

The Crystal Structure of Rubidium Ammonium Hydrogen Fluorocitrate Dihydrate

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The crystal structure of the racemate of rubidium ammonium hydrogen fluorocitrate dihydrate, $\text{RbNH}_4\text{H}(\text{C}_6\text{H}_4\text{O}_7\text{F}) \cdot 2\text{H}_2\text{O}$, containing the isomer of fluorocitrate that inhibits the enzyme aconitase, has been determined. The configurations of the two isomers in the racemate are 1*R*:2*R* and 1*S*:2*S* for 1-fluoro-2-hydroxy-1,2,3-propanetricarboxylic acid. The crystals are triclinic, space group $P\bar{1}$, $Z=2$, with unit-cell dimensions $a=8.458$ (2), $b=10.910$ (3), $c=7.516$ (2) Å, $\alpha=102.80^\circ$ (3), $\beta=115.90^\circ$ (3), $\gamma=80.50^\circ$ (4). The observed and calculated densities are 1.92 and 1.925 g cm⁻³ respectively. The structure was solved from the Patterson map and refined to an *R* value of 0.072. All hydrogen atoms, except that on a carboxyl group of the anion, were located from a difference electron-density map. The fluorocitrate ion forms a tridentate chelate with a rubidium ion, the fluorine atom being shared by two rubidium coordination polyhedra. There is disorder of rubidium ions with ammonium ions to an approximate extent of 84% and, corresponding to this, the fluorocitrate ion, also disordered to the extent of 84%, is presumed to pack so that in each case the fluorine atom is coordinated to the rubidium ion.

The plant *Dichapetalum cymosum* found in South Africa is toxic, often fatally so, to grazing cattle. The leaves have been found to contain fluoroacetate and the phenomenon of the toxicity has been studied extensively by Peters (1954). He suggested that a 'lethal synthesis' of fluorocitrate from the fluoroacetate in the ingested leaves had occurred within the body of the cattle, and that the product, fluorocitrate (and not the fluoroacetate itself), was the active principle, exerting its effect by blocking the action of the enzyme aconitase in the tricarboxylic cycle. It has also been suggested that the target of the lethal effect of fluorocitrate in the cell is the tricarboxylate carrier of mitochondria (Eanes, Skilleter & Kun, 1972).

Fluorocitric acid has two asymmetric carbon atoms so that there are four possible isomers. There is only one isomer of fluorocitric acid that is a strong inhibitor of aconitase, and this is the one formed from fluoroacetate by the enzyme citrate (*si*)-synthase. This isomer has been resolved by Dummel & Kun (1969) from a chemical preparation of fluorocitric acid. In order to determine the absolute configuration of this isomer, crystals of the rubidium ammonium salt were examined. Unfortunately, it appeared early in the investigation that the crystals were those of a racemate. However, it was felt that the resulting structure determination would be of interest, particularly in indicating the relative configuration of the fluorocitrate and in showing the relationship of the fluorine atom to the metal cation. A preliminary account of this work has been published (Carrell, Glusker, Villafranca, Mildvan, Dummel & Kun, 1970).

Experimental

Crystals of rubidium ammonium fluorocitrate were sent to us by Drs Dummel and Kun of the San Fran-

cisco Medical Center of the University of California. They were deliquescent and were clumped so that it was difficult to find a single crystal in the sample. They were found to be triclinic, space group $P1$ or $P\bar{1}$. Crystal data are given in Table 1.

Table 1. *Crystal data for rubidium ammonium hydrogen fluorocitrate dihydrate*

Formula: $\text{Rb}(\text{NH}_4)\text{H}(\text{C}_6\text{H}_4\text{O}_7\text{F}) \cdot 2\text{H}_2\text{O}$ F.W. 347.7
Crystal system: triclinic

$a = 8.458$ (2) Å $\alpha = 102.80$ (3)^c
 $b = 10.910$ (3) $\beta = 115.90$ (3)
 $c = 7.516$ (2) $\gamma = 80.50$ (4)

(From 12 reflections measured on a diffractometer with $\alpha_1\alpha_2$ peaks resolved, Cu $K\alpha_1$ radiation, $\lambda = 1.5405$ Å)

$V = 600.1$ Å³ $F(000) = 348$

$D_m = 1.92$ g cm⁻³ (measured in mixture of *m*-xylene and bromoform)

$D_x = 1.925$ g cm⁻³ $Z = 2$
 $\lambda(\text{Cu } K\alpha) = 1.5418$ Å $\mu(\text{Cu } K\alpha) = 68.5$ cm⁻¹

Space group: $P\bar{1}$ with disorder Systematic absences: none

The presence of fluorine in the sample and the activity with aconitase were checked on crystals from the same batch as that from which the crystal for data collection was chosen. Enzymatic studies were made with purified aconitase from pig heart by Drs Joseph J. Villafranca and Albert S. Mildvan of this Institute (Villafranca & Mildvan, 1971). They determined this salt to be a linear competitive inhibitor with respect to citrate. On prolonged incubation the salt inactivated the enzyme, as described by Kun (1969). The n.m.r. spectrum of the crystals dissolved in D₂O was measured on a 100 MHz instrument and revealed, with heating to 75°C, the two -CHF- peaks shown by

Fanshier, Gottwald & Kun (1964) for esters of fluorocitric acid in deuteriochloroform.

