# Metal Chelation versus Internal Hydrogen Bonding of the α-Hydroxy Carboxylate Group

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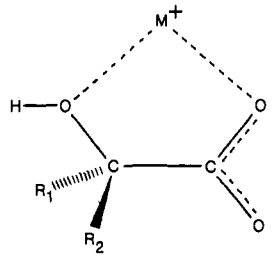
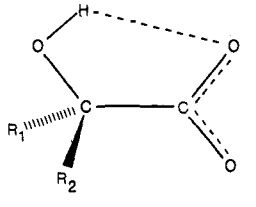
**Abstract:** This study analyzes metal chelation of the α-hydroxy carboxylate group and the effect of the replacement of the hydroxyl group by fluorine in some alkali-metal citrate salts. The structure of potassium citrate monohydrate shows that, unlike the case for sodium or many other salts of citrate, the metal ion does not span the α-hydroxy carboxylate group. In this case, as for free citric acid, the hydrogen atom from the hydroxyl group forms a hydrogen bond to an oxygen atom of the central carboxylate group. When no such hydroxyl hydrogen atom is available for hydrogen bonding, as in potassium 3-fluorodeoxycitrate, where the hydroxyl group is replaced by fluorine, the metal spans the α-fluoro carboxylate group so that the carbon-bound fluorine is part of the coordination sphere of the metal ion. This analysis involved crystal structure analyses by X-ray and neutron diffraction of tripotassium citrate monohydrate with unit cell dimensions $a$ = 13.795 (1), $b$ = 11.772 (1), $c$ = 7.092 (2) Å; $\beta$ = 112.57 (2)°; space group $P2_1/a$. In addition, X-ray diffraction studies were done on potassium 3-fluoro-3-deoxycitrate monohydrate, unit cell dimensions $a$ = 8.062 (2), $b$ = 10.215 (1), $c$ = 6.832 (1) Å; $\alpha$ = 96.27 (1), $\beta$ = 93.66 (1), $\gamma$ = 69.00 (1)°; space group $P\bar{1}$.

The α-hydroxy carboxylate grouping, found in citrates, malates, and many other naturally occurring organic salts, is an effective metal-chelating agent. In general, when the cation is of a suitable size, the entire chelate grouping (I) is approximately planar[1] and torsion angles between the hydroxyl and carboxyl groups are small.[2] The planarity of the α-hydroxy carboxylate group results




(I)




(II)

in a short nonbonded O···O distance (~2.6 Å with a van der Waals radius of 1.4 Å for oxygen). This short contact is not found to be relieved by rotation of the carboxyl group. No metal cation is present in the crystal structures of anhydrous citric acid[3,4] and its monohydrate.[5,6] In these structures the α-hydroxy carboxylate group is still planar. The hydrogen atom of the hydroxyl group forms a hydrogen bond to an oxygen atom of the carboxyl group of the α-hydroxy carboxylic acid and thus lies in a position related to that of the cation in metal citrates (II). This means that the hydroxyl group rotates in the free acid, as shown by comparison of I and II. These experimental results are in line with theoretical calculations[7] that show that rotations of the α-hydroxyl group cost less energy than distortions that lead to a nonplanar α-hydroxy carboxylate group.

We present here the X-ray and neutron structure determinations of a citrate, tripotassium citrate monohydrate, in which the metal cation does not span the α-hydroxy carboxylate group, and in the absence of this cation, the hydroxyl hydrogen atom forms a hydrogen bond to the α-carboxyl group, as found in the two forms of the free acid. However, in the case of potassium 3-fluorodeoxycitrate, the metal cation does span the α-fluoro carboxylate grouping. In this case the group adjacent to the carboxyl group has no available hydrogen atom to form a hydrogen bond. These results imply that either a hydrogen or a cation will span an α-hydroxy carboxylate or α-fluoro carboxylate group if at all possible.

## Experimental Section

**(a) Tripotassium Citrate Monohydrate. X-ray Study.** Crystals of potassium citrate monohydrate were grown from water layered with a small amount of ethanol. Unit cell dimensions have been described by Burns and Iball[8] [$a$ = 7.06 (3), $b$ = 11.72 (5), $c$ = 13.69 (7) Å; $\beta$ = 112.0°; $Z$ = 4, experimental density $D_x$ = 2.00 g·cm$^{-3}$; space group $P2_1/c$] and Love and Patterson[9] ($D_x$ = 1.991 g·cm$^{-3}$, pH 7.30). Our experimental measurements gave $a$ = 13.795 (1), $b$ = 11.772 (1), $c$ = 7.092 (2) Å; $\beta$ = 112.57 (2)°; the space group in this setting was $P2_1/a$.

