Experimental

Before making any deductions we want to describe the procedure used for the collection of 5 Å data from hexon. The experimental arrangement consisted of a Supper precession camera with a Philips fine-focus Cu tube. Monochromation of the radiation was effected by a plane graphite monochromator mounted in a crystal holder built at this institute as a somewhat modified version of a design by Rasmussen (1968). The diameter of the collimator was 0·3 mm, the crystal-to-film distance was 75 mm and the precession angle used for the screenless precession photographs was 1·25°. In order to diminish the splitting of the diffraction spots, the film casette was moved 2·5 mm toward the crystal along the film normal.

The diffraction data to 5 Å resolution have been collected on 19 film sets. It should be noted that because of the cubic space group $P2_13$ in which the hexon crystallizes it should have been possible to collect the unique reflexions on a smaller number of film sets. However, the crystals usually last more than 50 hours before any measurable radiation damage appears and each film set consisted of two photographs with exposure times 5 and 2 hours. This means that it was relatively easy to overdetermine the unique reflexions. With the strategy used, each unique reflexion was on the average recorded on three different film sets. Consequently, errors in individual reflexion measurements were more easily detected than if an optimized data-collection strategy had been used.

In order to make further checks of the three-dimensional cut-off effects two normal layer-line film sets (*hk*0 and *hkk*) with the precession angle 9·08° were included before the intensities were scaled together by the method of Monahan, Schiffer & Schiffer (1967). Only the equations (1) and (2) with $c=0{\cdot}3$ mm were used in order to avoid the integration of intensities form partially recorded reflexions.

A list of 2221 unique reflexions with non-zero intensities obtained from more than one film set was calculated. An inspection of the list showed no serious discrepances between the intensities obtained from the various screenless and normal layer-line film sets. Furthermore, no indications of any correlation between *d* spacings and deviations in integrated intensities were found.

At this stage a recalculation of the partially recorded reflexions was made. The equations

$$c=f\lambda|\zeta_{\max}-\zeta| \qquad (3)$$

and

$$c=f\lambda|\zeta_{\min}-\zeta| \qquad (4)$$

were included in order to extend the equations (1) and (2) for the cut-off effects in the z direction (*cf.* Fig. 2).

The calculations indicated that almost all reflexions with *d* spacings above 10 Å should have been partially recorded if the limit *c* was set to 0·3 mm. Even for a *c* limit equal to 0·1 mm a large number of these reflexions should have been only partially recorded. However, these calculations were clearly inconsistent with the measurements. This conclusion could be drawn not only from comparisons with the 9° normal layer-line precession photographs but also from comparisons between reflexions occurring at more and less favourable positions on different screenless photographs.

Theory

The reason why equations (1) and (2) cannot be extended to cover the three-dimensional situation is that a diffraction-spot size depends on the crystal size and the mosaic spread of the crystal but the cut-off phenomenon is only dependent on the mosaic spread. It is presupposed that the primary beam consists of

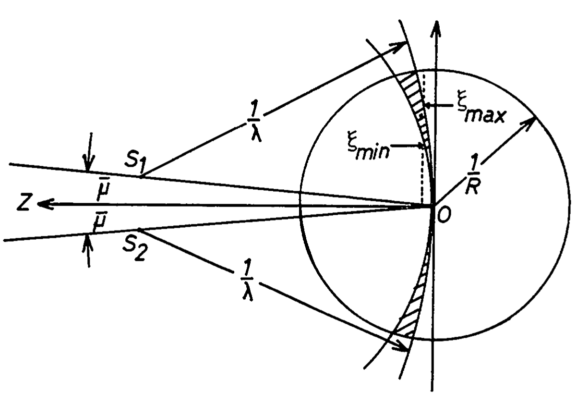

Fig. 1. Section through the origin of the reciprocal lattice drawn to illustrate the region recorded on one exposure with precession angle $\bar{\mu}$. Limit of resolution = R.

