Acta Cryst. (1972). B28, 419

X-ray Crystal Analysis of the Substrates of Aconitase. X. The Structure of Dipotassium *cis*-Aconitate*

BY JENNY PICKWORTH GLUSKER, W. OREHOWSKY JR, CAROL ANN CASCIATO AND H. L. CARRELL

The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111, *U.S.A.*

(*Received* 3 *May* 1971)

Dipotassium hydrogen *cis*-aconitate crystallizes in the monoclinic system in the space group $P2_1/c$. The unit-cell dimensions are $a = 9{\cdot}417 \pm 0{\cdot}007$, $b = 12{\cdot}421 \pm 0{\cdot}020$, $c = 7{\cdot}615 \pm 0{\cdot}009$ Å, $\beta = 96{\cdot}27 \pm 0{\cdot}07°$. There are four units of $K_2C_6O_6H_4$ in the unit cell. 2048 independent data, of which 170 were below the threshold of measurement, were collected on an automatic diffractometer with Mo $K\alpha$ radiation (to $2\theta = 55°$). The structure was solved by the symbolic addition method and refined to an R value of 0·025. All hydrogen atoms were located from a difference map and were refined. The structure contains a short internal hydrogen bond (2·425 Å). The hydrogen atom in this hydrogen bond appears as an elongated peak in a difference map. Each potassium ion is surrounded by seven oxygen atoms to a distance of 3·1 Å.

The enzyme aconitase catalyzes the interconversion of citrate, isocitrate and *cis*-aconitate in the Krebs cycle by elimination or addition of the elements of water in a stereospecific manner, or by direct isomerization of citrate and isocitrate. An analysis of the conformations of citrate and isocitrate ions in the crystalline state has led to a hypothesis on the mechanism of the action of the enzyme (Glusker, 1968) which accounts for the stereospecificity of the enzyme and involves a conformational change in the enzyme-ferrous-*cis*-aconitate complex. Therefore, in order to test this hypothesis, shown in Fig. 1, a study of the most likely conformation of the *cis*-aconitate ion was required. For this reason the structure of a crystalline potassium salt was determined.

* A preliminary account of this work was given at a meeting of the American Crystallographic Association, Tulane University, New Orleans, Louisiana, March 1-5, 1970. Paper F3.

The *cis*-aconitate ion is also a substrate of the enzymes aconitate isomerase (Rao & Altekar, 1961; Klinman & Rose, 1970) and *cis*-aconitic decarboxylase (Bentley, 1957) and results from crystallographic studies are also of interest in a study of the mechanism of action of these enzymes.

Experimental

The salt was prepared by mixing alcoholic solutions of potassium hydroxide and *cis*-aconitic acid and dissolving the precipitate in warm water, in which it is very soluble. Crystals were obtained by evaporation of the solution and had to be dried as soon as they were grown, otherwise they took up water.

The crystals were assayed enzymatically, with aconitase, by Dr I. A. Rose and Mr E. O'Connell. It was found that there were 225 micromoles of *cis*-aconitate

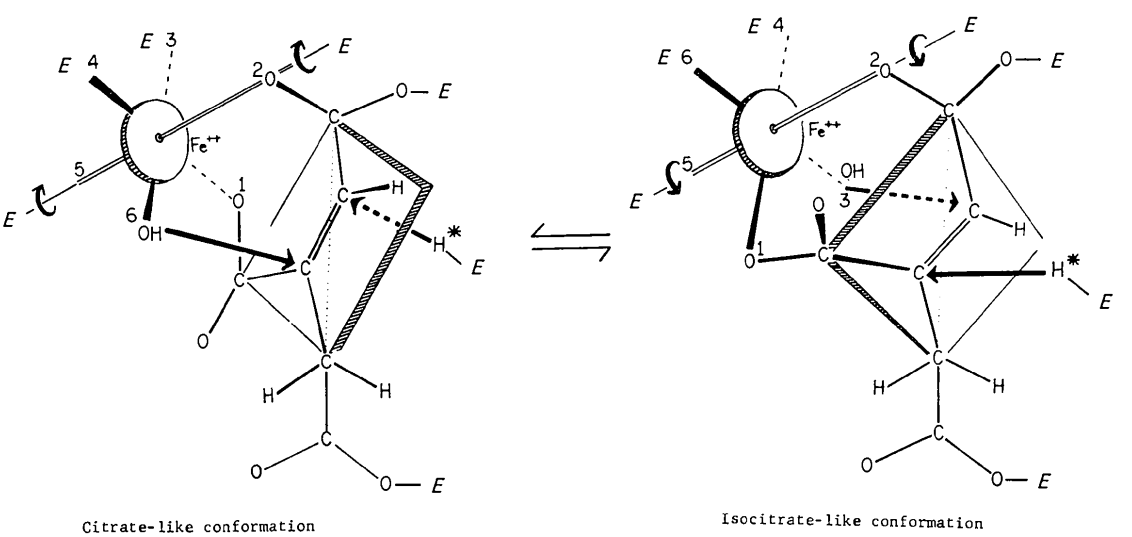


Fig. 1. Conformational changes in the enzyme-ferrous-*cis*-aconitate complex proposed in the action of aconitase. Points of attachment to the enzyme are denoted by *E*.

