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A Crystallographic Examination of 14 Straight Chain Alkyl Esters of Cholesterol

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The unit cell dimensions and space groups of the common *n*-alkyl esters of cholesterol (with the exception of the propanoate) have been determined by single crystal X-ray diffraction studies. No overall structural pattern is evident for this series but there are small groups of esters whose structures appear to form regular sequences. Using X-ray powder photography, the crystalline solid phases of the esters obtained from solution were compared with those obtained by cooling the melt. In the majority of cases there are no significant differences but for the methanoate, ethanoate, butanoate, pentanoate and dodecanoate there appears to be solid state polymorphism.

INTRODUCTION

Although the thermal properties of the cholesteryl esters have been extensively studied, no systematic crystallographic survey has been carried out. Only for isolated esters are crystallographic data available. Using single crystal X-ray techniques, the unit cell dimensions and space groups of cholesteryl dodecanoate and octadecanoate have been determined by Abrahamsson and Selin[1] and those of cholesteryl ethanoate by Schulze.[2] The unit cell dimensions and space group of the tetradecanoate and the octadecanoate have been deduced from the X-ray powder data by Wendorff and Price.[3] No X-ray diffraction data appear to have

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been published for any of the other esters of this series.

The upper temperature limit of stability of a phase is related to the stability of that phase and hence to its structure. A smooth variation of this transition temperature with alkyl chain length for a homologous series of compounds would imply some homology of structure–i.e. a situation where molecular pattern is either essentially the same in all of the structures or alternatively where the pattern changes steadily with chain length. This kind of behaviour is common for mesophase $\rightleftarrows$ isotropic liquid and for mesophase $\rightleftarrows$ mesophase transitions. It occurs sometimes for solid → mesophase transitions for the higher membranes of homologous series, but rarely for the lower members. For some homologous series, a regular alternation of the transition temperatures has been found, where the values lie on two smooth curves, one for the odd members and one for the even–implying that one type of structure is adopted by the old members and a different structure by the even. Examples of these various patterns of behaviour are given by Gray.[4]

The crystal to mesophase transition temperatures and enthalpies of the mesogenic cholesteryl alkanoates have been measured by a number of workers and the more recent experimental data have been collated in a review by Davis and Porter.[5] No overall pattern is evident in the way in which the transition temperatures vary with chain length and the series does not fall clearly into two halves, with one half corresponding to the esters up to C_8 which form cholesteric phases only (or no mesophase at all) and the other corresponding to the esters from C_9 onwards which form both smectic and cholesteric phases. There is an abrupt drop of 20° between C_8 and C_9 but there is also a comparable drop between C_{12} and C_{13}. The only regular feature is an indication of an odd/even effect for the higher esters, i.e. C_{13} to C_{20} but the temperature differences concerned are fairly small and are comparable with the spread of the values obtained by different workers for each measurement.

In contrast with the transition temperature, the enthalpy change at a transition is related to the structures of both phases and thus a smooth variation with chain length would suggest homology of structure of both phases involved. When the crystal → mesophase transition entropies are plotted against chain length (see Figure 1 in the review by Davis and Porter) there is a discontinuity between the octanoate and the nonanoate coinciding with the onset of smectic phase formation, but note that the data do not lie on smooth curves on either side of this point.

Some workers have preferred to discuss transition entropies (rather than transition enthalpies), regarding them as having more structural significance. Davis and Porter have suggested that the plot of these data against the alkyl chain length approximates to two straight lines of the same slope which are offset by about 10 cals/mole/°K, one line ending at the C_8 ester and the other commencing at the C_9 ester. Many of the points lie a long way from these two

lines and the spread of values obtained by different workers is large. The extent to which this assessment of the situation is justified is therefore in doubt.

The principal object of this investigation was to prepare single crystals of common alkyl esters of cholesterol and to determine the unit cell dimensions and space groups by X-ray study. These data should indicate the degree of structural homology in this series. In particular they should show whether there is an abrupt change of molecular arrangement in the crystal structures between the C_8 and the C_9 esters, which could explain the appearance of smectogenic properties at this point.

We shall refer to the esters by the number n where this is the number of carbon atoms in the parent carboxylic acid, i.e. it is the number directly corresponding to the IUPAC nomenclature. It is the number of methylene groups $+^2$; n refers to the ester $CH_3(CH_2)_{n-2}COO\,C_{27}H_{45}$.

