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The Crystal Structure of Creatine Monohydrate*

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The crystal structure of creatine monohydrate has been determined from two-dimensional projections. Hydrogen contributions and deviations from average thermal motion have been allowed for in F_c. The resulting standard deviations in atomic coordinates are comparable with those from the three-dimensional determination by Mendel & Hodgkin.

Introduction

There are in progress in these laboratories fundamental studies on the ultrastructure of biological systems. Creatine and phosphocreatine are of basic importance in understanding the function and structure of one such system, namely, muscle. This paper is to report the determination of the crystal structure of creatine monohydrate by two-dimensional projections. Near the end of this work it was learned through the courtesy of Dr C. Brink that Drs D. Hodgkin and H. Mendel had already determined the structure using three-dimensional data treated by least squares. Their work has appeared (Mendel & Hodgkin, 1954), but it seems worth-while to report the two-dimensional work and to compare the results.

Unit cell and space group

The parameters of the unit cell were determined from oscillation and Weissenberg photographs using Cu $K\alpha$ radiation and were found to be

$$a_0 = 12{\cdot}52,\ b_0 = 5{\cdot}03,\ c_0 = 12{\cdot}18\ \text{Å};\ \beta = 108{\cdot}8°.$$

The space group is $P2_1/c$ with four molecules in the unit cell.

Intensity data

Integrated intensity data for the three principal zones were collected using a Wiebenga-type integrating Weissenberg goniometer set to integrate along the film coordinate at right angles to the rotation axis. Integration along the film coordinate parallel to the rotation axis was done with a Moll-type recording microdensitometer (Bennett, Quinton & Mueller, 1953). With proper precautions, this method is capable of fair accuracy. Duplicate data for approximately 100 of the most intense $h0l$ reflections taken from different crystals, one medium sized crystal with both absorption and extinction present and one small crystal with negligible absorption, and extinction reduced, gave results with a standard deviation of 0·64.

Data were collected from a number of crystals none of which had a cross-sectional dimension greater than 0·3 mm. The most intense reflections, in particular 200, 011 and 013, were low, presumably owing to secondary extinction. An attempt was made on a small crystal, cross-section 0·1 × 0·1 mm., to eliminate extinction effects by repeated dipping in liquid nitrogen. However, 200 was still low by about 10%. Hence a powder sample was prepared and the observed values of the above three planes were determined densitometrically from the powder photograph and brought on to the same scale as the single-crystal data by comparison with 100. Of the 323 possible reflections, 269 were observed.

The preliminary scale and temperature factors were determined in the usual way (Wilson, 1942).

Determination of the structure

The method of attack on this structure was similar to Mendel & Hodgkin's but sufficiently different to warrant a brief description. In view of the short b axis and the occurrence of a number of relatively large U_{h0l} it seemed likely that the projection on (010) would be solvable by a direct approach. The signs of two F_{h0l} may be chosen arbitrarily. By applying inequalities and the equation $S_h S_{h'} = S_{h+h'}$ (Harker & Kasper, 1948; Zachariasen, 1952) the signs of most $U_{h0l} > 0{\cdot}3$ are determined in terms of them and three others, a, b and c. An attempt was made to eliminate these unknowns by extension to planes with smaller U, but only a trivial solution resulted.

Sayre's squaring method was applied in an effort to determine a, b and c (Sayre, 1952; Cochran & Penfold, 1952). The equations for about 50 of the most intense reflections were written out and, although the correct sign combination was not determined with certainty, two of the eight combinations appeared as possibilities. F_o syntheses gave, for both, peaks in positions such that a reasonable model of the molecule would fit, but peak heights were erratic and there was no certainty that either was correct.

Recourse was then taken to a detailed interpretation of the Patterson projection on (010) involving peaks

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within 2·5 Å from the origin. This led rapidly to a model in which the orientation of the molecule was that indicated by the most likely sign combinations from the direct method. The F's calculated from one of these gave satisfactory F_{h00}, F_{h06}, $F_{h,0,12}$ with poor agreement for other F_{h0l}'s. The other possibility, differing essentially only in the displacement of the molecule by $c_0/12$, resulted in F's with a satisfactory reliability index for the most intense reflections. Refinement proceeded through two F_o syntheses and five F_o-F_c syntheses.