The crystal used for the data collection was cut to a cube 0.15 mm along an edge. The intensity data were collected on a Picker automatic diffractometer. Nickel filtered Cu $K\alpha$ radiation was used and all data out to $2\theta = 130^\circ$ were measured. Even though the crystal was coated with ambroid cement, it still showed evidence of radiation damage on exposure to X-rays. (The intensities of the standard reflections fell off by 17% during the 24 hour data collection period.) Because of this

deterioration the data were collected using the stationary-crystal stationary-counter technique with a 20 sec counting time and were corrected by factors from an experimentally derived curve to give data corresponding to integrated intensities. Of 1975 independent reflections measured, 1799 were considered observed by the criterion $I \geq 2.33\sigma(I)$. The data were then corrected for Lorentz and polarization factors and a spherical absorption correction for a crystal with the same volume as that of the crystal studied was applied.

Since no appreciable intensity differences that could be attributed to anomalous dispersion effects ($\Delta f'' = 1.608$ for Rb with Cu radiation; Cromer & Liberman, 1970) were observed for equivalent reflections (hkl and $\bar{h}\bar{k}\bar{l}$), it was concluded that the structure was centrosymmetric, space group $P\bar{1}$, and averaged intensities were used for all subsequent calculations. Statistics on the data were intermediate between those for centrosymmetric and for non-centrosymmetric structures.

The structure was determined from an examination of the Patterson synthesis and confirmed by inspection of a Fourier synthesis phased on the positions of the rubidium atoms. The initial R value for the entire structure was 0.27 which, after six cycles of block-diagonal least-squares calculations (three cycles isotropic, three cycles anisotropic), was lowered to 0.097. At this point a difference map was computed and peaks corresponding to the hydrogen atoms associated with the hydrogen fluorocitrate anion were located, except for the proton on the unionized carboxyl group. In addition, the hydrogen atoms of the water molecules and the ammonium ion were located. This difference map also showed large negative regions at the rubidium and fluorine positions and a large positive peak at the ammonium ion position. One of the peaks assigned as a hydrogen atom on C(4) also had an anomalously high electron density.

Thus, at this point we were faced with two possible problems. One problem was that we might have been studying two crystals, one larger than the other and the second problem was that the structure might really have been acentric, $P1$, and that we had been studying an optically pure fluorocitrate.* The relative configurations of the anions are the same in each disordered state, the two forms of the fluorocitrate ion being related approximately by a mirror plane as shown in Fig. 1 [the mirror plane is halfway between C(2) and C(4) and between Rb^+ and NH_4^+ so that F is reflected into F(2)]. However, there is no 'image' of the water molecules across this approximate mirror plane, thus eliminating the idea that two crystals were being studied.

The problem of a centric but disordered structure versus a noncentric structure (still with disordered rubidium and ammonium cations) was then studied. Data on this are presented in Table 2. Of the 1500 Friedel

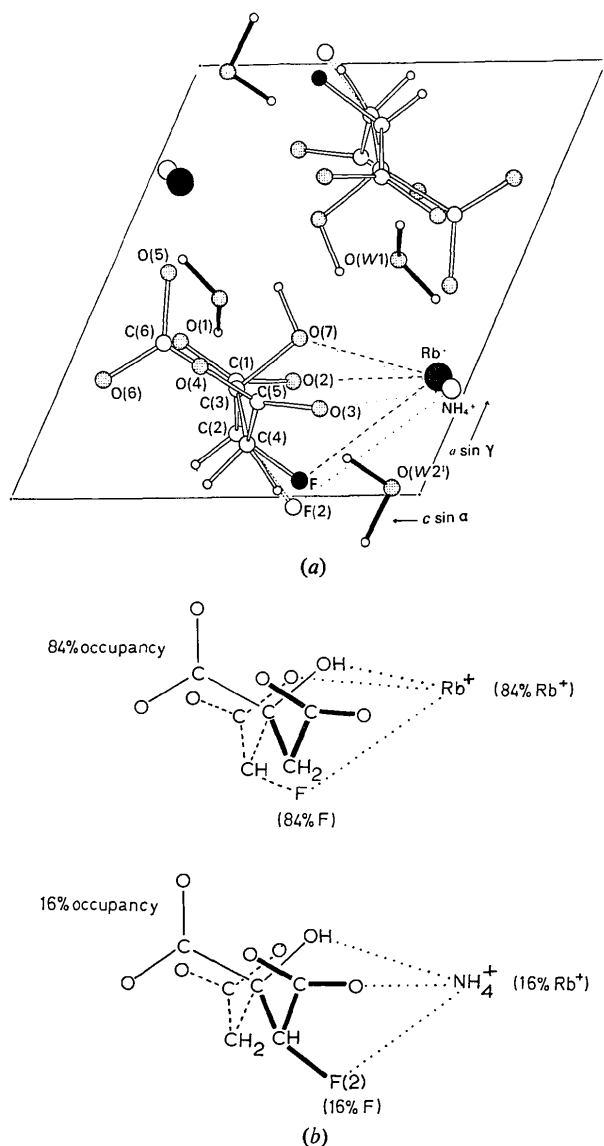


Fig. 1. General packing in the unit cell. This is a view down the b axis. Oxygen atoms are stippled and rubidium and fluorine atoms are black. The coordination to 84% Rb^+ is indicated by dashed lines and that to 16% Rb^+ (in the position of NH_4^+) by dotted lines. (b) Inset corresponding to lower anion in (a). In each case the relative arrangement of F and OH is the same.