ion per 56·5 mg of crystal, indicating that active *cis*-aconitate was associated with a formula weight of 251 ± 1. These data indicated that the crystals contained dipotassium hydrogen *cis*-aconitate or potassium dihydrogen *cis*-aconitate dihydrate.

The crystal data are given in Table 1. The cell dimensions of the salt (determined by the structure analysis to be dipotassium *cis*-aconitate) were measured on a G.E. XRD-5 and a Picker automatic diffractometer with Cu $K\alpha$ [$\lambda(K\alpha_1) = 1{\cdot}5405$ Å] radiation and on a Picker automatic diffractometer with Mo $K\alpha$ [$\lambda(K\alpha_1) = 0{\cdot}70926$ Å] radiation.

Table 1. *Crystal data for dipotassium cis-aconitate*

Dipotassium *cis*-aconitate
$K_2C_6O_6H_4$
Formula weight: 250·22
Cell dimensions: $a = 9{\cdot}417 \pm 0{\cdot}007$ Å
$b = 12{\cdot}421 \pm 0{\cdot}020$
$c = 7{\cdot}615 \pm 0{\cdot}009$
$\beta = 96{\cdot}27 \pm 0{\cdot}07°$
$V = 863 \pm 2$ Å^3
$Z = 4$
$D_m = 1{\cdot}86$ g.cm^{-3} (flotation in *m*-xylene and methylene iodide)
$D_x = 1{\cdot}88$ g.cm^{-3}
Space group: $P2_1/c$ from systematic absences ($h0l$, l odd and $0k0$, k odd).
$F(000) = 504$

Data were initially collected with nickel-filtered copper $K\alpha$ radiation to $2\theta = 160°$ with a General Electric XRD-5 diffractometer. However, in view of the large absorption correction required ($\mu = 93{\cdot}8$ cm^{-1}) the data were recollected, after the structure was determined, with monochromatized molybdenum $K\alpha$ radiation ($\mu = 10{\cdot}6$ cm^{-1}) to $2\theta = 55°$, on a Picker automatic diffractometer. The crystal dimensions were 0·20 × 0·18 × 0·25 mm. 2048 nonequivalent reflections were measured by the θ–2θ scan technique with a scan rate of 2° per min and a background measurement time of 40 sec. Of these reflections 170 were found to have intensity below the threshold value [$2{\cdot}33\sigma(I)$] and were marked as unobserved reflections. Lorentz and polarization corrections were made. Absorption corrections were made for an ellipsoid of revolution which approximated the crystal shape (Johnson, 1963).

Structure determination and refinement

The structure was solved by the symbolic-addition method and all atoms were located from peaks on the *E* map. The structure was then refined by three cycles

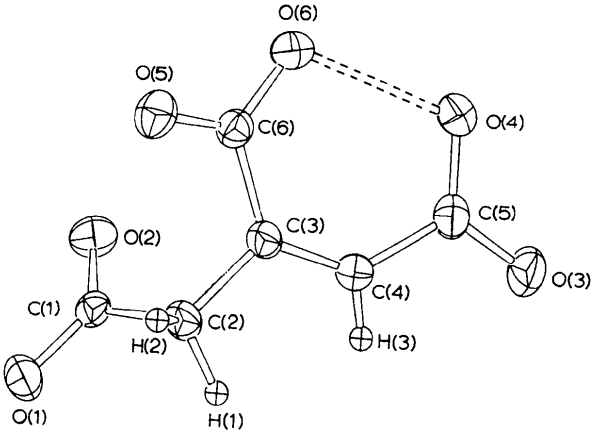


Fig. 2. Thermal ellipsoids for the *cis*-aconitate ion calculated with *ORTEP* (Johnson, 1965). The internal hydrogen bond is indicated by broken lines.

