values of δk and δB are $+0{\cdot}024$ and $+0{\cdot}074$ Å^2: their use in equation (4) gives differences of essentially the same pattern as quoted above, but slightly more than twice the magnitude. This value of δk is still sufficiently small not to affect seriously the estimate of K in thiophthen given by the earlier analysis of the S curve. However, the replacement of k for S there by the more correct $(k+\delta k)$ worsens rather than improves the agreement with k obtained from the C curve. Hence, it follows that the influence on scaling of even small dispersion effects (for the case of larger effects see Clark, Templeton & MacGillavry, 1958) needs consideration in any detailed structure analysis of a heteroatomic compound, particularly if experimental f-curve derivation is intended (Jellinek, 1958).

References

ABRAHAMS, S. C. (1955). *Acta Cryst.* **8**, 661.

BEECHAM, A. F., FRIDRICHSONS, J. & MATHIESON, A. McL. (1958). *J. Amer. Chem. Soc.* **80**, 4739.

BERGHUIS, J., HAANAPPEL, IJ. M., POTTERS, M., LOOPSTRA, B. O., MACGILLAVRY, C. H. & VEENENDAHL, A. L. (1955). *Acta Cryst.* **8**, 478.

CLARK, E. S., TEMPLETON, D. H. & MACGILLAVRY, C. H. (1958). *Acta Cryst.* **11**, 284.

COX, E. G., GILLOT, R. J. J. H. & JEFFREY, G. A. (1949). *Acta Cryst.* **2**, 356.

DAWSON, B. (1960). *Acta Cryst.* **13**. 403.

JAMES, R. W. (1948). *The Optical Principles of the Diffraction of X-rays*, pp. 159, 609. London: Bell.

JAMES, R. W. & BRINDLEY, G. W. (1931). *Phil. Mag.* (7), **12**, 81.

JELLINEK, F. (1958). *Acta Cryst.* **11**, 626.

MCWEENY, R. (1951). *Acta Cryst.* **4**, 513.

MCWEENY, R. (1954). *Acta Cryst.* **7**, 180.

MEGAW, H. D. (1958). *Rev. Mod. Phys.* **30**, 96.

VIERVOLL, H. & ØGRIM, O. (1949). *Acta Cryst.* **2**, 277.

WARREN, B. E. & BURWELL, J. T. (1935). *J. Chem. Phys.* **3**, 6.

Acta Cryst. (1960). **13**, 414

X-ray Crystal Analysis of the Substrates of Aconitase.*
I. Rubidium Dihydrogen Citrate

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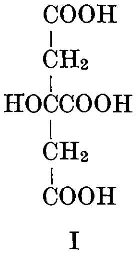
Rubidium dihydrogen citrate crystallizes in the space group $P2_1/a$ with 12 molecules in a cell of dimensions

$$a=14{\cdot}924,\quad b=9{\cdot}710,\quad c=19{\cdot}145\ \text{Å}\ (\pm 0{\cdot}2\%),\quad \beta=108{\cdot}63^\circ\ (\pm 0{\cdot}10^\circ)\ .$$

Reflections hkl with $l \neq 3n$ are very much weaker than those for $l=3n$. An approximate analysis was made on the basis of a cell for which $c=19{\cdot}145/3=6{\cdot}382$ Å. The structure was refined by difference Fourier methods on the $hk0$ and $h0l$ projections. A general description of the relative orientation of the groups within the citrate ion is given and the hydrogen bonding between ions is discussed. The rubidium ion is coordinated with nine oxygens and all oxygens take part in the coordination.

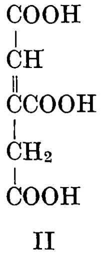
Introduction

It has been demonstrated (cf. e.g. Ochoa, 1951) that the enzyme aconitase catalyzes the establishment of equilibrium between the ions of citric acid (I), *cis*-aconitic acid (II), and *d*-isocitric acid (III). A particularly interesting aspect of this process lies in the fact that only one of the four possible optical isomers of the isocitrate ion is made from optically inactive citrate. Although it has been generally supposed that a single enzyme is responsible for this equilibrium there is evidence (Neilson, 1955) that in some organisms two enzymes may be involved. The transformation of citrate to isocitrate catalyzed by aconitase



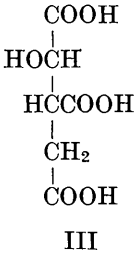
$$\begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{HOCCOOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{array}$$

I



$$\begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{CH}\\ \|\\ \mathrm{CCOOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{array}$$

II



$$\begin{array}{c}\mathrm{COOH}\\ |\\ \mathrm{HOCH}\\ |\\ \mathrm{HCCOOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{array}$$

III

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is an essential step in the Krebs cycle which in most biological systems is largely responsible for the conversion of the combustion energy of carbohydrates and fats into the form in which it can be utilized by the organism.