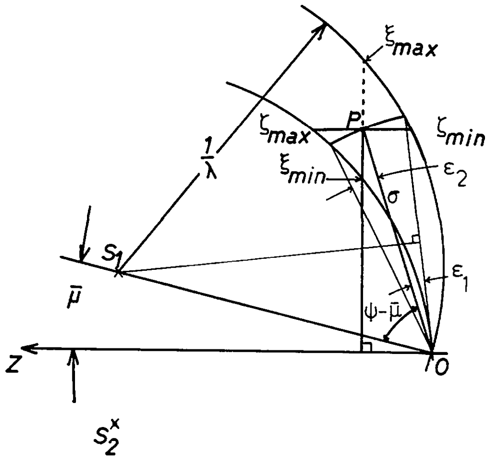

Fig. 2. Diffraction geometry for a recordable reciprocal-lattice point P showing the maximum allowed misalignments ε_1 and ε_2 for the mosaic blocks.

monochromated parallel X-rays. It should be noted that the mosaic spread gives rise to a more concentrated group of reciprocal-lattice points for an *hkl* representing a diffraction vector close to the origin than for a diffraction vector far away from the origin. As pointed out by Xuong & Freer (1971) Fig. 1 can be most easily understood if we consider the crystal and film to be fixed while the X-ray source, and hence the sphere of reflexion, precess about the **z** axis. Each mosaic block in the crystal, however, gives rise to a somewhat misaligned reciprocal lattice. In order to replace equations (1) and (2) by three-dimensionally valid equations we may proceed as follows.

With the notations of Fig. 2 we may write

$$\sigma=(\xi^2+\zeta^2)^{1/2}\,, \tag{5}$$

$$\tan\psi=\xi/\zeta \tag{6}$$

and

$$\cos(\psi-\bar{\mu}+\varepsilon_1)=\lambda\sigma/2. \tag{7}$$

Thus

$$\varepsilon_1=\bar{\mu}-\psi+\arctan[(2/\lambda\sigma)^2-1]^{1/2} \tag{8}$$

and for the corresponding angle ε_2 to the sphere of reflexion centred at S_2

$$\varepsilon_2=2\bar{\mu}-\varepsilon_1. \tag{9}$$

Now, ε_1 and ε_2 must both exceed half the angular mosaic spread of the crystal.

Application and discussion

For the hexon photographs usually less than five unique reflexions have ε_1 or ε_2 less than 0·25° unless the reflexion was already discarded by the conditions given by (1) and (2). In total, 71 unique reflexions having an ε less than 0·25° were measured, of which 53 with non-zero intensities were measured within 12 mm of the film centres on the 19 film sets. All but one of these 71 were also measured on other screenless or normal layer-line film sets. One reflexion with $\varepsilon_{\min}=0{\cdot}21°$ had an integrated intensity significantly less than the corresponding integrated intensities obtained from one normal layer-line and four other screenless film sets. This is the only indication of a possible cut-off in the **z** direction. By use of (1) and (2) with $c=0{\cdot}3$ mm about 350 unique reflexions with *d* spacings greater than 5·0 Å were excluded on each film set. About 10 of these reflexions had $\varepsilon_{\min}$ greater than 0·21°. All the others should also have been excluded if equations (8) and (9) with ε_1 and ε_2 less than 0·21° had been used as the definition of a partially recorded reflexion.

In this particular case the equations (1) and (2) have functioned relatively satisfactorily. However, a small number of reflexions may have been unnecessarily rejected. By use of equations (3) and (4) a large number of completely registered reflexions are needlessly rejected. It should be noted that these results are dependent on the precession angle and the actual spot dimensions. The equations (8) and (9) should be generally valid and independent of these parameters.

It is also an advantage that equations (8) and (9) can be applied without the introduction of the spot-size parameter *c* which is dependent on the shape and size of the actual crystal. As a result of this investigation we also conclude that even reflexions with *d* spacings above 35 Å can be correctly determined from screenless precession photographs obtained with a precession angle as small as 1·25°.

Finally, it may be noted that it is also possible to determine the conditions for partially recorded reflexions on oscillation photographs from the mosaic spread. However, a smaller volume of the reciprocal lattice is exposed on an oscillation photograph when the oscillation angle is the same as the precession angle. Therefore, it might be preferable to use the screenless precession method for low-angle reflexions from macromolecules.

The present investigation has received financial support from the Tri-Centennial Fund of the Bank of Sweden and from the Swedish National Science Research Council. We are indebted to Professor L. Philipson for the crystals used in this investigation. We should also like to express our gratitude to Dr S. Westman for his comments and to Professor P. Kierkegaard for his continuous interest in this work.

References

Franklin, R. M., Harrison, S. C., Pettersson, U., Philipson, L., Brändén, C. J. & Werner, P. -E. (1971). *Cold Spring Harbor Symposia on Quantitative Biology*. Vol. XXXVI, pp. 503–510.

Monahan, J. E., Schiffer, M. & Schiffer, J. P. (1967). *Acta Cryst.* **22**, 322.

Rasmussen, S. E. (1968). Private communication.

Xuong, N. H. & Freer, S. T. (1971). *Acta Cryst.* B**27**, 2380–2387.