Table 2. *Atomic parameters and their estimated standard deviations*

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

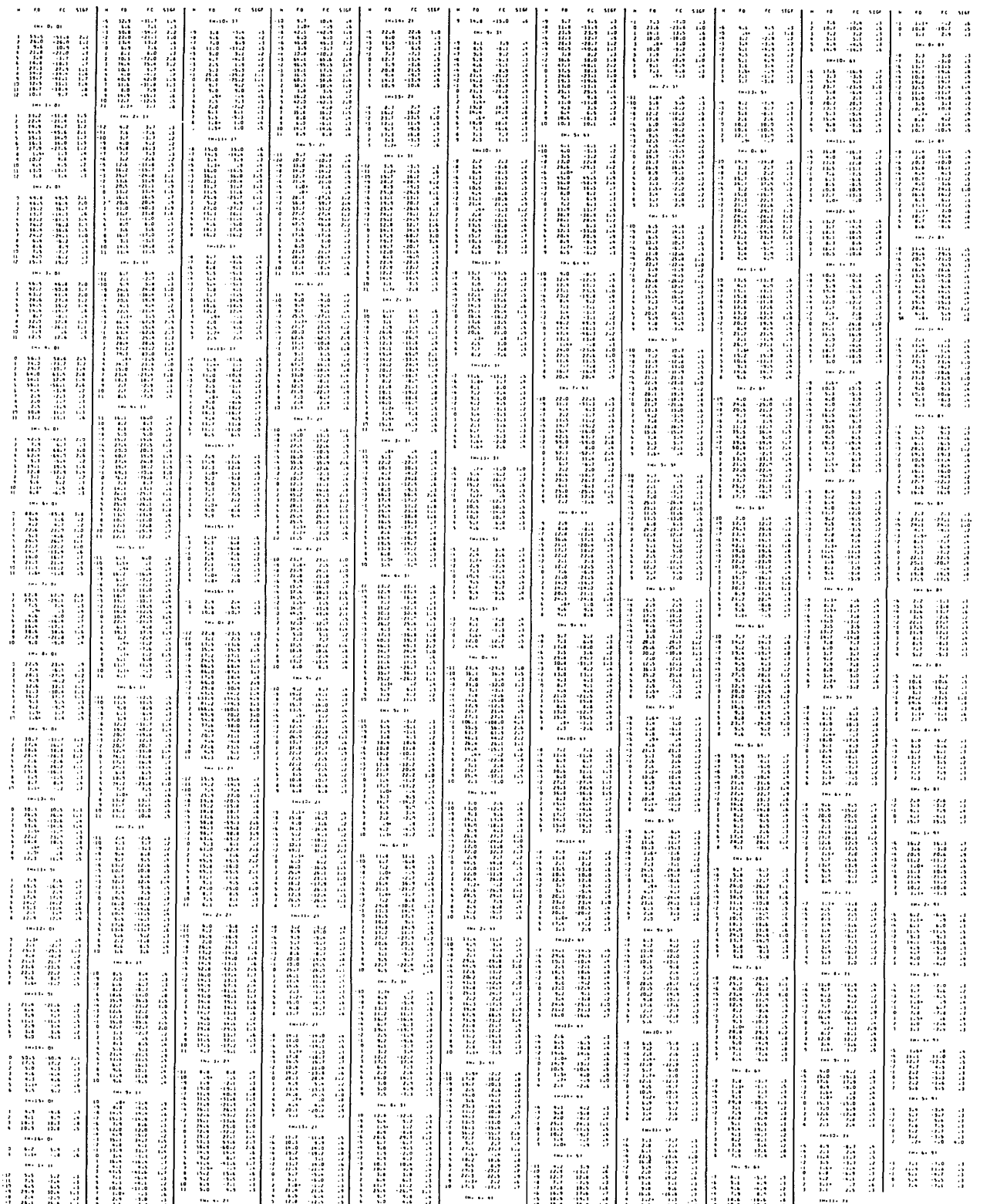
$$\exp(-b_{11}h^2 - b_{22}k^2 - b_{33}l^2 - b_{12}hk - b_{13}hl - b_{23}kl)$$

and all values listed should be multiplied by 10^{-4}. Isotropic temperature factors are of the form $\exp(-B\sin^2\theta/\lambda^2)$ with B values in Å^2. E.s.d.'s, determined from the inverted full matrices, are listed in parentheses beside each parameter with respect to the least significant digit of any parameter.