The studies reported in the present series of papers were undertaken to provide detailed stereochemical information for the three ions which are substrates for the aconitase system.* By such studies we hope to determine the exact spatial arrangement of each of these ions in various crystallographic environments. This will suggest probable configurations for the ions in solution. We also hope to obtain some indication of the order in which the carboxyl groups of these acids are ionized. It is believed that such information must be obtained before a detailed understanding of this enzyme reaction can be achieved.

The first paper of the series reports an analysis of rubidium dihydrogen citrate. Only an approximate analysis was undertaken owing to the crystallographic nature of this material. In the second paper a detailed analysis of anhydrous citric acid is reported. In later papers data will be presented on other salts of citric acid, and on other substrates of aconitase.

Experimental data

As a preliminary to the study of citric acid an investigation of one of the alkali dihydrogen citrates was undertaken. At the time we were unable to obtain good crystals of the sodium salt although they could be grown later (cf. Love & Patterson, 1960). The potassium salt was found to be triclinic with eight molecules in the unit cell. Rubidium dihydrogen citrate was found to crystallize with 12 molecules in a monoclinic cell of dimensions:

$$a = 14{\cdot}924,\ b = 9{\cdot}710,\ c = 19{\cdot}145\ \text{Å};\ \beta = 108{\cdot}63°.$$

The standard deviation for the cell dimensions is about $\pm 0{\cdot}2\%$ and for the beta angle about $\pm 0{\cdot}10°$. The extinctions were those of the space group $P2_1/a$ but since this would have an asymmetric unit containing 3 molecules the allocation cannot be considered unambiguous (cf. Templeton, 1956). From the precession photographs it was clear that the intensities of $h, k, 3n$ were strong while all other reflections were very weak. An analysis was therefore carried out for the 'average' structure on the cell of dimensions:

$$a = 14{\cdot}924,\ b = 9{\cdot}710,\ c = 6{\cdot}382\ \text{Å};\ \beta = 108{\cdot}63°$$

with four molecules in the cell and under the assumption of $P2_1/a$ space group symmetry. Although such an analysis cannot be anything more than approximate its results seemed of sufficient interest to merit publication.

Intensities were recorded for the $hk0$ and $h0l$ zones using the integrating Weissenberg camera of Wiebenga & Smits (1950) with copper radiation. The intensities were measured with a recording microphotometer calibrated in terms of a set of timed exposures. Observations were made of 114 $hk0$ reflections, 108 $h03n$ reflections, and in addition 95 reflections in $h0l$ were observed for $l \neq 3n$.

Determination of the structure

The structure analysis followed the classical 'heavy atom' technique of Robertson. Patterson maps of the