* We are grateful to the referee who suggested that this second problem existed.

pairs measured, only 36 had values of $|F_+|_{\text{calc}}$ and $|F_-|_{\text{calc}}$ differing by 1.5 or more, the greatest difference being 4.5 (calculated on a model in *P1* with disordered 'rubidium' atoms). Of these 36 pairs of reflections only 6 had observed differences in F^2 that were greater than $8\sigma(F^2)$. Two of these indicated one model and 4 the other. We conclude that, while the data are not very good, the model with 50% occupancy in the fluorine positions (or the *P1* model) does not give the best fit. The data indicate 84% rubidium and 16% ammonium in one cation position and 16% rubidium and 84% ammonium in the other position. The same proportions apply to the fluorine atom. The fact that these occupancies are similar (from the least-squares refinement) suggests that the fluorine atom is always coordinated to the rubidium rather than the ammonium ion. Love & Patterson (1960) reported cell constants for a citrate of composition $(\text{NH}_4)_{5/4} \text{Rb}_{3/4} \text{H}(\text{C}_6\text{H}_5\text{O}_7)$.

Table 2. Determination of the site occupancies of the rubidium, ammonium and fluorine positions

(a) Integrated electron density for peaks on a Fourier map Centric case

	Peak count	Occupancy*	
Rb(1)	31.5e	94% Rb ⁺	6% NH ₄ ⁺
Rb(2)	8.0	6 Rb ⁺	94 NH ₄ ⁺
F(1)	5.76	72 F	28 H
F(2)	2.74	28 F	72 H

* If p is the fraction of Rb and q the fraction of F in a peak (*i.e.*, the occupancy) then the electron count in a rubidium peak is $29p+7$ and in a fluorine peak $8q+1$. The total count $\text{Rb}(1)+\text{Rb}(2)$ was increased to 43 and $\text{F}(1)+\text{F}(2)$ to 10.

(b) Peaks in a difference map calculated with disordered cations

	Input	Output (peaks)
F(1)	100%	0e
F(1b)	0	3
F(2)	0	0
F(2b)	100	-3

Molecule b is at $-x, -y, -z$.

(c) Full-matrix least-squares refinement

P1 refinement $R=0.118$

	Population parameter	B	Occupancy (p)
Rb(1)	0.974 (14)	2.38 (9)	0.86
Rb(2)	0.402 (21)	5.29 (49)	0.79
Rb(1b)	0.935 (15)	2.23 (8)	0.82
Rb(2b)	0.304 (17)	3.42 (18)	0.90
F(1)	1.000	3.34 (18)	
F(2b)	1.000	8.92 (47)	

Troubles

C(2)	1.000	0.22 (36)
C(1b)	1.000	0.46 (27)

P1 refinement $R=0.105$

	Population parameter	B	Occupancy (p or q)
Rb(1)	0.919 (9)	2.52 (4)	} $p=0.86$
Rb(2)	0.316 (6)	4.24 (14)	
F(1)	0.739 (27)	2.53 (24)	} $q=0.85$
F(2)	0.213 (31)	4.86 (127)	

It is possible that a type of disordering of ammonium and rubidium ions similar to that found in the fluorocitrate also occurs in this structure.

The thermal motion of the carboxyl group containing atoms C(5), O(3) and O(4) is high and is indicative of further disorder in the structure, but it was felt that the data did not justify further refinement. This disorder probably occurs because the $-\text{CF}-\text{COOH}$ grouping is nearly planar while there is no such constraint for a $-\text{CH}_2-\text{COOH}$ group.

Table 3. Atomic parameters for rubidium ammonium fluorocitrate

Positional parameters are expressed as fractions of cell edges. Anisotropic temperature factors are expressed as

$$\exp[-(\frac{1}{4})(B_{11}h^2a^{*2} + \dots + 2B_{12}hka^*b^* + \dots)]$$

and isotropic temperature factors as

$$\exp(-B \sin^2 \theta/\lambda^2)$$

with B values given in \AA^2 . Estimated standard deviations, determined from the inverted full matrices, are listed beside each parameter with respect to the last decimal place given. Hydrogen atom positions listed were determined from a difference map but were not refined.

(a) Non-hydrogen atomic coordinates ($\times 10^4$).

	x	y	z
Rb*	2703.7 (8)	-34.7 (7)	778 (1)
N†	2431 (3)	5615 (2)	340 (5)
O(W1)	5423 (6)	3066 (4)	3007 (7)
O(W2)	157 (7)	7913 (6)	760 (8)
C(1)	2661 (6)	-238 (5)	5705 (9)
C(2)	1454 (7)	962 (5)	5145 (9)
C(3)	2480 (7)	2105 (5)	5607 (9)
C(4)	1212 (9)	3235 (6)	4774 (12)
C(5)	2190 (11)	4319 (6)	4975 (14)
C(6)	3552 (8)	2467 (6)	7898 (10)
O(1)	3586 (6)	-231 (4)	7501 (7)
O(2)	2639 (6)	-1118 (5)	4303 (8)
O(3)	2003 (11)	4617 (7)	3362 (14)
O(4)	3011 (9)	4866 (6)	6754 (12)
O(5)	5195 (6)	2412 (5)	8536 (7)
O(6)	2728 (6)	2787 (5)	8985 (8)
O(7)	3625 (5)	1787 (4)	4605 (7)
F(1)‡	345 (6)	779 (5)	3098 (8)

* 84% Rb⁺, 16% NH₄⁺.

† 16% Rb⁺, 84% NH₄⁺.

‡ 84% F, 16% H.