	x	y	z	b_{11} or B	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
K(1)	0·29709 (4)	0·24583 (3)	0·44970 (4)	69·4 (6)	43·1 (3)	91·1 (9)	−12·5 (4)	24·5 (9)	5·3 (5)
K(2)	0·59668 (4)	0·04365 (3)	0·27360 (5)	69·4 (6)	34·8 (3)	105·2 (2)	2·9 (4)	49·1 (9)	−5·4 (5)
C(1)	0·5887 (2)	0·3212 (1)	0·2118 (2)	51 (2)	29 (1)	80 (2)	9 (2)	30 (3)	−9 (2)
C(2)	0·7265 (2)	0·3173 (1)	0·3396 (2)	56 (2)	34 (1)	125 (3)	−8 (2)	3 (3)	37 (3)
C(3)	0·8287 (2)	0·4062 (1)	0·3105 (2)	51 (2)	29 (1)	97 (2)	−4 (2)	1 (3)	12 (2)
C(4)	0·9517 (2)	0·3808 (1)	0·2457 (2)	59 (2)	30 (1)	140 (3)	−2 (2)	23 (4)	−18 (3)
C(5)	1·0768 (2)	0·4457 (1)	0·2061 (2)	52 (2)	43 (1)	108 (3)	−3 (2)	23 (3)	−9 (3)
C(6)	0·7805 (2)	0·5177 (1)	0·3611 (2)	55 (2)	34 (1)	94 (2)	6 (2)	8 (3)	0 (2)
O(1)	0·5008 (2)	0·2467 (1)	0·2291 (2)	61 (1)	44 (1)	144 (3)	−21 (2)	10 (3)	22 (2)
O(2)	0·5718 (1)	0·3953 (1)	0·1020 (2)	92 (2)	37 (1)	96 (2)	13 (2)	5 (3)	22 (2)
O(3)	1·1823 (2)	0·3960 (1)	0·1668 (2)	62 (1)	55 (1)	172 (3)	8 (2)	64 (3)	−38 (3)
O(4)	1·0750 (2)	0·5494 (1)	0·2166 (2)	69 (1)	38 (1)	210 (3)	−13 (2)	77 (3)	7 (2)
O(5)	0·6763 (1)	0·5254 (1)	0·4448 (2)	76 (1)	52 (1)	118 (2)	13 (2)	56 (3)	−17 (2)
O(6)	0·8483 (2)	0·6012 (1)	0·3150 (2)	83 (2)	30 (1)	203 (3)	8 (2)	76 (3)	4 (2)
H(1)	0·779 (3)	0·247 (2)	0·322 (3)	3·2 (5)					
H(2)	0·701 (3)	0·315 (2)	0·466 (4)	3·9 (5)					
H(3)	0·965 (3)	0·314 (2)	0·221 (3)	3·2 (5)					
H(4)	0·971 (8)	0·568 (7)	0·273 (9)	16·7 (22)					

Table 3. *Observed and calculated structure factors*

Each entry lists, in order, h, $|F_o|$, F_c and $\sigma|F_o|$. Unobserved reflections are denoted by an asterisk.



of isotropic full-matrix least squares. The R value dropped from 0·32 for the trial structure from the E map to 0·12. The structure was refined anisotropically for the heavier atoms, and isotropically for hydrogen atoms to an R value of 0·072 for the copper data. These data were not used further because of possible problems due to errors in correction for absorption.

The refinement by full-matrix least-squares methods, was continued with the data collected with molybdenum radiation. The four hydrogen atoms were located from a difference map. Parameters for the potassium, oxygen and carbon atoms were refined anisotropically and those for the hydrogen atoms refined isotropically. The final R value was 0·025 and the parameters are listed in Table 2. An extinction correction, $\alpha = 1{\cdot}7 \times 10^{-8}$, was applied (Zachariasen, 1963*a*, *b*) with the formula $F_{corr} = F_{obs}(1 + \alpha\beta I_{obs})$. Observed and calculated structure factors are listed in Table 3. The thermal motion is illustrated in Fig. 2 (Johnson, 1965).

The hydrogen atom, H(4), in an internal hydrogen bond, refined to a B value of 16·7 Å^2. The appearance of this hydrogen atom in a difference map is illustrated in Fig. 3, which shows a section through a plane of the atoms O(4), O(6), C(5), and C(6). It may be compared with the appearance of H(3) which is also shown at the lower right hand corner of Fig. 3. Since the peak for H(4) is so elongated, when the refinement was complete, two half-hydrogen atoms, corresponding to two positions along this peak, were refined isotropically, together with the four nearest heavier atoms which were refined anisotropically. The half-hydrogen atomic positions refined to positions 0·61 and 0·70 Å from O(4) and O(6) respectively (*i.e.* about 1·22 Å from each other), with B values of 3·5 and 8·1 Å^2. However, since the coordinates for H(4), given in Table 2, lie within one standard deviation of the midpoint of O(4) and O(6), and since the results of refining two separate half-hydrogen atoms are less convincing (in view of the short O---H distances) than those for the refinement of a hydrogen atom in a symmetrical hydrogen bond it is concluded that the latter model is better with much temperature motion along the hydrogen bond direction. This situation was found for the similar short internal hydrogen bond in potassium hydrogen maleate (Darlow & Cochran, 1961) which was shown by neutron diffraction studies to be statistically symmetrical (Peterson & Levy, 1958).