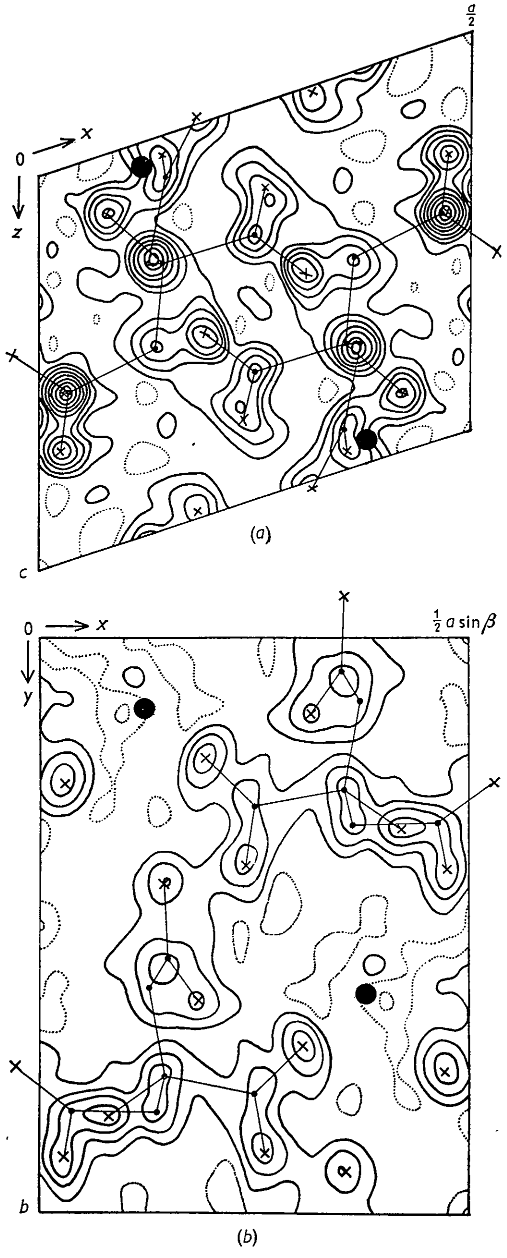

Fig. 1. Rubidium dihydrogen citrate: (a) $h0l$ projection, (b) $hk0$ projection. Rb contribution removed. Contour interval 3e. Å^{-2}. Rb: large full circles; C: small full circles; O: crosses.

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* We use this term to include the possibility that aconitase is not a single enzyme.

two zones exhibited strong Rb–Rb peaks which established positions for the rubidium ion. Fourier syntheses on $h0l$ and $hk0$, using signs calculated for rubidium alone, located most of the oxygen and carbon atoms and the remaining atoms were found on the second cycle of refinement. The final electron-density maps from which the rubidium contributions have been subtracted are shown in Fig. 1.

The refinement of the atomic coordinates was carried out by a succession of (F_o-F_c) syntheses. Between these syntheses the scale constants and the temperature factors were adjusted by a least-squares procedure. The structure factors of McWeeny (1951) were used for oxygen and carbon while those of the *Internationale Tabellen* were used for the rubidium ion.

Table 1. *Temperature factors and discrepancies*

Zone	B_{Rb}	B_{I}	R	R'
$h0l$	0·90 Å^2	1·28 Å^2	0·13	0·36
$hk0$	1·61	2·52	0·10	0·38

$R=\Sigma|F_o-F_c|/\Sigma|F_o|$; $R'=\Sigma|F_o-F_c|/\Sigma|F_o-F_{\text{Rb}}|$; observed only.

The first (F_o-F_c) syntheses indicated that the temperature constant B_{Rb} for the rubidium atom and B_{I} for the citrate ion differed from each other and furthermore that these constants had different values in the two projections. It was felt that these differences were expressions of apparent disorder in the 'average' structure due to the presence of the superstructure. In addition there was possibly an effect due to absorption. The two zones were therefore analyzed independently. Table 1 lists the B values and the R values for the two projections.

The final atomic parameters are listed in Table 2.

Table 2. *Atomic coordinates*

	x	y	z
Rb$^+$	0·122	0·123	0·061
O_1	0·356	−0·071	0·953
O_2	0·316	0·131	1·018
O_3	0·530	0·249	0·568
O_4	0·473	0·401	0·289
O_5	0·191	0·208	0·546
O_6	0·238	0·396	0·784
O_7	0·421	0·331	0·850
C_1	0·352	0·056	0·894
C_2	0·373	0·109	0·695
C_3	0·355	0·261	0·679
C_4	0·365	0·323	0·469
C_5	0·465	0·320	0·431
C_6	0·250	0·292	0·672

While we have made no statistical study of the error of these coordinates we feel that the rubidium coordinates have a probable error less than ±0·02 Å while that for the oxygen and carbon atoms may be as large as ±0·07 Å. We have made no attempt to locate hydrogen atoms.

