

The chemical changes caused by irradiation of our crystal are still unknown. Ultimately, after long exposure at room temperature a yellowish insoluble, presumably polymeric material is obtained with extensive loss of crystallinity.

## References

Alexander, L. F., and Smith, G. S. (1962). *Acta Crystallogr.* **15**, 983.

Blake, C. C. F., and Phillips, D. C. (1962). Symp. on Biological Effects of Ionizing Radiation at the Molecular Level, p. 183 (International Atomic Energy Agency: Vienna).

Blundell, T. L., and Johnson, L. N. (1976). 'Protein Crystallography', p. 330 (Academic: New York).

Denne, W. A. (1977). *Acta Crystallogr.* A **33**, 438.

Dunitz, J. D., and Seiler, P. (1983). *J. Am. Chem. Soc.* **105**, 7056.

McCandlish, L. E., Stout, G. H., and Andrews, L. C. (1975). *Acta Crystallogr.* A **31**, 245.

Ohashi, Y., Uchida, A., Sasada, Y., and Ohgo, Y. (1983). *Acta Crystallogr.* B **39**, 54.

Ohashi, Y., Yanagi, K., Kurihara, T., Sasada, Y., and Ohgo, Y. (1981). *J. Am. Chem. Soc.* **103**, 5805.

Ohashi, Y., Yanagi, K., Kurihara, T., Sasada, Y., and Ohgo, Y. (1982). *J. Am. Chem. Soc.* **104**, 6353.

Stout, G. H., and Jensen, L. H. (1968). 'X-ray Structure Determination', p. 194 (Macmillan: New York and London).

von Girsewald, C., and Silgens, H. (1921). *Ber. Dtsch. Chem. Ges.* **54**, 492.

Whittleton, S. N., Seiler, P., and Dunitz, J. D. (1981). *Helv. Chim. Acta* **64**, 2614.