(b) Hydrogen atomic coordinates and isotropic temperature factors

	x	y	z	B
H(1)	0.075	0.102	0.583	2.1
H(2)	0.030	0.334	0.533	3.4
H(3)	0.014	0.293	0.355	3.4
H(7)	0.483	0.162	0.567	2.8
H(W11)	0.450	0.295	0.167	4.4
H(W12)	0.622	0.390	0.333	4.4
H(W21)	0.086	0.832	0.217	3.3
H(W22)	-0.110	0.788	0.083	3.3
H(N1)	0.15	0.62	0.05	4
H(N2)	0.35	0.57	0.10	4
H(N3)	0.25	0.52	-0.10	4
H(N4)	0.22	0.54	0.13	4
F(2)§	-0.025 (3)	0.287 (2)	0.297 (3)	3.8 (4)

§ 16% F, 84% H.

Table 3 (cont.)

(c) Thermal parameters for non-hydrogen atoms

	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Rb	2.20 (3)	2.81 (3)	1.54 (3)	-0.27 (2)	0.88 (2)	0.31 (2)
N	4.95 (9)	1.98 (9)	4.57 (11)	-0.22 (8)	3.06 (12)	0.31 (9)
O(<i>W</i> 1)	4.69 (17)	2.51 (17)	3.25 (17)	-0.62 (15)	1.83 (19)	0.60 (22)
O(<i>W</i> 2)	4.16 (19)	6.15 (30)	3.45 (20)	-0.62 (20)	1.81 (21)	-0.18 (22)
C(1)	2.04 (17)	2.32 (22)	2.70 (22)	-0.42 (17)	1.06 (20)	0.50 (20)
C(2)	2.36 (18)	2.28 (22)	2.08 (19)	-0.19 (17)	1.10 (20)	0.16 (19)
C(3)	2.83 (18)	1.39 (20)	2.88 (21)	0.20 (16)	1.48 (21)	0.50 (19)
C(4)	2.88 (24)	2.70 (25)	4.28 (30)	0.09 (21)	0.53 (26)	1.31 (25)
C(5)	7.28 (30)	1.98 (25)	5.97 (31)	1.18 (23)	4.41 (40)	1.49 (28)
C(6)	2.96 (20)	1.73 (22)	3.07 (23)	-0.28 (18)	1.41 (23)	-0.27 (20)
O(1)	3.54 (17)	3.57 (19)	2.79 (17)	-0.09 (15)	1.05 (18)	1.29 (17)
O(2)	3.61 (18)	2.90 (20)	3.41 (20)	0.21 (16)	1.07 (18)	-0.97 (17)
O(3)	18.98 (36)	4.07 (27)	10.11 (31)	-0.41 (30)	11.27 (65)	1.37 (35)
O(4)	9.28 (32)	4.19 (25)	6.42 (33)	-2.93 (28)	2.61 (34)	0.99 (28)
O(5)	2.93 (16)	3.42 (20)	3.15 (19)	-0.65 (15)	0.91 (17)	-0.06 (17)
O(6)	3.95 (18)	4.74 (25)	3.27 (19)	-0.73 (18)	1.94 (20)	-0.64 (19)
O(7)	2.77 (13)	3.07 (18)	2.94 (15)	-0.33 (14)	1.53 (16)	0.74 (16)
F(1)	3.31 (18)	3.46 (22)	3.37 (20)	-0.30 (17)	0.81 (19)	0.18 (18)

The final refinement of the structure was carried out using full-matrix least-squares techniques. All the non-hydrogen atoms were refined with anisotropic temperature factors. The hydrogen atoms were included in the structure factor calculations but were not refined. The final R value for the observed reflections is 0.072 and the weighted R value is 0.099. The atomic parameters are presented in Table 3 and observed and calculated structure factors in Table 4.

Computations

Modified versions of the block-diagonal least-squares program of Harris (1966) and the full-matrix least-squares program of Gantzel, Sparks, Long & True-

blood (*UCLALS4*; 1965), both of which minimize $\sum \omega[|F_o| - |F_c|]^2$, were used. The least-squares calculations on the population parameters (occupancy factors) were carried out using the *X-RAY 70* System of programs (Stewart, Kundell & Baldwin, 1970) and *CLINUS*, a version of the least-squares program *ORFLS* of Busing, Martin & Levy (1962) at Brookhaven National Laboratory (Hamilton, 1972, unpublished). The program to compute interatomic distances, planes and torsion angles (*ALLD*) was written by H. L. Carrell and A. Caron. Figs. 1, 2 and 4, apart from the lettering, were prepared by the program *ORTEP* (Johnson, 1965). Atomic scattering factor curves were taken from *International Tables for X-ray Crystallography* (1962), the curve for Rb^+ being used for the rubidium ion with $\Delta f' = -0.574$, $\Delta f'' = 1.608$ (Cromer & Liberman, 1970). Scattering factors for hydrogen were the values given by Stewart, Davidson & Simpson (1965).

Discussion of the structure

This analysis has shown that the isomers provided by Drs Dummel and Kun have the 1*R*:2*R* and 1*S*:2*S* configurations for 1-fluoro-2-hydroxy-1,2,3-propanetricarboxylic acid (Cahn, Ingold & Prelog, 1966) and this is illustrated in Fig. 2 which is a view down the C(2)–C(3) bond (positions 1 and 2 respectively in the above formula). This result, that the active isomer is the *erythro*-isomer, had been deduced from chemical studies (Fanshier *et al.*, 1964). The structure analysis has also shown that the ion forms a tridentate chelate to the rubidium ion through F, O(2) of the carboxylic acid group adjacent to the fluorine, and O(7) of the hydroxyl group. The fact that the fluorine atom is on the coordination sphere around the rubidium ion is of interest because it implies that fluorine in no way behaves like a hydrogen atom in this salt but, by virtue of its electronegativity, is chelated to this metal, so that the chelation is not like that of citrate.