Computations

The data reduction and symbolic addition procedure listing was performed on an IBM 1620, 20K memory with programs written in this laboratory (for a list see Gabe, Glusker, Minkin & Patterson, 1967). The full-matrix least-squares program was run on a CDC 6600 computer at New York University, *via* a UNIVAC DCT 2000, with the program of Gantzel, Sparks, Long & Trueblood (1969) (*UCLALS* 4) modified by H.L.C. All other computations were done on a UNIVAC 1108 *via* a DCT 2000 terminal. The program to compute distances and angles was written by H.L.C. and A. Caron. In the least-squares refinements the quantity minimized was $\sum w|kF_o| - |F_c|)^2$ where the weights, w, were assigned as $(1/\sigma_F^2)$. The values of σ_F were calculated from counting statistics and instrumental uncertainties. The 170 unobserved data were given a weight of zero in the refinement.

Scattering factors were taken from *International Tables for X-ray Crystallography* (1962) except for those for hydrogen, for which the values of Stewart, Davidson & Simpson (1965) were used. The scattering factors of potassium were corrected for the real component of anomalous dispersion ($\Delta f' = +0{\cdot}2$).

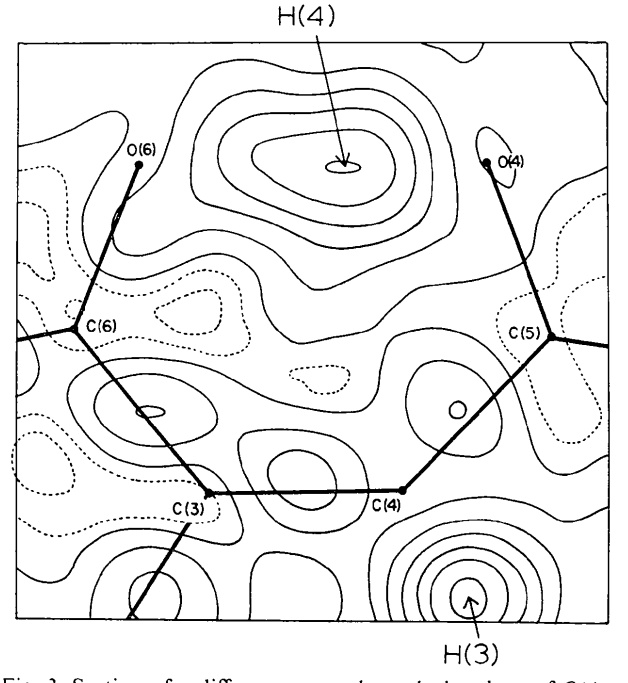


Fig. 3. Section of a difference map through the plane of O(4), O(6), C(5), and C(6) showing H(4) (elongated) and H(3) (spherical). The contour interval is 0·1 e.Å^{-3} with negative contours indicated by broken lines.

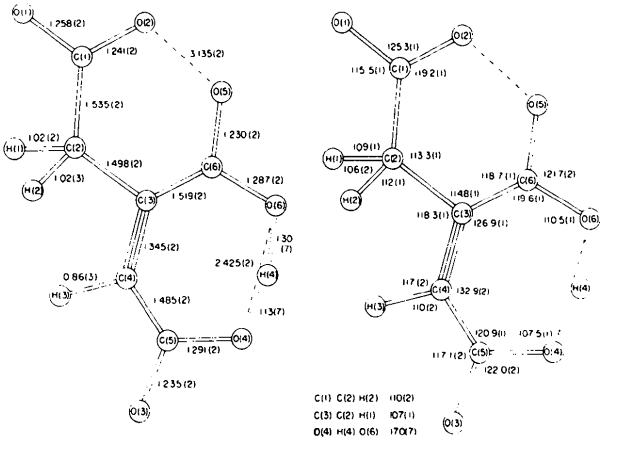


Fig. 4. Bond distances and interbond angles.

Discussion of the structure

A striking feature of this structure, for which bond distances and interbond angles are shown in Fig. 4, is the very short internal hydrogen bond (2·425 Å) which is probably symmetrical. A similar short hydrogen bond was also found in potassium hydrogen maleate (Peterson & Levy, 1958; Darlow & Cochran, 1961) and potassium hydrogen chloromaleate (Ellison & Levy, 1965). This hydrogen bond must confer much stability to the anion and this fact may have significance in those enzyme mechanisms involving the *cis*-aconitate ion at *p*H values at which the anion is not triionized. An estimate of the pK_3 values for *cis*- and *trans*-aconitic acid was made by Pratt & Smith (1967). They studied the effect of *p*H on the positions of the lines of the methylene and methyne protons in the nuclear magnetic resonance spectra. They derived approximate values of 6·5 for pK_3 for *cis*-aconitic acid and 4 for *trans*-aconitic acid. Therefore, the *cis*-aconitate ion is fully ionized at *p*H values 7–8 and diionized at *p*H 5–6. The *p*H optimum for *cis*-aconitic decarboxylase is 5·6–5·9 and it has been shown (Bentley & Thiessen, 1955; Bentley, 1957) that the terminal carboxyl group adjacent to the methylene group is decarboxylated. The enzyme is thus stereospecific for the ion containing a ring by virtue of internal hydrogen bonding and acts only on the one carboxyl group. Since the *p*H optima of aconitase and *cis*-aconitate isomerase are 7·5–8·6 and 8·5, respectively, this study does not give direct information on the conformation of the anion when it is a substrate of these enzymes.