Preparation of crystals

Previous workers have used ethanol or an ethanol/acetic acid mixture as a solvent for the preparation of crystalline cholesteryl esters. We have found that n-pentanol (which Davis and Porter[6] have described as a regular solvent for these compounds, in the light of the thermodynamic properties of the solutions) is in many instances preferable, giving larger and better formed crystals. The morphologies of the crystals grown from solution are given below. Unless otherwise stated, the solvent was n-pentanol.

In many cases the crystals grew as thin elongated plates whose crosssections were parallelograms. This shape is referred to as a monoclinic lath and is shown in Figure 1 (a). For some esters the laths have a simple rectangular profile. In

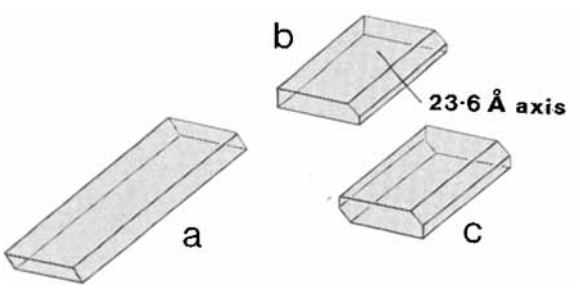

FIGURE 1 The idealised morphologies of the crystals of the cholesteryl alkanoates, grown from solution.

(a) The monoclinic lath shape: the most common crystal morphology of the esters in this series.

(b) The characteristic morphology of cholesteryl butanoate crystals.

(c) The characteristic morphology of cholesteryl octanoate crystals.

the molecules in the unit cell from the cell dimensions and space groups. Bernal *et al.* [11] have used considerations of this type to deduce the molecular arrangements in the crystals of a large number of sterol derivatives (with smaller adherent groups). Where the molecules lie along an axis, for homologous structures this will result in the relevant cell dimension increasing steadily with chain length whilst the other two dimensions remain constant. This appears to be the situation for the two pairs of esters with n = 10 and 12 and n = 14 and 18. Alternatively where the molecules lie parallel to the side of the unit cell, but not parallel to an axis, two dimensions will change and the third will remain constant. This appears to be the situation for esters with n = 6, 7, 8 and 9.

In Figure 3, although there is no overall structural pattern, there are nevertheless certain spacings which occur repeatedly and which are most probably directly related to the dimensions of the molecules. This suggests that the sort of packing considerations used by Bernal *et al.* may be viable for this series of structures. A detailed discussion of this kind of the crystal structures of the individual members of this series will be presented in a separate paper.

The smallest unit cell dimensions lie in the 6Å-7Å range–which presumably correspond to a molecular width. The spacings in the 9Å-10Å range may repre-

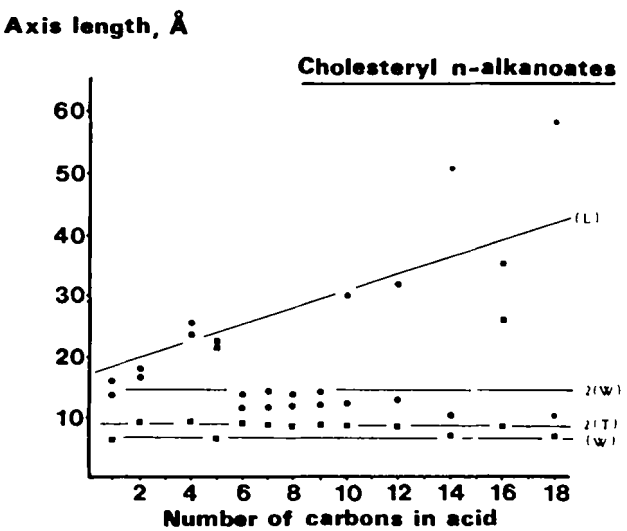

FIGURE 3 The unit cell axial lengths of the cholesteryl *n*-alkanoates, plotted against the total number of carbon atoms in the parent carboxylic acids. Cell dimensions correspondings to 2_1 screw axes in the crystals are indicated by ■. Those which do not correspond to a symmetry axis are indicated by ●. The dimensions of the molecular length (L) and multiples of the molecular width (W) and thickness (T) are indicated.