Discussion of the structure

The bond lengths and bond angles in the dihydrogen citrate ion are shown in Fig. 2. The atoms C_1, C_2, C_3, C_4 in the main carbon chain lie approximately in a plane with the carboxyl $O_1C_1O_2$ while the carboxyl $O_3C_5O_4$ is turned out of this plane. The central carboxyl $O_5C_6O_6$ makes an angle of approximately 15° with the plane $C_6C_3O_7$ which contains the hydroxyl oxygen O_7. Thus the carboxyl group is nearly coplanar with the α-hydroxyl despite the close approach (2·70 Å) between O_6 and O_7. This type of configuration has been found in a number of oxalates and tartarates and in the corresponding acids and has been discussed by

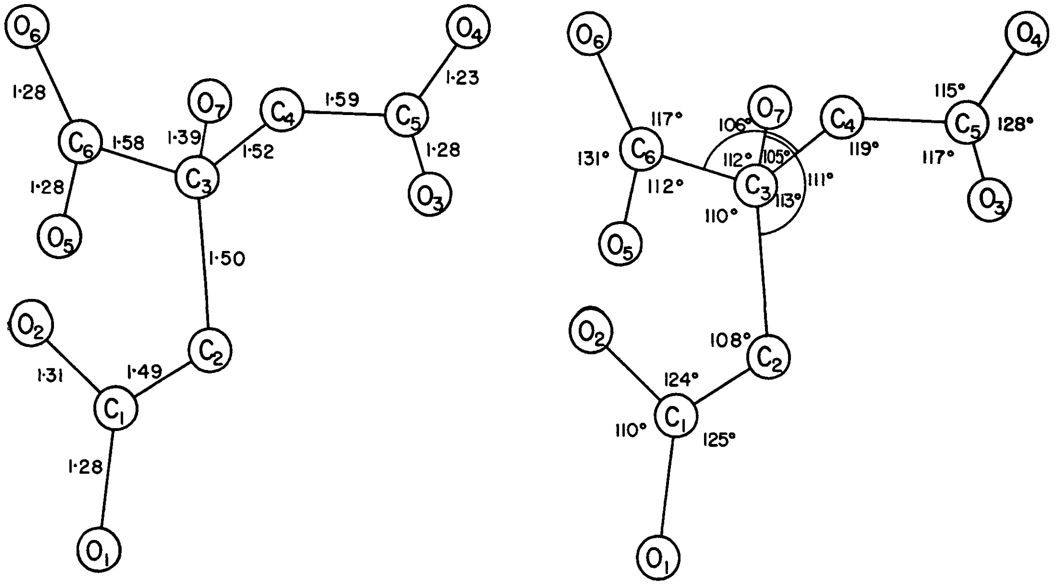

Fig. 2. Bond lengths (±0·1 Å) and bond angles (±2°) in the dihydrogen citrate ion.

Jeffrey & Parry (1952, 1955). In addition to the cases discussed by them a similar configuration has been found in the gluconate ion (Littleton, 1953), ammonium hydrogen D-tartarate (van Bommel & Bijvoet, 1958), and citric acid (Nordman, Weldon & Patterson, 1960).

In view of the large experimental error the differences between the bond lengths in the carboxyl groups are not significant and it is therefore impossible to distinguish the hydroxyl groups from the carbonyl groups. However, it is possible that the shortness of two of the hydrogen bonds is a contributing factor to this similarity. Within experimental error, the carboxyl groups are coplanar with their α-carbons. Our work locates the oxygen atom O_2 at a distance of 2·63 Å from the carboxyl carbon atom C_6 (in the same molecule) in a direction approximately perpendicular to the plane of the carboxyl group $O_5O_6C_6C_3$. The distance O_2–C_3 is 2·72 Å. While these distances may be in error by 0·1 Å it seems that a close approach between O_2 and the central carboxyl group is indicated.

Fig. 3 exhibits the surroundings of the rubidium ion. There are nine oxygen aroms at distances between 2·90 and 3·25 Å, the next nearest oxygen being at a distance of 3·83 Å. The nine-fold coordination is not unexpected for the radius ratio and the Rb–O distance is in accord with established ionic radii. The coordination spheres occur in pairs with a center of symmetry between them. Four oxygen atoms (O_4 and O_7 and their equivalents) are common to the two spheres. The Rb–Rb distance between the metal atoms in the pair of spheres is 4·21 Å as compared with the next closest approaches at 6·37 Å and 6·38 Å. It will be noted that all oxygens are coordinated with rubidium.