The first F_o-F_c synthesis indicated clearly the approximate position of ten of the eleven hydrogen atoms and showed that the thermal motion differed considerably for the various atoms and was quite anisotropic for some. The remaining hydrogen atom, H_9, Fig. 1, was not located with certainty until after the third F_o-F_c synthesis. Allowance for anisotropic thermal motion was made after this difference synthesis (Cochran, 1951) and final temperature-factor parameters were extrapolated (or interpolated) from the values used in the fourth and fifth difference syntheses. Fig. 2(*a*) shows the final F_o-F_c synthesis on (010) with all atoms and the scattering factor parameters of Table 1 included in F_c. Fig. 2(*b*) shows the positions of the hydrogens and Fig. 2(*c*) the difference of thermal motion from that corresponding to a temperature factor of $\exp[-2{\cdot}0 \sin^2 \theta/\lambda^2]$.

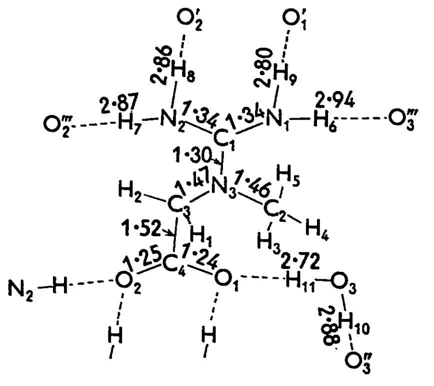

Fig. 1. Structural formula for creatine monohydrate and numbering of atoms.

Table 1. *Final temperature factors*, $\exp[-(B+C\cos^2\varphi)\sin^2\theta/\lambda^2]$, *for* F_{h0l}

Atom	B (Å²)	C (Å²)
C_1	2·0	—
C_2	3·5	—
C_3	2·0	1·2
C_4	1·6	0·8
N_1	2·0	0·5
N_2	2·0	1·0
N_3	2·0	—
O_1	2·4	1·2
O_2	1·4	2·0
O_3	3·0	1·0

φ = angle between normal to plane and direction of maximum vibration taken as +41° from c axis.