Three-dimensional X-ray diffraction data were collected on a Syntex $P2_1$ diffractometer with monochromatic Mo Kα radiation (λ = 0.71069 Å) by the $\theta/2\theta$ scan technique. Intensities were measured for 4725 unique reflections in the range sin $\theta/\lambda$ = 0–0.81 Å$^{-1}$. Values for $\sigma(I)$ were derived from counting statistics. There were 3904 reflections for which the measured intensity, $I_0$, was greater than $1.50\sigma(I)$, and these were considered to be above the threshold of observation. Values of $\sigma(F)$ for observed data were calculated from the formula $\sigma(F) = (F/2)[\sigma^2(I)/I^2 + \delta^2]^{1/2}$, where $\delta$ is a measured instrumental uncertainty, 0.0320,

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Table I. Atomic Coordinates as Fractions of Unit Cell Edges with Estimated Standard Deviations from the Least-Squares Refinement[a]

| | x | y | z | | x | y | z |
|---|---|---|---|---|---|---|---|
| (a) Tripotassium Chloride Monohydrate[a] | | | | | | | |
| K(1) X | 0.11410 (2) | 0.16003 (2) | 0.10135 (4) | C(2) X | 0.1659 (1) | 0.7897 (1) | 0.3681 (2) |
| K(1) N | 0.1155 (2) | 0.1620 (2) | 0.1027 (5) | C(2) N | 0.1651 (1) | 0.7911 (1) | 0.3667 (2) |
| K(2) X | 0.38614 (2) | 0.44331 (2) | 0.27360 (4) | C(3) X | 0.1513 (1) | 0.6811 (1) | 0.2420 (2) |
| K(2) N | 0.3861 (2) | 0.4408 (3) | 0.2691 (5) | C(3) N | 0.1507 (1) | 0.6825 (1) | 0.2407 (2) |
| K(3) X | 0.11469 (2) | 0.42940 (2) | 0.78263 (4) | C(4) X | 0.1801 (1) | 0.5812 (1) | 0.3959 (2) |
| K(3) N | 0.1148 (2) | 0.4311 (2) | 0.7845 (4) | C(4) N | 0.1790 (1) | 0.5827 (1) | 0.3932 (2) |
| O(W) X | 0.0382 (1) | 0.2097 (1) | 0.4014 (2) | C(5) X | 0.1353 (1) | 0.4676 (1) | 0.2982 (2) |
| O(W) N | 0.0398 (2) | 0.2114 (2) | 0.4056 (3) | C(5) N | 0.1354 (1) | 0.4687 (1) | 0.2972 (2) |
| O(1) X | 0.1758 (1) | 0.9267 (1) | 0.1272 (2) | C(6) X | 0.2207 (1) | 0.6768 (1) | 0.1133 (2) |
| O(1) N | 0.1760 (2) | 0.9285 (2) | 0.1309 (3) | C(6) N | 0.2201 (1) | 0.6790 (1) | 0.1141 (2) |
| O(2) X | 0.0610 (1) | 0.9568 (1) | 0.2747 (2) | H(1) X | 0.122 (2) | 0.783 (2) | 0.451 (3) |
| O(2) N | 0.0620 (2) | 0.9574 (2) | 0.2755 (4) | H(1) N | 0.1178 (4) | 0.7800 (3) | 0.4589 (6) |
| O(3) X | 0.1775 (1) | 0.4174 (1) | 0.1931 (2) | H(2) X | 0.238 (2) | 0.788 (2) | 0.451 (4) |
| O(3) N | 0.1778 (1) | 0.4201 (1) | 0.1932 (3) | H(2) N | 0.2474 (3) | 0.7978 (3) | 0.4654 (6) |
| O(4) X | 0.0567 (1) | 0.4314 (1) | 0.3316 (2) | H(3) X | 0.154 (2) | 0.599 (2) | 0.506 (3) |
| O(4) N | 0.0586 (2) | 0.4320 (2) | 0.3317 (4) | H(3) N | 0.1471 (4) | 0.6028 (3) | 0.5096 (5) |
| O(5) X | 0.3190 (1) | 0.6883 (1) | 0.2090 (2) | H(4) X | 0.255 (2) | 0.587 (2) | 0.460 (4) |
| O(5) N | 0.3174 (1) | 0.6891 (2) | 0.2080 (3) | H(4) N | 0.2653 (3) | 0.5779 (3) | 0.4673 (5) |
| O(6) X | 0.1756 (1) | 0.6585 (1) | −0.0736 (2) | H(5) X | 0.043 (2) | 0.662 (2) | 0.001 (4) |
| O(6) N | 0.1757 (1) | 0.6627 (2) | −0.0705 (3) | H(5) N | 0.0438 (3) | 0.6502 (3) | −0.0220 (5) |
| O(7) X | 0.0431 (1) | 0.6734 (1) | 0.1072 (2) | H(WA) X | 0.081 (2) | 0.200 (2) | 0.515 (4) |
| O(7) N | 0.0436 (1) | 0.6745 (1) | 0.1076 (2) | H(WA) N | 0.0929 (3) | 0.2027 (3) | 0.5418 (6) |
| C(1) X | 0.1312 (1) | 0.8992 (1) | 0.2459 (2) | H(W2) X | 0.037 (2) | 0.281 (2) | 0.383 (4) |
| C(1) N | 0.1312 (1) | 0.9008 (1) | 0.2468 (2) | H(WB) N | 0.0391 (3) | 0.2918 (3) | 0.3719 (5) |
| (b) Dipotassium 3-Fluoro-3-deoxycitrate Monohydrate | | | | | | | |
| K(1) | 0.67309 (7) | 0.43725 (6) | 0.24947 (8) | C(3) | 0.1264 (3) | 0.7855 (3) | 0.1972 (4) |
| K(2) | 0.23271 (7) | 0.30906 (6) | 0.14966 (8) | C(4) | 0.1743 (3) | 0.9164 (3) | 0.1993 (4) |
| F | 0.0693 (2) | 0.7552 (2) | 0.0015 (2) | C(5) | 0.3272 (4) | 0.9007 (3) | 0.0691 (4) |
| O(W) | 0.5729 (5) | 0.8445 (4) | 0.5325 (5) | C(6) | 0.2848 (3) | 0.6565 (3) | 0.2567 (4) |
| O(1) | 0.0023 (3) | 0.6202 (2) | 0.5130 (3) | HW(1) | 0.597* | 0.893* | 0.667* |
| O(2) | −0.1373 (3) | 0.6242 (3) | 0.2214 (3) | HW(2) | 0.504 (7) | 0.810 (5) | 0.498 (8) |
| O(3) | 0.3561 (3) | 1.0159 (2) | 0.0650 (4) | H(O1) | 0.000** | 0.500** | 0.500** |
| O(4) | 0.4123 (3) | 0.7890 (2) | −0.0198 (4) | H(O3) | 0.500** | 1.000** | 0.000** |
| O(5) | 0.3692 (3) | 0.6774 (2) | 0.4095 (3) | H(1C2) | −0.001 (4) | 0.848 (3) | 0.453 (5) |
| O(6) | 0.3110 (3) | 0.5422 (2) | 0.1549 (3) | H(2C2) | −0.153 (4) | 0.886 (3) | 0.266 (5) |
| C(1) | −0.0638 (3) | 0.6772 (3) | 0.3507 (4) | H(1C4) | 0.218 (4) | 0.943 (3) | 0.341 (5) |
| C(2) | −0.0322 (4) | 0.8127 (3) | 0.3287 (4) | H(2C4) | 0.075 (4) | 0.994 (3) | 0.171 (5) |