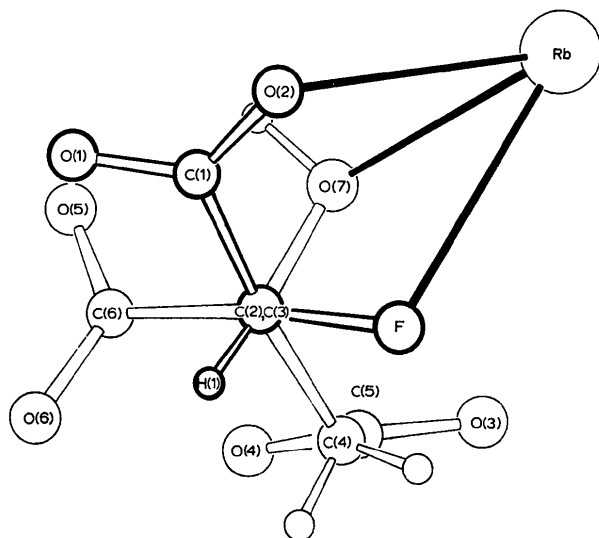


Fig. 2. View of the fluorocitrate anion down the C(2)–C(3) bond showing the chelation to a rubidium ion. Note that the central carboxyl group [involving O(5) and O(6)] is not involved in this chelation (contrary to the case for citrates and isocitrates). Only one optical isomer in the racemate is shown.

Interatomic distances and interbond angles are illustrated in Fig. 3 and a stereoview of the anion is given in Fig. 4. The planarities of various groupings

are shown in Table 5. The hydroxyl group is coplanar with the carboxyl group adjacent to it (plane 3) while the fluorine atom is 0.156 Å from the plane of the ad-

Table 4. Observed and calculated structure factors

Each entry lists, in order, k, |F_o|, and F_c. Unobserved reflections are denoted by an asterisk. Values of |F_o| have been corrected for absorption.

Table with multiple columns containing reflection indices (h, k, l), observed structure factors (|F_o|), and calculated structure factors (F_c). The table is organized into several sections based on the reflection indices, such as 0k0, 0k1, 0k2, etc., and includes various numerical values and signs.

adjacent carboxyl group (planes 1 and 4) so that there is an angle of 7.1° between the plane of the carboxyl group and the plane containing C(1) C(2) F. As seen in Table 3 and Fig. 4 the thermal motion of O(3) and O(4) is high. At this end of the anion there are two

possible conformations, one being present to the extent of 16% so that the grouping O(3) C(5) C(4) F(2) is nearly planar and the other, which is present to the extent of 84%, with differing positions for the group C(4) C(5) O(3) O(4).

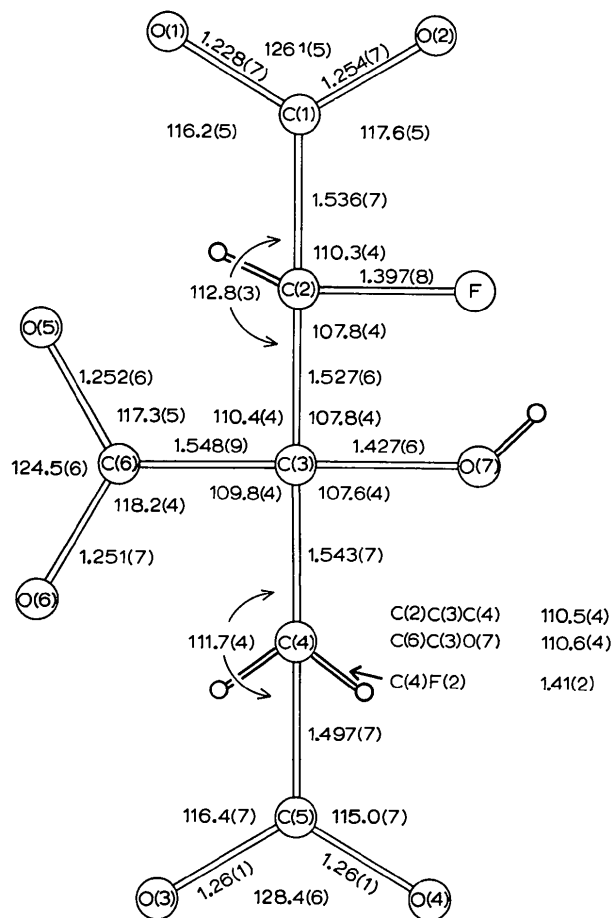


Fig. 3. Interatomic distances and interbond angles in the fluorocitrate anion. E.s.d. values are given in parentheses for the last digit shown. F(2) is attached to C(4), replacing a hydrogen atom, in 16% of unit cells.

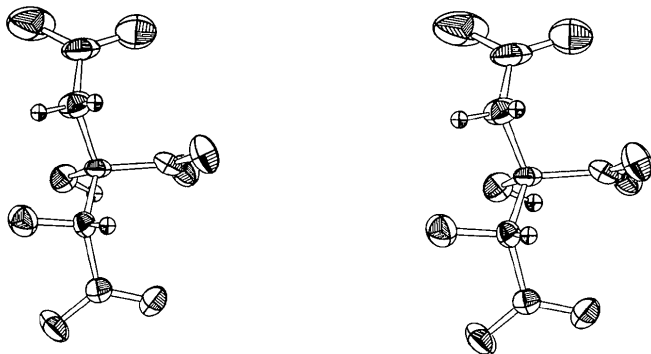


Fig. 4. Stereoview of the fluorocitrate ion (drawn by ORTEP).