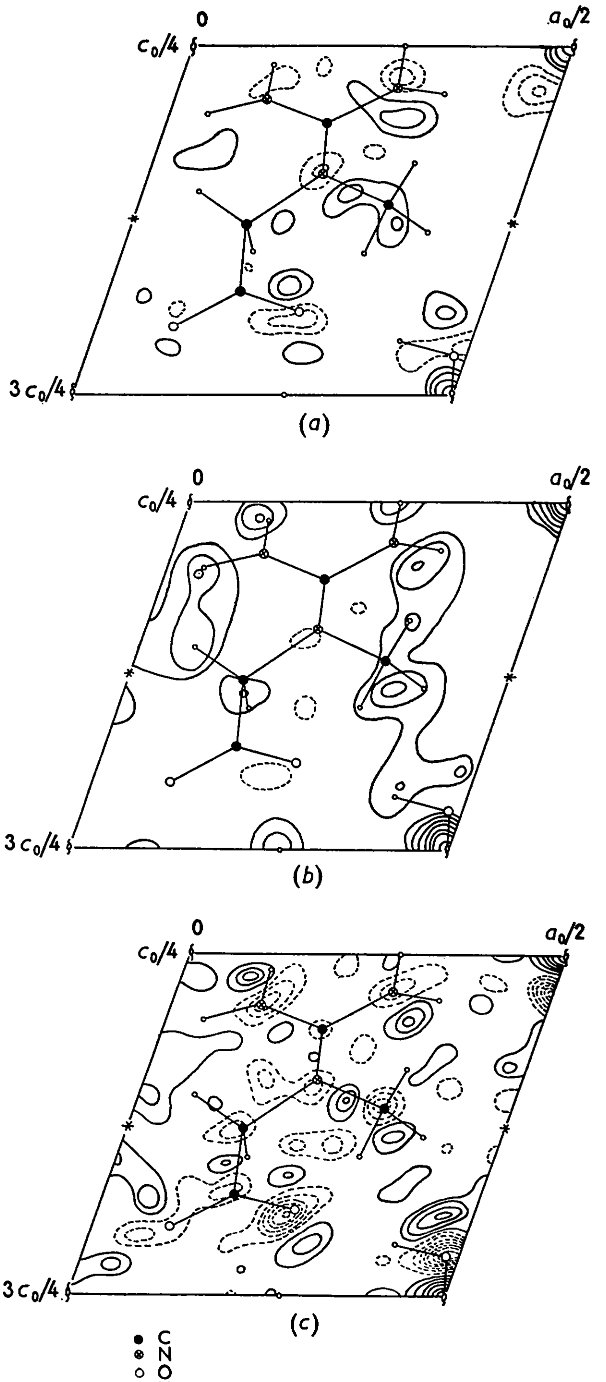

Fig. 2. (*a*) Fifth F_o-F_c synthesis on (010). Final δx_i and δz_i taken from this map.

(*b*) F_o-F_c synthesis on (010) showing hydrogens.

(*c*) F_o-F_c synthesis on (010) showing difference of thermal motion from that corresponding to $\exp[-2{\cdot}0 \sin^2 \theta/\lambda^2]$.

Contours at intervals of 0·25 e.Å^{-2}, zero contour omitted, negative contours broken.

The Patterson projection along [100] was most readily interpreted as indicating the molecule to be

V-shaped in this projection. The very intense 013 showed the approximate position of the molecule, and the oxygen of the water was placed at $y = \frac{1}{2}$. Refinement by difference syntheses was slow because of the rather poor y coordinates assumed and the appreciable overlap of six of the ten atoms. Seven difference syntheses, resulting in a reliability index of 17·4%, were run before data for ($hk0$) were available. In projection along [001] all atoms except two are quite well resolved and these are resolved in projection along [100]. Refinement now proceeded by alternate use of data from ($hk0$) and ($0kl$). No attempt was made to include anisotropic scattering factors in F_{hk0} or F_{0kl}, but partial allowance for differences of thermal motion was made by including the individual isotropic temperature-factor parameters listed in Table 2.

Table 2. *Final temperature factors*, $\exp[-B \sin^2\theta/\lambda^2]$, *for* F_{hk0} *and* F_{0kl}

Atom	B_{hk0} (Å^2)	B_{0kl} (Å^2)
C_1	2·5	2·5
C_2	3·7	3·5
C_3	3·0	2·5
C_4	2·5	3·0
N_1	2·5	2·5
N_2	2·5	3·0
N_3	3·0	2·5
O_1	3·4	3·5
O_2	3·7	3·0
O_3	3·7	5·0

The y coordinates for the hydrogen atoms were chosen on the basis of their (x, z) coordinates and an assumed bond length of 1·0 Å. Final difference syntheses along [100] and [001] appear in Fig. 3.

The x and z coordinates were taken from the fifth difference synthesis on (010) and the y coordinates were averaged from the seventh difference synthesis along [001] and the eleventh along [100]. Final coordinates appear in Table 3 and the bond lengths calculated from them in Table 4. The four figures to which the x and z coordinates and bond lengths are recorded is not meant to imply an accuracy greater than Mendel & Hodgkin's, but rather to indicate how the results round off for comparative purposes.