[a] The results of the X-ray study are designated X and of the neutron study are designated N. Different crystals and conditions apply to the X-ray and neutron studies. We have used neutron coordinates for hydrogen atoms and X-ray coordinates for other atoms. Key: *, computed position; **, coordinates (with half-occupancy) set by symmetry.

determined from the variation in the intensities of the standard reflections. The intensity falloff during data collection, as indicated by the periodically measured standard reflections, was negligible. The data were converted to structure amplitudes by application of Lorentz and polarization factors and placed on an absolute scale with a Wilson plot.

The structure was solved by use of a sharpened three-dimensional Patterson map to find potassium ions and some oxygen atoms. Fourier syntheses were used to find the other oxygen and carbon atoms, and a Fourier difference map was used to find hydrogen atoms. Scattering factors were taken from ref[10] and anomalous scattering factors were those listed by Cromer and Liberman.[11] The final $R$ value was 0.036 for 3904 reflections (weighted $R$ value 0.042). Atomic coordinates are listed in Table Ia, and temperature factors and observed and calculated structure factors (Tables A–C) are in the supplementary material.

**(b) Tripotassium Citrate Monohydrate. Neutron Study.** In order to locate hydrogen atoms with certainty a neutron diffraction study was also done. The crystal, an approximate rectangular prism 5 × 3 × 2 mm, was grown by evaporation of a solution in pure water over a 4-month period. It was then sealed with a coating of formvar.

Neutron data were collected at 294 (1) K with the 2TANA four-circle diffractometer at the 10 MW HIFAR ("DIDO") research reactor, Lucas Heights, Australia (hole 2 tan $A$). The wavelength used was 1.237 (2) Å. Each peak was scanned by the $2\theta$–$\omega$ method (in steps of 0.01°) over 2° in $2\theta$. The intensity of one standard every 25 reflections remained within $\pm 6\sigma$ of the overall standard average; trends within this range were detected and the corresponding reflection intensities corrected by a cumulative intensity technique.[12] Of the 4044 reflections measured to a maximum $\sin\theta/\lambda = 0.70$ Å$^{-1}$, 3707 were considered to be above the threshold of observation; these were weighted by the function $1/(\sigma^2(F) + 0.002F^2)$. No absorption correction was applied. Scattering lengths used were $b_c = 6.6484$, $b_H = -3.7423$, $b_k = 3.67$, $b_o = 5.805$ fm.[13]

Structural parameters were least squares refined starting from values determined by X-ray analysis. All hydrogen atom positions were readily obtained. The final $R$ values were 0.059 (weighted $R$ value 0.064). Atomic coordinates are listed in Table Ia, and temperature factors and observed and calculated structure factors (Tables D and E) are in the supplementary material.