TABLE 1

Cholesteryl *n*-Alkanoates

n	Ester	Space Group	a (Å)	b (Å)	c (Å)	β (°)	molecules per unit cell	molecules per asymmetric unit	ρ meas. (g/ml)	ρ calc. ‡ (g/ml)
1	METHANOATE	Monoclinic $P2_1$	15.65	6.06	13.53	96.0	2	1	1.051	1.027
2	ETHANOATE	Monoclinic $P2_1$	17.51	9.44	16.4	105.5	4	2	1.056	1.023
4	BUTANOATE	Monoclinic $P2_1$	25.36	9.55	23.60	95.0	8	4	1.037	1.064
5	PENTANOATE	Orthorhombic $P2_12_12_1$	21.45	21.5	6.40	–	4	1	(1.035) †	1.014
6	HEXANOATE	Monoclinic $P2_1$	13.67	9.30	12.19	92.0	2	1	1.034	1.028
7	HEPTANOATE	Monoclinic $P2_1$	14.02	9.23	12.54	92.0	2	1	1.032	1.062
8	OCTANOATE	Monoclinic $P2_1$	13.95	9.20	12.67	94.0	2	1	1.042	1.034
9	NONANOATE	Monoclinic $P2_1$	14.44	9.33	12.81	95.5	2	1	(1.035) †	1.051
10	DECANOATE	Monoclinic $P2_1$	30.00	9.05	12.85	92.0	4	2	1.020	1.001
12	DODECANOATE	Monoclinic $P2_1$	31.80	8.92	12.92	93.0	4	2	(1.035) †	1.023
14	TETRADECANOATE	Monoclinic $P2_1$	50.30	7.50	10.18	92.5	4	2	(1.035) †	1.061
16	HEXDECANOATE	Orthorhombic $P2_12_12_1$	25.53	34.9	9.00	–	8	2	1.066	1.103
18	OCTADECANOATE	Monoclinic $P2_1$	57.50	7.55	10.20	96	4	2	(1.035) †	1.086

† Estimated values for the densities.

‡ Densities calculated from the unit cell columns and the molecular weights.

The estimated errors in the unit cell parameters are of 2% for a, b and c and of 0.5° for the β angles of the monoclinic cells. The estimated errors of the measured densities are 3%. The differences between ρ meas. and ρ calc. lie within the range of experimental errors and do not appear to be significant

densities are listed in Table 1 and estimated values are shown in parentheses. The differences between them lie within the experimental error and we do not regard them as being significant.

X-ray single crystal investigation

Rotation and Weissenberg photographs of the zero and first layers were taken about at least two of the principal crystallographic axes for each crystal. Nickel filtered copper K-radiation was used and the photographs were calibrated with aluminium powder lines. The unit cell lengths were measured from the axial reflection positions and the interaxial angles of the monoclinic crystals from the separation of the axes on the appropriate Weissenberg photographs. The only systematic absences found for this series of compounds were for reflections of odd index along some axes, indicating 2_1 screw axes. The observed cell dimensions and space groups are shown in Table 1. With the exceptions of the pentanoate and hexadecanoate which formed orthorhombic crystals with space group $P2_12_12_1$, all of the esters formed monoclinic crystals with space group $P2_1$ – there is, however, a considerable diversity in the number of molecules per unit cell and this clearly therefore does not imply any widespread structural homology.

In Table 1 the penultimate column gives the measured (or estimated) densities and the final column gives the values of the corresponding quantities calculated from the unit cell volumes and molecular weights, for comparison.

The results of previous crystallographic studies of the cholesteryl esters are summarised in Table 2. The values which we have obtained for the cell dimensions of cholesteryl ethanoate, dodecanoate and octadecanoate agree, to within the experimental error, with the values obtained by Abrahamsson and Selin[1] and Schulze[2], who also used single crystal methods. There is, however, only partial agreement between the values which Wendorff and Price[3] have obtained for the octadecanoate and the values obtained by us and by Abrahamsson and Selin. There is also only partial agreement between Wendorff and Price's data and ours for the tetradecanoate. It is possible that this arises because of solid state polymorphism, but note that Wendorff and Price used trial and error indexing of powder diffraction data which must be regarded as a less certain approach than single crystal methods, where each cell parameter is measured independently. It is perhaps significant that in both cases it is the largest dimension for which there is no disagreement, and it is the parameter which can be estimated with most certainty from powder data.