Table 5. Planes through the molecule and deviations from these planes

Plane 1		Plane 2	
C(2)	0.060 Å	C(4)	-0.006 Å
F	-0.048	C(5)	0.023
C(1)	0.002	O(3)	-0.008
O(1)	-0.035	O(4)	-0.008
O(2)	0.022		
Plane 3		Plane 4	
O(5)	-0.004 Å	O(1)	-0.007 Å
O(6)	0.005	O(2)	0.002
O(7)	0.007	C(1)	0.002
C(6)	0.000	C(2)	0.002
C(3)	-0.009	F*	-0.156

* Not included in calculation of plane.

The coordinations of oxygen and fluorine atoms around the rubidium and ammonium ions are shown in Table 6 and Fig. 5. There are six oxygen atoms and two fluorine atoms around each rubidium ion and five oxygen atoms around each ammonium ion.

Table 6. Coordination around the rubidium cation and the hydrogen bonding

Rubidium coordinated to	Rb...O distance (Å)	Code	x	y	z
O(1 ⁱⁱ)	2.826 (4)	i	-x	-y	-z
O(1 ⁱⁱⁱ)	2.863 (4)	ii	x	y	-1+z
O(5 ⁱⁱⁱ)	2.902 (4)	iii	1-x	-y	1-z
O(7)	2.975 (4)	iv	-x	1-y	-z
F ⁱ	2.979 (5)	v	1-x	1-y	1-z
O(W2 ^{iv})	3.073 (5)	vi	x	1+y	z
F	3.095 (4)	vii	-x	1-y	1-z
O(2)	3.156 (5)	viii	x	y	1+z

Donor	Acceptor	O...O distance (Å)
O(W1)-H(W12)...	O(4 ^y)	2.735 (6)
O(W1)-H(W11)...	O(6 ⁱⁱ)	2.869 (7)
O(W2)-H(W21)...	O(2 ^{vi})	2.687 (7)
O(W2)-H(W22)...	O(6 ^{vi})	2.768 (5)
O(7)-H(7)...	O(2 ⁱⁱⁱ)	2.903 (5)

	N...O distance (Å)	
N-H(N1)...	O(W2)	2.945 (5)
N-H(N2)...	O(5 ^y)	2.918 (4)
N-H(N3)...	O(4 ⁱⁱ)	2.867 (8)
N-H(N4)...	O(3)	2.901 (8)
N.....	O(6 ⁱⁱ)*	3.032 (5)

* Not a hydrogen bond.

The crystal packing is such that an infinite chain of rubidium cations, alternatively coordinated through two fluorine atoms and two oxygen atoms, is formed

approximately parallel to the *a* direction. The distance between rubidium ions coordinated through the fluorine atoms is 4.189 Å while the distances between the rubidium ions that share the two oxygen atoms is 4.535 Å. In addition, the coordination sphere of the

two rubidium ions that share fluorine atoms are held together by a hydrogen bond between O(*W*2) and O(2). These infinite chains of coordination spheres are then hydrogen bonded to adjacent chains through O(*W*2), O(*W*1) and the ammonium cation.

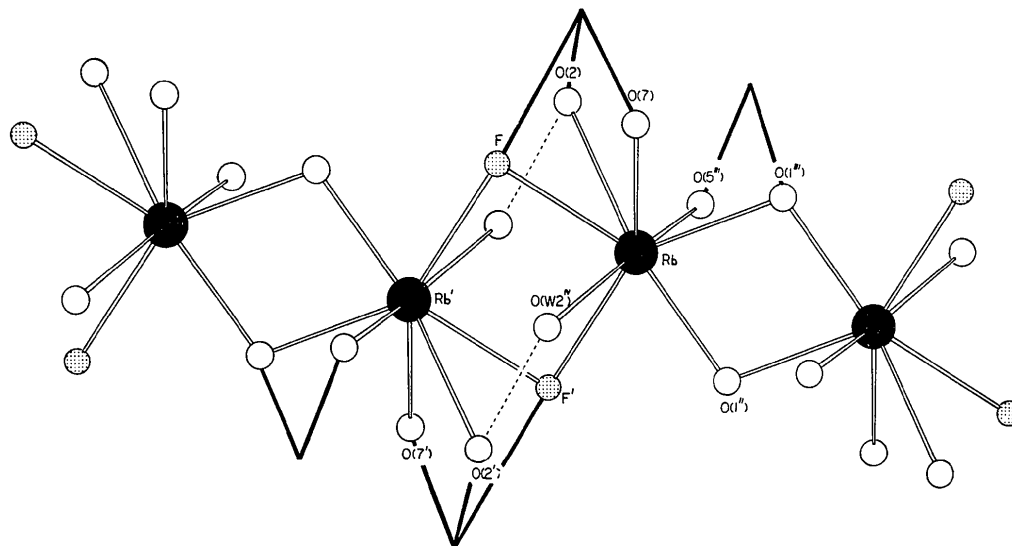


Fig. 5. Coordination around rubidium ions. The meanings of the superscripts are given in Table 5. The chains of rubidium polyhedra extend through the crystal almost parallel to the *a* axis.