**(c) Preparation of Dipotassium 3-Fluorodeoxycitrate Monohydrate.** 3-Fluorodeoxycitric acid was prepared at the Merck Sharp and Dohme Research Laboratories by Drs. J. Kollonitsch and S. Marburg[14] by fluorodehydroxylation of citric acid. The method, called "fluorodehydroxylation" has been described by Kollonitsch, Marburg, and Perkins.[15] 3-Fluorodeoxycitric acid was isolated as its crystalline bis(dicyclohexylamine) salt. The dicyclohexylamine salt of 3-fluoro-3-deoxycitric acid (25 mg) was dissolved in methanolic KOH in a 12-mL screw-cap vial and warmed to 40 °C for $^1/_2$ h. After cooling, diethyl ether (5 mL) was carefully layered on top of this solution, and the vial was closed. Crystals formed over 8 days.

**(d) Dipotassium 3-Fluoro-3-deoxycitrate Monohydrate. X-ray Study.** The crystals were triclinic, space group $P\bar{1}$. Cell parameters, measured from 14 centered reflections with $24° > \theta < 47°$, were $a = 8.062$ (2), $b = 10.215$ (1), $c = 6.832$ (1) Å; $\alpha = 96.27$ (1), $\beta = 93.66$ (1), $\gamma = 69.00$ (1)°; $V = 521.95$ (15) Å$^3$; $Z = 2$, $D_x = 1.840$ Mg·m$^{-3}$.

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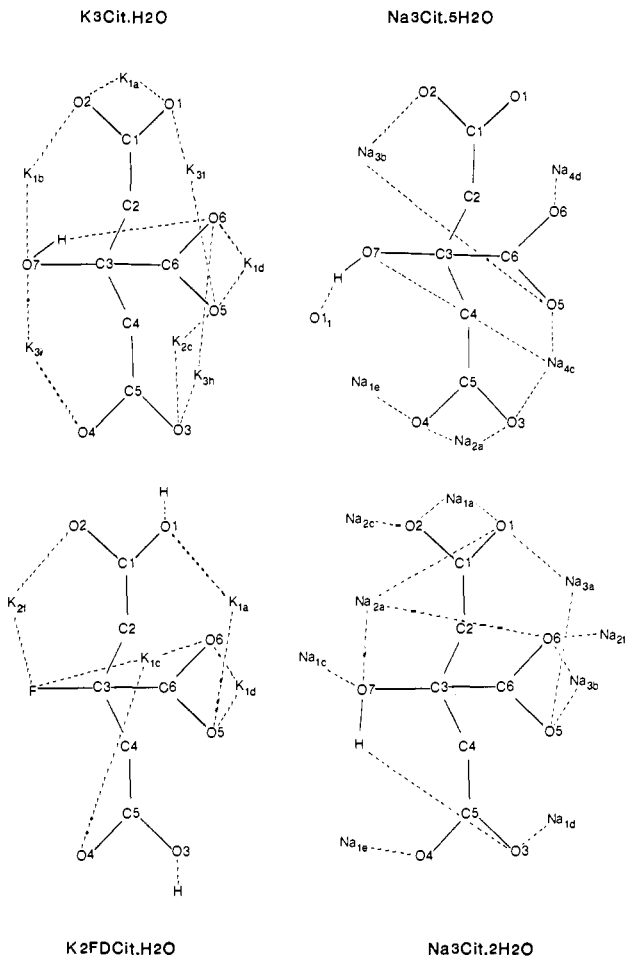


**Figure 1.** Coordination schemes of tripotassium citrate monohydrate ($K_3Cit·H_2O$), dipotassium 3-fluoro-3-deoxycitrate monohydrate ($K_2FDCit·H_2O$), and two forms of sodium citrate ($Na_3Cit·5H_2O$ and $Na_3Cit·2H_2O$) showing the ways by which metal is chelated. Designations *a*–*i* are given in Table II.