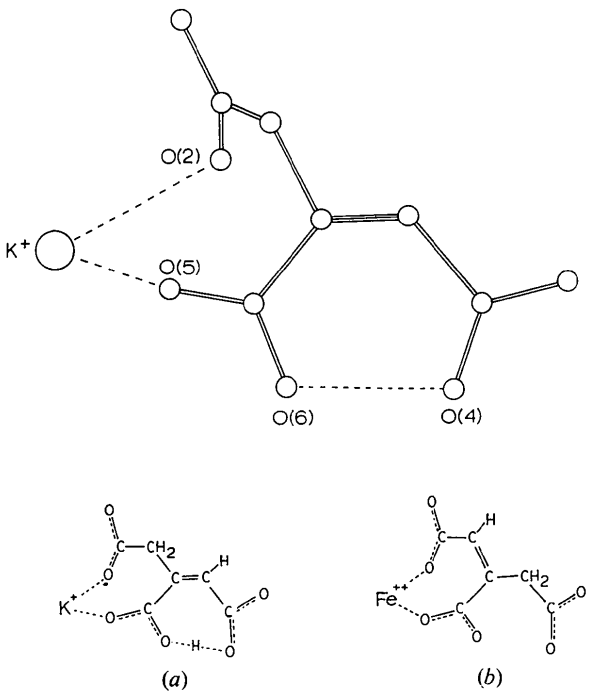


Fig. 5. The differing chelations of (*a*) diionized *cis*-aconitate *p*H 5-6, (this work) and that proposed for the (*b*) triionized anion, *p*H 7-8, as a substrate of aconitase.

Deviations of atoms from some least-squares planes through the molecule are listed in Table 4, in which it can be seen that the entire system of atoms, O(6), C(6), C(3), C(4), C(5), O(4) is almost exactly planar [with C(2) and O(3) also almost in this plane]. The angle C(3)–C(4)–C(5) of 132·9° is larger than the expected values of around 125° [126·9° for C(6)–C(3)–C(4) and 126·2° and 125·4° for analogous angles in potassium dihydrogen *trans*-aconitate (Dargay, Berman, Carrell & Glusker, 1972)] so that some distortion has possibly occurred in order to accommodate the hydrogen atom, H(4), in the planar system. This same situation occurs in potassium hydrogen maleate (Darlow, 1961). The anion is in the isocitrate-like conformation illustrated in Fig. 1.

The packing in the crystal is determined by packing of the *cis*-aconitate anions with potassium ions. The only hydrogen atom available for hydrogen bonding is involved in the intramolecular hydrogen bond. Some potassium–oxygen distances around the potassium ion

Table 4. *Deviations of atoms from least-squares planes through portions of the molecule*

Atoms through which the plane was computed are denoted by an asterisk. All distances are in Å.

K(1)	2·693	−0·428	3·626	−0·636	−1·012	−0·088	2·162	−1·632
K(2)	0·486	−0·325	1·772	−0·723	−1·222	−0·480	−1·623	−1·120
C(1)	*−0·133	−1·264	*0·354	−1·388	−1·640	−1·097	*0·000	−1·707
C(2)	*0·315	0·040	*0·500	−0·074	−0·313	0·149	*0·000	−0·264
C(3)	*−0·322	*0·005	*−0·537	*−0·016	−0·159	0·144	−0·033	*0.000
C(4)	−1·214	*−0·005	−1·470	*−0·046	−0·189	*0·002	−1·149	0·218
C(5)	−1·993	*0·002	−2·597	*0·032	*−0·032	*−0·007	−1·515	0·544
C(6)	*0·140	*−0·002	*−0·398	*0·088	*0·033	0·318	1·240	*0·002
O(1)	0·357	−1·289	1·189	−1·492	−1·825	−1·148	*0·000	−1·981
O(2)	−0·928	−2·193	*−0·550	−2·249	−2·434	−1·959	*0·000	−2·500
O(3)	−2·620	0·154	−3·187	0·140	0·050	*0·003	−2·577	0·848
O(4)	−1·983	−0·117	−2·918	*0·017	*0·043	*0·003	−0·733	0·534
O(5)	1·116	0·226	*0·631	0·321	0·252	0·650	2·245	*−0·001
O(6)	−0·496	−0·251	−1·352	*−0·075	*−0·044	0·111	1·243	*−0·001
H(1)	0·049	0·208	0·395	0·026	−0·266	0·191	−0·834	−0·076
H(2)	1·329	0·822	1·444	0·710	0·459	0·983	0·788	0·381
H(3)	−1·393	−0·007	−1·439	−0·115	−0·314	−0·097	−1·807	0·180
H(4)	−1·219	−0·096	−2·113	0·052	0·076	0·132	0·197	0·358