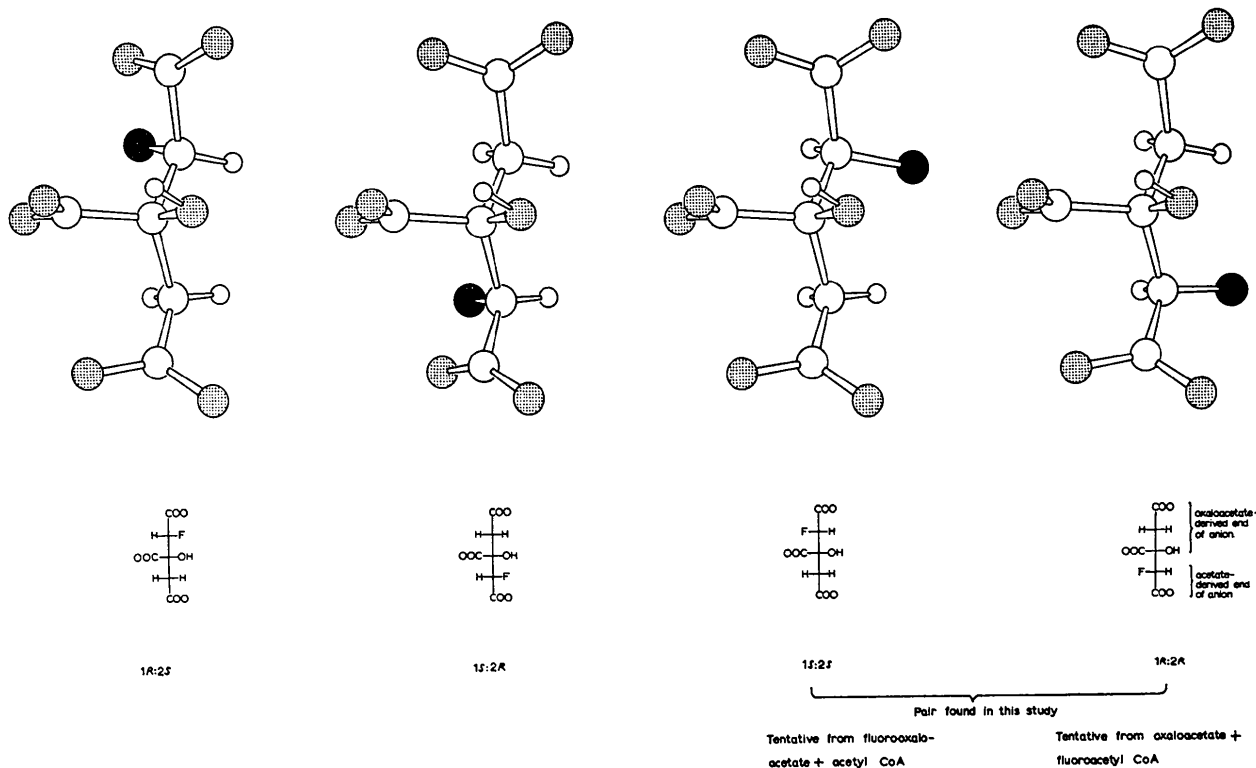


Fig. 6. The four isomers of fluorocitric acid. Direct views of the molecule and Fischer formulas are shown. The deduced absolute configuration of the isomer of fluorocitrate that inhibits and inactivates aconitase and that is formed *in vivo* from fluoroacetate in plants is shown.

The general packing of the molecule is shown in Fig. 1 which illustrates the disorder. The approximate mirror plane through O(7) C(3) C(6) O(5) O(6) is apparent in this figure so that 84% of the time O(7), O(2) and F are chelated to Rb^+ (dashed lines) and 16% of the time O(7), O(3) and F(2) are chelated to Rb^+ (in the position for NH_4^+). Details of hydrogen bonding are given in Table 5. The hydrogen atoms of the ammonium ion were located from peaks in the difference map. The ammonium ion has five nearest neighbors excluding the disordered fluorine atom.

The isomer of fluorocitrate that inhibits aconitase is formed *in vivo* from fluoroacetate in the reaction catalyzed by citrate (*si*)-synthase. This enzyme introduces the acetate group into the position of citrate which, on replacement of fluorine by hydrogen, would be in the lower half of each of the formulas for fluorocitrate illustrated in Fig. 6. If we assume that the stereochem-

istry for citrate (*si*)-synthase is the same for fluoroacetate as for acetate, then the active isomer must have a fluorine atom in one of the $2R$ configurations. This crystallographic study has demonstrated the $1R:2R$ and the $1S:2S$ configurations for the racemate. Thus it is concluded, with the above assumption that the action of citrate (*si*)-synthase yields the $2R$ -isomer, that the inhibitory isomer has the $1R:2R$ configuration.

It is found that only the $1R:2R$ isomer of fluorocitrate can fit in the active site of the enzyme aconitase in a manner similar to that proposed for citrate or isocitrate in an enzyme-ferrous-substrate ternary complex (Glusker, 1968). A comparison of the crystallographically determined conformations of the metal chelates of citrate, isocitrate, and the fluorocitrate is illustrated in Fig. 7. It is seen that the isocitrate and fluorocitrate chelates are similar in appearance. This may explain the competitive inhibition of aconitase by