X-ray diffraction data were collected at room temperature (293 K) on a single crystal (a thin prism, 0.3 × 0.2 × 0.08 mm) with a Nicolet P3 diffractometer and graphite-monochromated Cu Kα radiation (λ = 1.5418 Å). The θ–2θ scan mode was employed. A Ψ-scan absorption correction was applied. 2123 reflections were measured in 2θ range 0–139°, $(\sin\theta/\lambda)_{max}$ = 0.6075 Å$^{-1}$. Four standard reflections were checked every 92 reflections; no significant intensity deviation was observed during the data collection, and $R_{int}$ = 0.012 for averaged equivalent reflections. A total of 1972 unique reflections with $I \geq 2.00\sigma(I)$ was used in subsequent analysis.

The structure was solved by Patterson and Fourier methods and refined by a full-matrix least-squares anisotropic refinement on *F*. Hydrogen atom positions were determined from difference Fourier maps and were refined isotropically. One of the hydrogen atoms of the water molecule was not clearly defined in the difference map, and therefore, its position was computed to be along a most probable hydrogen-bonding direction; its position is marked by an asterisk in the tables. The final *R* value was 0.060 (weighted *R* 0.082).

Atomic coordinates are listed in Table Ib, and temperature factors and observed and calculated structure factors (Tables F–H) are in the supplementary materials.

## Results

Bond lengths and interbond angles in potassium citrate and potassium fluorodeoxycitrate are illustrated in Figure A (supplementary material). The coordination schemes of tripotassium citrate monohydrate and dipotassium 3-fluoro-3-deoxycitrate monohydrate are illustrated in Figure 1 and are compared with those of two forms of sodium citrate. The coordination spheres for the potassium ions are compared in Table II. Only in the fluoro compound does the potassium ion span the α-hydroxy carboxylate group; in potassium citrate the hydrogen atom of the hydroxyl spans this group. Coordination of metal ions by fluorine in a C–F bond has been noted in several cases involving alkali-metal cations and appears to be a general phenomenon.[16] An

**Table II.** Analysis of Charge Using the Method of Brown[a]

| | K(H)···O, Å | sym-metry | positive charge on O | total |
|---|---|---|---|---|
| (a) Tripotassium Citrate (X-ray Study) | | | | |
| K(1)···O(1) | 2.862 | *a* | 0.12 | |
| O(2) | 2.902 | *a* | 0.11 | |
| O(2) | 3.147 | *b* | 0.05 | |
| O(3) | 3.151 | *c* | 0.05 | |
| O(5) | 2.713 | *d* | 0.20 | |
| O(6) | 2.983 | *d* | 0.09 | |
| O(7) | 2.878 | *b* | 0.12 | |
| O(W) | 2.773 | *c* | 0.17 | 0.91 |
| K(2)···O(1) | 2.645 | *d* | 0.26 | |
| O(2) | 2.679 | *e* | 0.23 | |
| O(2) | 3.007 | *f* | 0.08 | |
| O(3) | 2.728 | *c* | 0.19 | |
| O(5) | 3.014 | *c* | 0.08 | |
| O(W) | 2.647 | *g* | 0.25 | 1.09 |
| K(3)···O(1) | 2.705 | *f* | 0.21 | |
| O(3) | 2.702 | *h* | 0.21 | |
| O(4) | 2.984 | *c* | 0.08 | |
| O(4) | 2.735 | *i* | 0.19 | |
| O(5) | 2.971 | *f* | 0.09 | |
| O(6) | 2.889 | *h* | 0.11 | |
| O(7) | 2.849 | *i* | 0.13 | 1.02 |
| H(O7)···O(6) | 1.98 | *c* | 0.16 | |
| H(W1)···O(5) | 1.74 | *d* | 0.22 | |
| H(W2)···O(4) | 1.71 | *c* | 0.23 | |
| (b) Dipotassium Fluorodeoxycitrate | | | | |
| K(1)···O(1) | 2.897 | *a* | 0.11 | |
| O(2) | 2.819 | *b* | 0.14 | |
| O(4) | 2.913 | *c* | 0.11 | |
| O(5) | 2.810 | *a* | 0.15 | |
| O(5) | 2.944 | *d* | 0.10 | |
| O(6) | 2.785 | *d* | 0.16 | |
| O(6) | 2.808 | *c* | 0.15 | |
| F | 2.812 | *c* | 0.11 | 1.03 |
| K(2)···O(1) | 2.939 | *e* | 0.10 | |
| O(2) | 2.690 | *f* | 0.22 | |
| O(3) | 2.802 | *g* | 0.15 | |
| O(4) | 2.838 | *c* | 0.13 | |
| O(6) | 2.670 | *d* | 0.23 | |
| F | 2.846 | *f* | 0.10 | 0.93 |
| H(O1)···O(1) | 1.23 | *e* | 0.47 | |
| H(O3)···O(3) | 1.24 | *i* | 0.46 | |
| H(W1)···O(3) | 2.02[b] | *h* | 0.16 | |
| H(W2)···O(5) | 2.03 | *c* | 0.16 | |