are listed in Table 5. Each potassium ion is surrounded by seven oxygen atoms within a distance of 3·1 Å. In one *cis*-aconitate ion the oxygen atoms O(5) and O(6) are chelated to a K(1) ion and the atoms O(2) and O(5) to a K(2) ion. The chelation of the *cis*-aconitate ion to the potassium ion is of interest with respect to the proposed manner in which aconitase works (Glusker, 1968, 1971). It has been suggested, as shown in Fig. 1, that in the action of aconitase the *cis*-aconitate ion binds to the enzyme in such a way that the double bond of the *cis*-aconitate ion is part of the chelation ring to the enzyme-bound metal ion (Fe^{2+}). At lower *p*H values, as found in this study, the double bond is in a ring formed as a result of an internal hydrogen bond, and in this conformation it cannot bind with the double bond near the metal as required for a substrate of the enzyme aconitase. This is illustrated in Fig. 5. The packing in the unit cell is shown in Fig. 6. The observed cleavage parallel to the *a* axis is explained by the packing illustrated here.

Table 5. *Some interatomic distances around the metal ions*

(*a*) K–O distances				
K(1)–O(1)	2·685 (1) Å	x	y	z
O(1)	2·706 (1)	x	$\frac{1}{2}-y$	$\frac{1}{2}+z$
O(3)	2·963 (2)	$x-1$	y	z
O(3)	2·719 (1)	$x-1$	$\frac{1}{2}-y$	$\frac{1}{2}+z$
O(5)	2·957 (1)	$1-x$	$1-y$	$1-z$
O(6)	3·038 (1)	$1-x$	$1-y$	$1-z$
O(6)	2·927 (1)	$1-x$	$y-\frac{1}{2}$	$\frac{1}{2}-z$
K(2)–O(1)	2·688 (1)	x	y	z
O(2)	2·648 (1)	x	$\frac{1}{2}-y$	$\frac{1}{2}+z$
O(2)	2·672 (1)	$1-x$	$y-\frac{1}{2}$	$\frac{1}{2}-z$
O(3)	2·774 (1)	$-x$	$y-\frac{1}{2}$	$\frac{1}{2}-z$
O(4)	3·085 (1)	$-x$	$y-\frac{1}{2}$	$\frac{1}{2}-z$
O(5)	2·824 (1)	x	$\frac{1}{2}-y$	$z-\frac{1}{2}$
O(5)	2·914 (1)	$1-x$	$y-\frac{1}{2}$	$\frac{1}{2}-z$
(*b*) K–K distances				
K(1)–K(1)	3·809	x	$\frac{1}{2}-y$	$\frac{1}{2}+z$
	3·809	x	$\frac{1}{2}-y$	$z-\frac{1}{2}$
K(1)–K(2)	4·110	x	y	z
	4·110	$1-x$	$-y$	$-z$
	4·232	$1-x$	$-y$	$1-z$
	4·237	$1-x$	$\frac{1}{2}+y$	$\frac{1}{2}-z$
	4·400	x	$\frac{1}{2}-y$	$\frac{1}{2}+z$
K(2)–K(2)	4·213	$1-x$	$-y$	$1-z$
	4·494	$1-x$	$-y$	$-z$

We are grateful to Mr F. B. Soule who helped with the data collection, and Drs Judith Klinman, Helen M. Berman and J. J. Villafranca for helpful discussions. We also thank Professor Jerry Donohue for the use of the automatic diffractometer at the University of Pennsylvania, Philadelphia, Pennsylvania.

This research was supported by U.S.P.H.S. grants CA-10925, CA-06927 and RR-05539 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania.