TABLE 2

Cholesteryl *n*-Alkanoates

n	Ester	Space Group	a (Å)	b (Å)	c (Å)	β (°)	molecules per unit cell	molecules per asymmetric unit		
1	ETHANOATE	Monoclinic $P2_1$	17.5	9.35	16.3	106.4	4	2	Schulze[2]	From single crystal studies
12	DODECANOATE	Monoclinic $P2_1$	32.63	9.02	13.13	91.0*	4	2	Abrahamsson and Selinc[1]	
18	OCTADECANOATE	Monoclinic $P2_1$	55.2	10.57	7.70	96.2	4	2		
14	TETRADECANOATE	Monoclinic $P2_1$	55.5	11.3	7.60	116	4	2	Wendorff and Price[3]	From powder diffraction patterns
18	OCTADECANOATE	Monoclinic $P2_1$	62.8	7.60	11.35	116	4	2		

* These values are expressed in terms of the current conventions. The cell dimensions quoted by Abrahamsson and Selin are a = 34.9, b = 9.02, c = 13.13, $\beta = 110.9°$ corresponding to $10\bar{1}$, $0\bar{1}0$, $00\bar{1}$.

Polymorphism

Solid state polymorphism may be a fairly common feature of the cholesteryl esters. Merritt *et al.*[7] nave found two solid crystalline forms of cholesteryl methanoate and of cholesteryl butanoate using X-ray powder diffraction, positron annihilation and thermal analysis. Barrall and Vogel[8] have reported two crystalline forms of heptadecanoate (a compound which we have not studied) and on the basis of density measurements Price and Wendorff[9] have reported three crystalline forms of cholesteryl ethanoate.

We have used X-ray powder photography in a systematic survey of those esters which we had attempted to examine as single crystals in order to examine whether crystallisation from solution and crystallisation from the melt tend to produce different crystal polymorphs.

Each ester was crystallised from solution in *n*-pentonol, filtered and washed with fresh *n*-pentanol and dried under vacuum at room temperature. The material was lightly ground in a pestle and mortar and loaded into 0.5 mm Lindemann glass tubes for X-ray powder photography with a Debye-Scherrer camera. Filtered Cu *K*-radiation was used. The material was then melted and allowed to recrystallise whilst still in the capillary and re-examined with X-rays. The powder patterns obtained from the esters crystallised from solvent are shown in Figure 2A and those obtained from material recrystallised from the melt are shown for comparison in Figure 2H. The speckled appearance of some of the powder photographs in Figure 2A indicates that the powder is rather too coarse. Rigorous grinding of the material was deliberately avoided because we have found that for at least one cholesteryl derivative, this can initiate a solid $\longrightarrow$ solid phase change.[10]. The samples obtained from the melt were found to be virtually ideal for X-ray powder photography; the crystallites were of suitable size and orientation to give diffraction patterns with sharp continuous lines.

For the esters with n = 3, 6, 7, 8, 9, 10, 14, 16, 18 there appear to be no significant differences between the two sets of photographs in terms of the positions and relative intensities of the lines. For the esters with n = 1, 2, 4, 5 and 12 there do appear to be significant differences, indicating that the different crystallisation processes have produced different crystalline modifications. Note that it is for the esters where n = 1, 2 and 4 that previous workers have observed solid state polymorphism. The lack of overall structural homology is apparent in both sets of powder photographs – although there are limited sequences where the photographs are similar:

(a)	n = 6, 7, 8 and 9	for both series
(b)	n = 10 and 12	
(c)	n = 14, 16 and 18	
(d)	n = 2 and 3	for the melt series only