| tripotassium citrate | accum charges | dipotassium fluorodeoxycitrate | accum charges |
|---|---|---|---|
| O(1) | 0.59 | O(1)–H | 0.68 |
| O(2)$^-$ | 0.47 | O(2) | 0.36 |
| O(3)$^-$ | 0.45 | O(3)–H | 0.77 |
| O(4)$^-$ | 0.50 | O(4) | 0.24 |
| O(5)$^-$ | 0.59 | O(5)$^-$ | 0.41 |
| O(6)$^-$ | 0.36 | O(6)$^-$ | 0.53 |
| O(7) | 0.25 | O(7) | |
| F | | F | 0.21 |

| symmetry | tripotassium citrate | dipotassium fluorodeoxycitrate |
|---|---|---|
| *a* | *x*, *y* − 1, *z* | 1 − *x*, 1 − *y*, 1 − *z* |
| *b* | −*x*, 1 − *y*, −*z* | 1 − *x*, *y*, *z* |
| *c* | *x*, *y*, *z* | 1 − *x*, 1 − *y*, −*z* |
| *d* | $^1/_2 - x, y - {}^1/_2, -z$ | *x*, *y*, *z* |
| *e* | $^1/_2 - x, y - {}^1/_2, 1 - z$ | −*x*, 1 − *y*, 1 − *z* |
| *f* | $^1/_2 + x, {}^3/_2 - y, z$ | −*x*, 1 − *y*, −*z* |
| *g* | $^1/_2 + x, {}^1/_2 - y, z$ | *x*, *y* − 1, *z* |
| *h* | *x*, *y*, *z* + 1 | 1 − *x*, 2 − *y*, 1 − *z* |
| *i* | −*x*, 1 − *y*, 1 − *z* | 1 − *x*, 2 − *y*, −*z* |

[a] $S = (R/R_o)^{-N}$ where, for bonds to oxygen, $R_o$ = 2.276 for K, *N* = 9.1; for bonds to F, $R_o$ = 1.420 and *N* = 3.27; and for H, $R_o$ = 0.87, *N* = 2.2. [b] Computed position for H(W1).

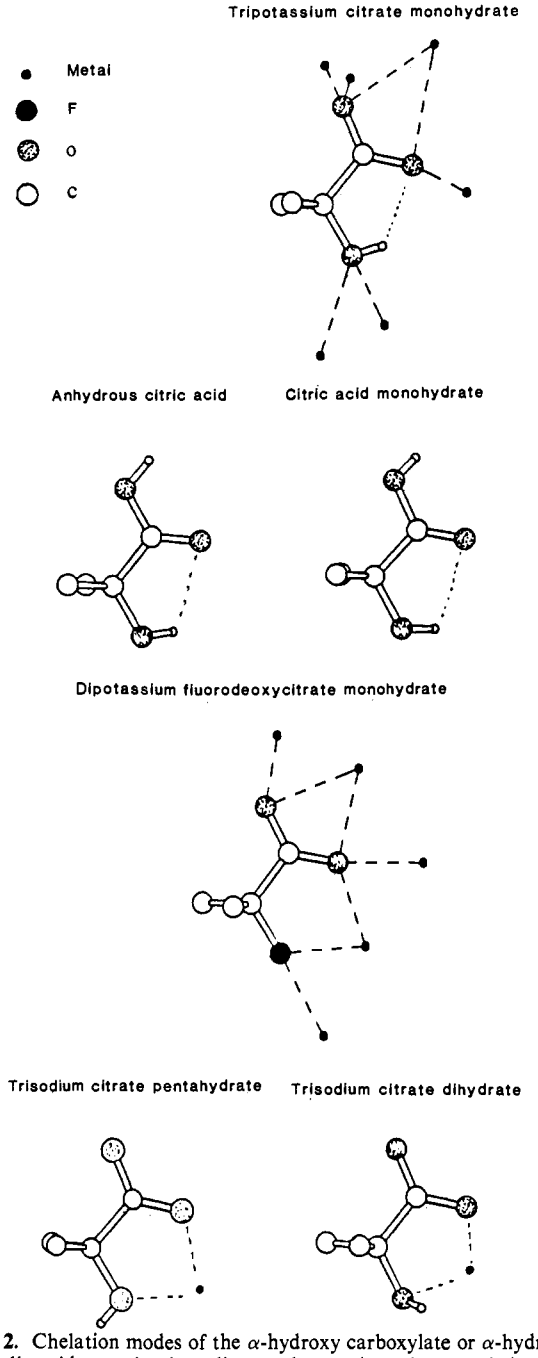


**Figure 2.** Chelation modes of the $\alpha$-hydroxy carboxylate or $\alpha$-hydroxy carboxylic acid grouping in sodium and potassium citrates, citric acid, and potassium fluorodeoxycitrate. The views are onto the plane through O(5), O(6), and O(7).