Table 3. *Atomic coordinates*

	x	y	z
C_1	0·2111	0·522	0·3597
C_2	0·3300	0·870	0·4731
C_3	0·1495	0·741	0·5039
C_4	0·1713	0·540	0·6014
N_1	0·2884	0·472	0·3072
N_2	0·1196	0·366	0·3275
N_3	0·2260	0·715	0·4348
O_1	0·2565	0·396	0·6265
O_2	0·0979	0·523	0·6509
O_3	0·4854	0·360	0·6896
H_1	0·170	0·914	0·546
H_2	0·073	0·734	0·457
H_3	0·320	0·012	0·548
H_4	0·396	0·754	0·516
H_5	0·344	0·917	0·415
H_6	0·352	0·527	0·317
H_7	0·048	0·381	0·348
H_8	0·116	0·214	0·272
H_9	0·280	0·341	0·250
H_{10}	0·500	0·206	0·750
H_{11}	0·408	0·367	0·675

The reliability index neglecting unobserved reflections is 6·5, 7·3 and 7·5% respectively for ($0kl$), ($h0l$) and ($hk0$) planes. (For the sake of brevity, Table 5

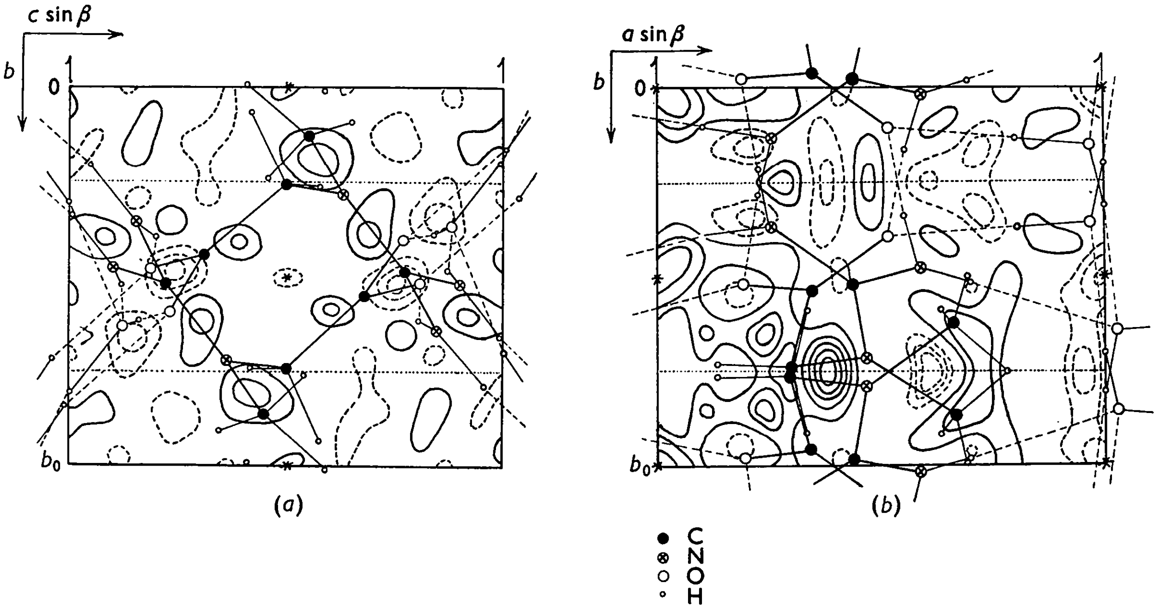

Fig. 3. (a) Eleventh F_o-F_c synthesis along [100] and (b) seventh along [001]. Final δy_i averaged from these maps. Contours at intervals of 0·50 e.Å^{-2}, zero contours omitted, negative coutours broken.

Table 4. *Bond lengths and angles*

C_1–N_1	1·344 Å	C_4–O_2	1·254 Å
C_1–N_2	1·339	O_1–O_3	2·723
C_1–N_3	1·304	O_3–O_3''	2·877
C_2–N_3	1·459	N_1–O_1'	2·804
C_3–N_3	1·471	N_1–O_3'''	2·944
C_3–C_4	1·515	N_2–O_2'	2·856
C_4–O_1	1·243	N_2–O_2'''	2·873
$N_1C_1N_2$	115·5°	$C_1N_3C_3$	119·7°
$N_1C_1N_3$	120·7	$C_2N_3C_3$	116·6
$N_2C_1N_3$	123·9	$C_3C_4O_1$	120·3
$N_3C_3C_4$	113·7	$C_3C_4O_2$	116·4
$C_1N_3C_2$	122·2	$O_1C_4O_2$	123·1

listing values of F_o and F_c has been omitted. A copy may be obtained by application to the author.)