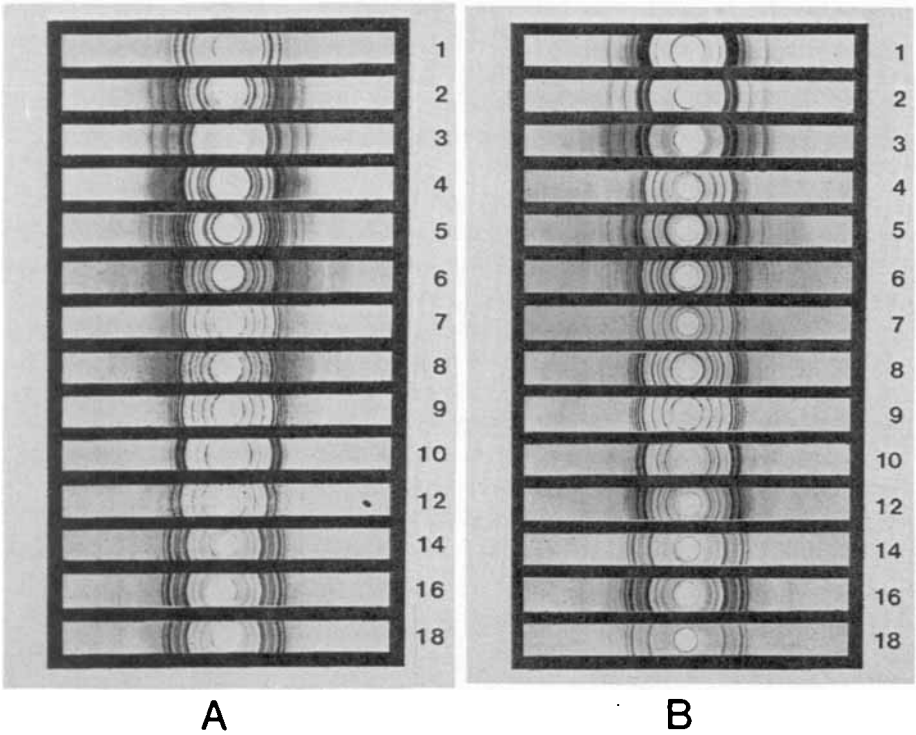

FIGURE 2 X-ray powder photographs of the cholesteryl *n*-alkanoates. The numbers indicate the total number of carbon atoms in the parent carboxylic acids.
(A) Samples obtained from solution in *n*-pentanol.
(B) Samples recrystallised from the melt.

Discussion

The unit cell axial lengths plotted against the alkyl chain lengths are shown in Figure 3. It is evident that there is no overall structural pattern. Nor do the structures fall into two distinct groups corresponding to those esters which form both smectic and cholesteric phases on the one hand and those which form only cholesteric phases, or no mesophase at all, on the other.

The sterol group approximates to a rectangular slab 18Å long, 7Å wide and $4\frac{1}{2}$Å thick. It is usually assumed that the alkyl chains are rigid and fully extended in the solid phase of the cholesteryl alkanoates. If this is the case then the molecules are shaped like flattened Indian clubs where the head is the sterol group and the alkyl chain handle is 4Å by $3\frac{1}{2}$Å in crosssection. For rigid elongated molecules of this type it is possible in many cases to infer the disposition of

the molecules in the unit cell from the cell dimensions and space groups. Bernal *et al.* [11] have used considerations of this type to deduce the molecular arrangements in the crystals of a large number of sterol derivatives (with smaller adherent groups). Where the molecules lie along an axis, for homologous structures this will result in the relevant cell dimension increasing steadily with chain length whilst the other two dimensions remain constant. This appears to be the situation for the two pairs of esters with n = 10 and 12 and n = 14 and 18. Alternatively where the molecules lie parallel to the side of the unit cell, but not parallel to an axis, two dimensions will change and the third will remain constant. This appears to be the situation for esters with n = 6, 7, 8 and 9.

In Figure 3, although there is no overall structural pattern, there are nevertheless certain spacings which occur repeatedly and which are most probably directly related to the dimensions of the molecules. This suggests that the sort of packing considerations used by Bernal *et al.* may be viable for this series of structures. A detailed discussion of this kind of the crystal structures of the individual members of this series will be presented in a separate paper.