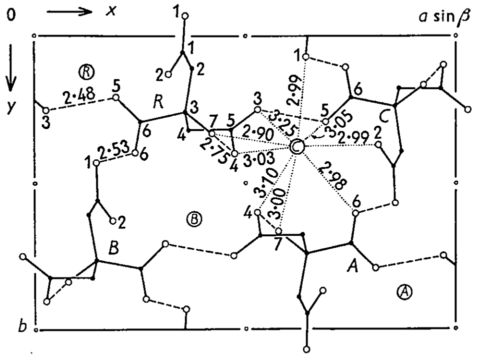

Fig. 3. Packing diagram for rubidium dihydrogen citrate. Dotted lines indicate rubidium coordination. Dashed lines correspond to hydrogen bonds. Numbering of atoms corresponds to that of Table 1 which locates the reference molecule *R*. Molecule *A* is related to *R* by a center of symmetry at $\frac{1}{2}, \frac{1}{2}, \frac{1}{2}$. Molecule *B* is related to *R* by the screw axis $\frac{1}{4}, v, 1$ with translation $b/2$. The molecule *C* is the glide plane $(u, \frac{1}{4}, w)$ equivalent with translation $+a/2$. The closest intermolecular approaches other than hydrogen bonds are: O_4R–O_4A, 3·20; O_6R–O_2B, 2·84; O_3R–C_6C, 3;16; O_2R–C_4Rc, 3·31 Å; and their equivalents to molecules $B\bar{b}$, $C\bar{a}$, and $R\bar{c}$, where Rc and $R\bar{c}$ etc., refer to the molecule *R* translated by c and $-c$ etc.

Fig. 3 also exhibits the hydrogen bonding of the structure. There is one bond of length 2·48 Å between the carboxyl oxygen O_3 of one molecule and the carboxyl oxygen O_5 of its glide plane equivalent molecule. A second bond length 2·53 Å occurs between the carboxyl oxygen O_6 of one molecule and the carboxyl oxygen O_1 of the screw axis related molecule. A third bond of length 2·74 Å lies between the hydroxyl O_7 and the carboxyl oxygen O_4 on the *c* translational equivalent. A fourth close approach of 2·84 Å occurs between O_6 and O_2 on the same carboxyl group as the O_1 involved in the 2·53 Å hydrogen bond. The next nearest intermolecular approach appears to be 3·16 Å.

An attempt to resolve the difference Patterson map for the superstructure was not successful. We have observed an ellipticity of the shape of the rubidium ion in the *y* direction of the *hk*0 projection which may be related to the tripling, but this alone could not explain the phenomenon. The radial average of the superstructure lines increases with respect to that of the average cell reflections as $\sin^2 \theta/\lambda^2$ increases. This lends support (Mackay, 1953) to our belief that the superstructure arises from small displacements of one or more atoms in going from one sub-cell to the next.

Since the work reported here was concluded, one of us (C. E. N.) has observed that on raising the temperature the superstructure disappears in the neighborhood of 35 °C. The nature of this transformation is being investigated.

References

Bommel, A. J. van, & Bijvoet, J. M. (1958). *Acta Cryst.* **11**, 61.

Jeffrey, G. A. & Parry, G. S. (1952). *Nature, Lond.* **169**, 1105.

Jeffrey, G. A. & Parry, G. S. (1954). *J. Amer. Chem. Soc.* **76**, 5283.

Littleton, C. D. (1953). *Acta Cryst.* **6**, 775.

Love, W. E. & Patterson, A. L. (1960). *Acta Cryst.* **13**, 426.

Mackay, A. L. (1953). *Acta Cryst.* **6**, 214.

McWeeny, R. (1951). *Acta Cryst.* **4**, 513.

Neilson, N. E. (1955). *Biochem. Biophys. Acta,* **17**, 139.

Nordman, C. E., Weldon, A. S. & Patterson, A. L. (1960). *Acta Cryst.* **13**, 418.

Ochoa, S. (1951). *Fumarase and Aconitase* in Sumner, J. B. & Myrbäck, K. *The Enzymes*, Vol. I, Part 2, pp. 1217–1236. New York: Academic Press.

Templeton, D. H. (1956). *Acta Cryst.* **9**, 199.

Wiebenga, E. H. & Smits, D. W. (1950). *Acta Cryst.* **3**, 265.