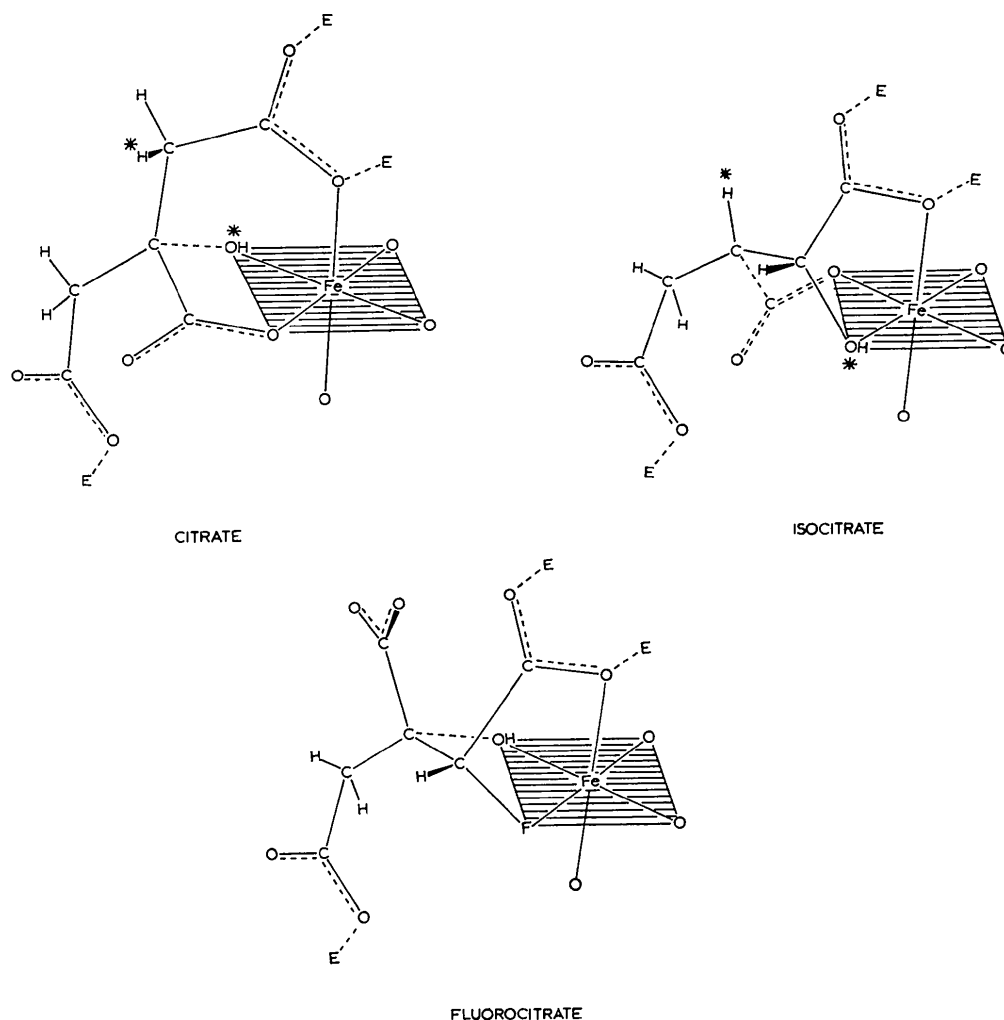


Fig. 7. A comparison of the binding of citrate and isocitrate as substrates to fluorocitrate as a competitive inhibitor of aconitase. An asterisk marks the hydrogen and hydroxyl group extracted during the enzymatic dehydration. *E* represents portions of the aconitase molecule. The conformations were determined from crystallographic studies of lithium ammonium hydrogen citrate monohydrate and potassium dihydrogen isocitrate, with that of the isomer of fluorocitrate described in this paper.

fluorocitrate when it chelates to the enzyme-bound ferrous iron in a manner similar to that of isocitrate.

The inactivation of aconitase with time is more complicated. It has been suggested that it is possibly due to alkylation at the active site following, for instance, cleavage of the C-F bond (Kun, 1969; Carrell *et al.*, 1970). Alternatively the central carboxyl group of fluorocitrate may hydrogen-bond to the hydrogen abstracting site. Further biochemical studies on this problem and on the effect of fluorocitrate on the tri-carboxylate carrier of mitochondria (Eanes *et al.*, 1972) are now in progress in various laboratories.

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The Crystal Structure of Lithium Formate Monohydrate

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The structure of $\text{LiHCOO} \cdot \text{H}_2\text{O}$ has been determined. The space group is $Pbn2_1$ with 4 molecules per unit cell. 1027 Mo $K\alpha$ reflexions gave a final R value of 4.9%. All hydrogen atoms were found.

Introduction

Though crystallographic data for lithium formate monohydrate were published long ago (Nitta, 1928), the structure was not known. Raman studies with polarized light (Cadène, 1970) suggested a tetrahedrally coordinated Li ion and formate ions in planes parallel to (100), forming hydrogen bonds 2.74 Å long. This is confirmed by the present work.

Experimental

Lithium formate was prepared by neutralizing a solution of formic acid in water with lithium carbonate.

After several days, long transparent needles separated from the solution. By cutting and etching with water a crystal approximately $0.3 \times 0.3 \times 0.4$ mm was obtained and sealed in a Lindemann-glass capillary tube to protect it from moisture. Data were collected on a Nonius automatic diffractometer at 20°C, using Zr-filtered Mo $K\alpha$ radiation. 1027 reflexions with $\theta < 40^\circ$ were measured using the $\theta/2\theta$ scanning technique; 166 had an intensity smaller than twice the standard deviation as estimated from counting statistics. The crystal was rotated about [001].

The systematic absences were: $0kl$ with $k = 2n + 1$; $h0l$ with $h + l = 2n + 1$, indicating the space group