analysis of the charge on oxygen and fluorine atoms in the various areas of the anion, computed from the distances to the cation, has been made using the formulas of Brown.[17] These numbers were computed by using the fact that a cation of charge +1 will coordinate around itself oxygen (and fluorine) atoms with an accumulated charge of −1. The distance of each atom to the cation gives a measure of partial charge that this atom contributes to the sum; shorter distances imply more of the share of negative charge on that atom. The results, which are very approximate, are listed in Table II. When the carboxylate is ionized, the charge

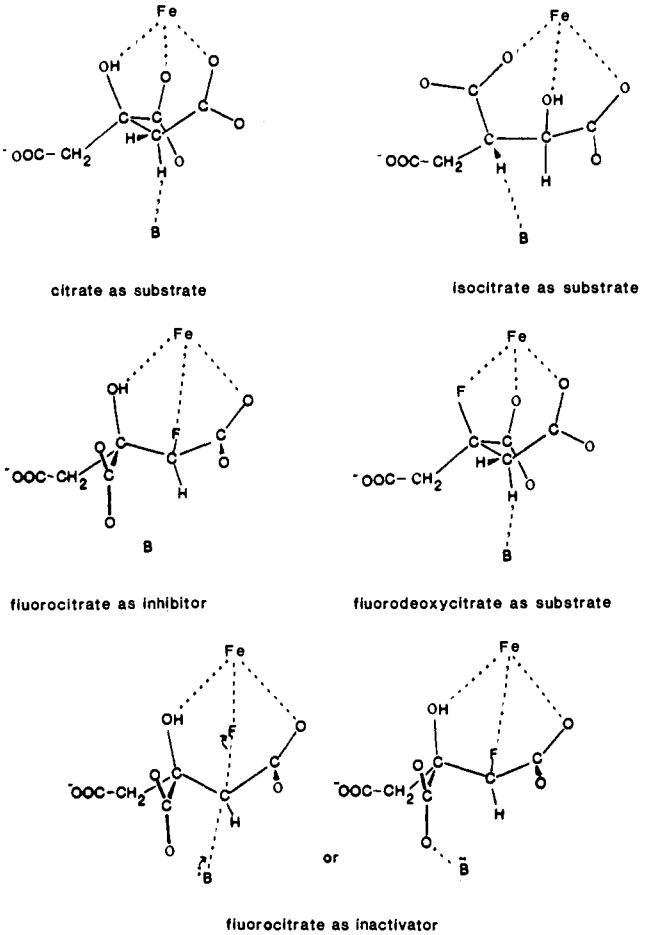


**Figure 3.** Modes of chelation of citrate, isocitrate, 2-fluorocitrate, and 3-fluorodeoxycitrate as envisioned in the active site of the enzyme aconitase.

on each oxygen atom is approximately 0.5e; the charges on O(7) and F are low but similar.

Each structure contains water of crystallization, and the hydrogen-bonding schemes are listed in Table III. It is interesting that in the citrate, where the hydrogen atoms have been fairly precisely located by the neutron diffraction analysis, the hydrogen H(5) of the hydroxyl group is near two oxygen atoms; these are one of the oxygen atoms [O(6)] of the same $\alpha$-hydroxy carboxylate group (1.97 Å) and one of the oxygen atoms of a terminal carboxyl group [O(4)] of another molecule (2.32 Å).[18] The system is approximately planar, with H(5) only 0.02 Å from the plane of the three oxygen atoms. In the fluorodeoxycitrate there are pairs of short hydrogen bonds across centers of symmetry; we have positioned the hydrogen atoms (at 50% occupancy per asymmetric unit) at the center of symmetry although they may, in fact, be slightly displaced from these locations.

## Discussion

We have shown that in citrates the $\alpha$-hydroxy carboxylate group accommodates a cation that may be chelated at two points, at the hydroxyl group and at one of the carboxyl oxygen atoms; it may also bind at an additional point, such as an oxygen atom of a terminal carboxyl group. If the $\alpha$-hydroxy carboxylate group does not bind a cation, as is necessarily the case of the free acid (anhydrous and monohydrate), then the hydrogen atom of the hydroxyl group is found to form an internal hydrogen bond across the $\alpha$-hydroxy carboxylate group. A comparison of the geometry of this chelation in a series of citrates is documented in Figure 2. Our data so far only apply, in the case of the fluoro compounds, to alkali-metal salts.