Discussion and comparison

Limitations of two-dimensional syntheses become apparent in projection along [100] and [001]. For such projections, δx_i cannot be taken as proportional to the slope of the difference map at the assumed atomic position but may be approximated from details at a distance. It is particularly important in such instances to include hydrogen and individual isotropic or anisotropic temperature factors in F_c.

Approximately the errors in atomic coordinates due to neglect of hydrogen may be computed by assuming $\varrho(r) = k_1 \exp[-k_2 r^2]$, where r is the distance from $\varrho_{\text{max.}}$. If in two-dimensional projections the maxima are taken as 1 and 8 e.Å^{-2} for hydrogen and carbon respectively, one calculates a maximum displacement of a carbon atom of 0·023 Å by a hydrogen 0·4 Å distant. Comparison of Figs. 2(*b*) and 2(*c*) indicates that neglect of deviations from the mean thermal motion may result in displacement of twice this amount in creatine monohydrate.

The carboxyl group may be regarded as constituted of two equally contributing resonance forms with a formal charge of $-\frac{1}{2}$ on each oxygen; the guanidine group as constituted of three approximately equally contributing resonance forms with a formal charge of $+\frac{1}{3}$ on each nitrogen. Hence it would seem reasonable to use as scattering factor for oxygen, $(17/16)f_{\text{O}}$, and for nitrogen, $(20/21)f_{\text{N}}$. This was tried for F_{h0l} after the fourth difference synthesis, but the reliability index rose from 9·7 to 10·0%. The increase can hardly be considered significant. However, two low-index F's, namely F_{201} and $F_{20\bar{1}}$, with F_o's of 27·2 and 6·1, were calculated 27·7 and 6·9 using conventional (McWeeny, 1951) scattering factors but 21·6 and 11·9 with modified f's. These F_o's were measured several times on different crystals and the ΔF's are considerably larger than expected observational errors. This would seem to indicate that the electron density at considerable distance from the atomic centers is not well represented by the modified f's, and that some other electronic distribution, e.g. that corresponding to conventional f's with H_6 and H_9 moved away from N_1 and N_2 and toward O_1 and O_2, would more closely approximate that actually present.

Both bond lengths and bond angles agree well with the three-dimensional results, the standard deviations, assuming equal accuracy for the two determinations, being 0·011 Å in bond length and 1·1° in bond angle within the creatine molecule and 0·010 Å for the hydrogen bonds. Application of Cruickshank's equations (1949) to $F_o - F_c$ gives

$$\sigma(A_h) = 0{\cdot}534,\ \sigma(A_k) = 0{\cdot}861,\ \sigma(A_l) = 0{\cdot}570,$$

which, with $\partial^2\varrho/\partial x^2 = 67{\cdot}2$ e.Å^{-4} for a carbon atom, gives

$$\sigma(x) = 0{\cdot}0083\ \text{Å},\ \sigma(y) = 0{\cdot}0128\ \text{Å},\ \sigma(z) = 0{\cdot}0090\ \text{Å}$$

with a r.m.s. value of 0·0102 Å and a standard deviation for a C–C bond of 0·014 Å.

The two determinations agree in indicating the equality of the two C–O bonds. C_1–N_3 is, however, surprisingly short. The difference between its value, 1·304 Å, and the average of C_1–N_1 and C_1–N_2, 1·342 Å, would fall in the 'probably significant' range, using the more conservative estimate for the standard deviation of a bond length. One would rather expect C_1–N_3 to be longer since N_1 and N_2 are planar, bonding orbitals sp^2 hybrids, while the bonding orbitals of pyramidal N_3 would have more p character. C_2–N_3 and C_3–N_3 are equal within experimental error and only slightly less than the usually accepted C–N single-bond length.

For further details of the structure and a full discussion the reader is referred to Mendel and Hodgkin's paper (1954).

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