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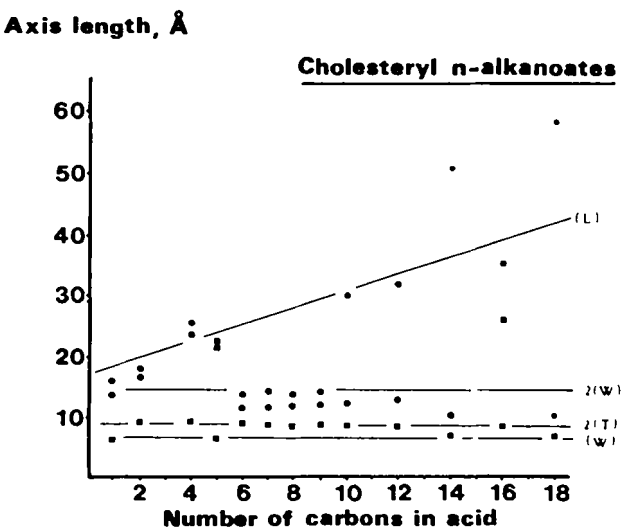

FIGURE 3 The unit cell axial lengths of the cholesteryl *n*-alkanoates, plotted against the total number of carbon atoms in the parent carboxylic acids. Cell dimensions correspondings to 2_1 screw axes in the crystals are indicated by ■. Those which do not correspond to a symmetry axis are indicated by ●. The dimensions of the molecular length (L) and multiples of the molecular width (W) and thickness (T) are indicated.

sent twice the molecular thickness or alternatively they may correspond to the combined width of a sterol group and the alkyl chain of the next molecule. Spacings in the 14Å-15Å range may correspond to twice the molecular width.

For those esters with n = 10 and 12 the largest unit cell dimension is comparable with the length of the molecule and the simple monoclinic structure is suggested, where the molecules are packed head to tail about the twofold axis. For the esters with n = 14 and 18 the largest unit cell dimension is greater than the length of the molecule.

The unit cell dimensions and powder photographs of the esters with n = 6, 7, 8 and 9 are all very similar, suggesting at first sight a close structural homology. This may perhaps not be the case. The incremental increases in the unit cell dimensions resulting from the increasing length of the molecules do not appear to be constant (but they are comparable with the experimental errors and no very definite conclusions can be drawn at this stage). Note also in this context that the transition temperatures and enthalpies of this group of esters vary irregularly with alkyl chain length.

The only instance where the indications of structural similarity suggested by the powder photographs and the single crystal data are clearly at variance is for the hexadecanoate. The powder photographs suggest a similarity with the tetradecanoate and the octadecanoate, whereas the space group and cell dimensions are very different. Note, however, that the single crystals were grown from dioxan (the only solvent found to give suitable single crystals) whereas the powder sample was obtained from *n*-pentanol. It is possible, therefore, that the single crystal data and the powder photographs correspond to different solid polymorphs

The unit cell dimensions of the two orthorhombic crystals do not suggest any close similarity between them. We have no data from which to comment on the possible odd/even effect for the longer-chain esters, suggested by the thermal data.

To summarise: the crystallography of the cholesteryl *n* alkanoates is complex; there are at least eight different types of crystal structure represented in this series and there is no readily apparent correlation between the crystal structures and the mesogenic properties.

References

1. Abrahamsson, S. and Selin, K., Rome, (1963). Abstract in Acta Cryst. Supplement, A58 (further details unpublished).
2. Schulze, G. E. R., *Z. Phys. Chem.* A171, 436 (1935).
3. Wendorff, J. H. and Price, F. P., *Mol. Cryst. Liquid Cryst.* to be published.
4. Gray, G. W., "Molecular Structure and the Properties of Liquid Crystals", Academic Press, New York (1962).
5. Davis, G. J. and Porter, R. S., *Mol. Cryst. Liquid Cryst.*, 11, 319 (1970).

6. Davis, G. J. and Porter, R. S., *Mol. Cryst. Liquid Cryst.*, 6, 377 (1970).
7. Merritt, W. G., Cole, G. D. and Walker, W. W., *Mol. Cryst. Liquid Cryst.*, 15, 105 (1971).
8. Barrall II, E. M. and Vogel, M. J., *Thermochim. Acta*, 1, 127 (1970).
9. Price, F. P. and Wendorff, J. H., *J. Phys. Chem.* 75, 2849 (1971).
10. Barnard, J. A. W. and Lydon, J. E., *Mol. Cryst. Liquid Cryst.* In preparation.
11. Bernal, J. D., Crowfoot, D. and Fankuchen, I., *Phil. Trans. Roy. Soc.*, 239A, 134 (1946).