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Table III. Hydrogen Bonding

| atoms D-H···A[a] | equiv posn of A | length, Å H···A | length, Å D···A | angle ∠D-H···A, deg |
|---|---|---|---|---|
| Potassium Citrate Hydrate | | | | |
| O(7)-H(5)···O(6) | $x, y, z$ | 1.965 | 2.564 | 118.4 |
| O(7)-H(5)···O(4) | $-x, 1-y, -z$ | 2.319 | 3.144 | 143.7 |
| O(W)-H(W1)···O(5) | $^1/_2-x, y-^1/_2, -z$ | 1.740 | 2.709 | 176.6 |
| O(W)-H(W2)···O(4) | $x, y, z$ | 1.705 | 2.669 | 171.1 |
| Potassium Fluorodeoxycitrate Hydrate | | | | |
| O(1)-HO(1)*···O(1′) | $-x, 1-y, 1-z$ | 1.23 | 2.454 | (180) |
| O(3)-HO(3)*···O(3′) | $1-x, 2-y, -z$ | 1.24 | 2.480 | (180) |
| O(W)-HW(1)**···O(3) | $1-x, 1-y, 1-z$ | 2.02 | 3.048 | (178) |
| O(W)-HW(2)···O(5) | $x, y, z$ | 2.03 | 2.774 | 167 |

[a] Key: *, symmetrical positions; **, calculated position.

The action of 3-fluorodeoxycitrate on the enzyme aconitase, for which citrate and isocitrate are substrates, is of interest.[19,20] The fluorodeoxycitrate is acted on by this enzyme and thus appears to act somewhat like a substrate; the C-F bond is broken to yield fluoride and *cis*-aconitate. However, the C-F bond is not formed again (unlike the case of C-OH bond breaking and remaking in citrate). As a result, fluorodeoxycitrate cannot be described as a true substrate of the enzyme. These findings, it is suggested,[19] favor a carbanion mechanism for aconitase action with proton abstraction as the first step, followed by hydroxyl elimination. This is in line without our proposals on a mode of action of the enzyme[21,22] as illustrated in Figure 3. It appears that some group in the enzyme must chelate the fluorodeoxycitrate and citrate in an analogous manner. Our work on another fluorocitrate, which is a powerful inhibitor, also backs up this idea and, as illustrated in Figure 3, explains why only one of four possible stereoisomers is such a potent inhibitor.[16] Our experimental observations of tridentate chelation of fluorocitrate and fluorodeoxycitrate are crucial to these ideas.

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**Supplementary Material Available:** Tables of X-ray and neutron data of atomic coordinates and temperature factors and representations of bond lengths and interbond angles in potassium citrate and potassium 3-fluoro-3-deoxycitrate (23 pages); tables of observed and calculated structure factors (55 pages). Ordering information is given on any current masthead page.

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# Synthesis and Evaluation of Phospholipid Analogues as Inhibitors of Cobra Venom Phospholipase $A_2$

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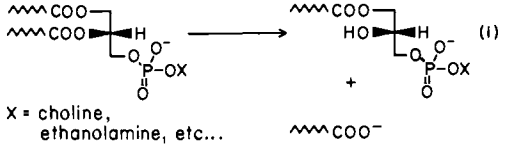
**Abstract:** Analogues of phospholipids that contain fluoro ketone, ketone, and alcohol replacements for the ester at the 2-position of the glycerol backbone have been prepared and analyzed as inhibitors of phospholipase $A_2$ from *Naja naja naja* venom. Phospholipid analogues were studied that contain two alkyl chains as well as single chain compounds that lack carbon-1 of the glycerol backbone and the attached acyl unit. Compounds that contain both long and medium length alkyl chains were studied. All of the potential inhibitors were tested in a well-defined mixed micelle system in which both the substrates and the inhibitors have been incorporated into Triton X-100 micelles. Surprisingly, the best inhibitors studied were the single chain fluoro ketones despite the fact that the enzyme has a strong preference for two-chain lipids. The most potent compound was found to have a dissociation constant some 600–3000-fold lower than the Michaelis constant for dipalmitoyl phosphatidylcholine substrate. $^{19}F$ NMR studies of the fluoro ketone phospholipid analogues in micelles show that whereas the single chain compounds are partially in the hydrated-ketone form, the two-chain compounds are less than 0.1% hydrated. In every case studied, potent inhibition of phospholipase $A_2$ was observed only with those compounds that are significantly hydrated in the micelle, and it is suggested that the hydrated fluoro ketone containing phospholipid analogues are the species responsible for the inhibition. In addition, the single chain fluoro ketones were better inhibitors than single and double chain alcohol and ketone analogues. Previous studies have shown that the cobra venom enzyme is activated by choline-containing lipids, and evidence is presented for the binding of the hydrated fluoro ketone inhibitors selectively to the activated enzyme.

Phospholipase $A_2$ catalyzes the hydrolysis of phospholipids at the 2-position of the glycerol backbone to produce a free fatty acid and a *lyso*-phospholipid (eq 1). The enzymes from pancreas




(1)

and snake venoms have been studied extensively[2] including the determination of the three-dimensional structure by X-ray diffraction for three enzymes.[3] All of these extracellular enzymes require millimolar concentrations of calcium for optimal activity.

There is considerable recent interest in intracellular phospholipases $A_2$ since these enzymes are currently thought to control

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