References

BENTLEY, R. (1957). *Proc. Chem. Soc.* p. 20.
BENTLEY, R. & THIESSEN, C. P. (1955). *Science*, **122**, 330.
DARGAY, J. M., BERMAN, H. M., CARRELL, H. L. & GLUSKER, J. P. (1972). *Acta Cryst.* In the press.
DARLOW, S. F. (1961). *Acta Cryst.* **14**, 1257.
DARLOW, S. F. & COCHRAN, W. (1961). *Acta Cryst.* **14**, 1250.
ELLISON, R. D. & LEVY. H. A. (1965). *Acta Cryst.* **19**, 260.
GABE, E. J., GLUSKER, J. P., MINKIN, J. A. & PATTERSON, A. L. (1967). *Acta Cryst.* **22**, 366.
GANTZEL, P. K., SPARKS, R. A., LONG, R. E. & TRUEBLOOD, K. N. (1969). *UCLALS* 4 Program in Fortran IV.
GLUSKER, J. P. (1968). *J. Mol. Biol.* **38**, 149.
GLUSKER, J. P. (1971). *Aconitase.* Chapter in *The Enzymes*, Vol. V. Edited by P. D. BOYER. New York and London: Academic Press. In the press.
International Tables for X-ray Crystallography (1962), Vol. III, p. 201. Birmingham: Kynoch Press.

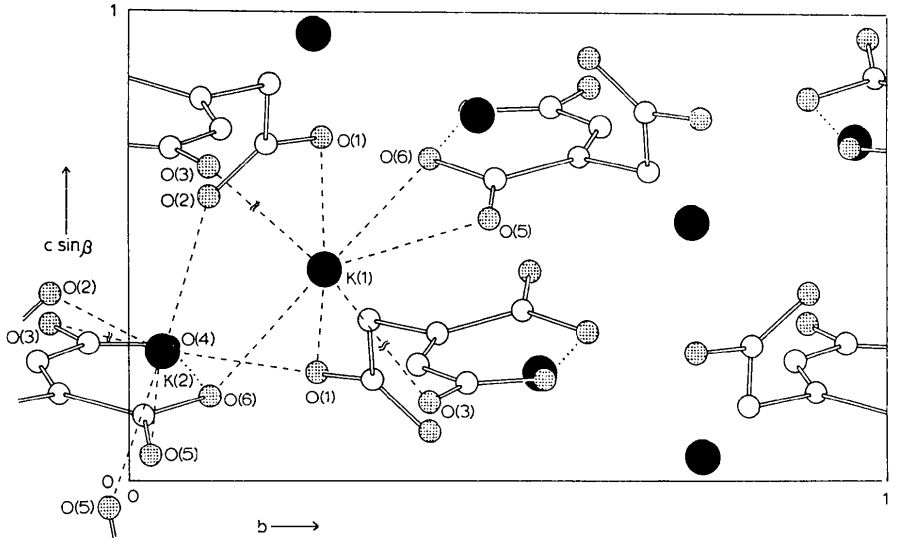


Fig. 6. Projection down the *a* axis showing the packing in the unit cell. Oxygen atoms are stippled and potassium ions are black.

JOHNSON, C. K. (1963). 1620 Programs from I.C.R. *I.C.R.* 10. *Absorption Factors for a Crystal Ground as an Ellipsoid of Revolution.*
JOHNSON, C. K. (1965). *ORTEP: A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations.* ORNL-3794. Oak Ridge National Laboratory, Oak Ridge, Tennessee.
KLINMAN, J. P. & ROSE, I. A. (1971). *Biochemistry.* **10**, 2259.
PETERSON, S. W. & LEVY, H. A. (1958). *J. Chem. Phys.* **29**, 948.
PRATT, L. & SMITH, B. B. (1967). *Trans. Faraday Soc.* **63**, 2858.
RAO, M. R. R. & ALTEKAR, W. W. (1961). *Biochem. Biophys. Res. Commun.* **4**, 101.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175.
ZACHARIASEN, W. H. (1963*a*). *Acta Cryst.* **16**, 1139.
ZACHARIASEN, W. H. (1963*b*). Paper G-2, Amer. Cryst. Assoc. Meeting, M.I.T., Cambridge, Mass., March 28–30.

Acta Cryst. (1972). B**28**, 425

The Crystal and Molecular Structure of Planteose Dihydrate

BY D. C. ROHRER

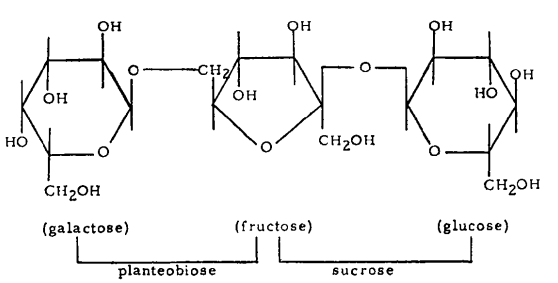
Department of Crystallography, University of Pittsburgh, Pittsburgh, Pa. 15213, *U.S.A.*

(*Received* 3 *March* 1971)

The crystal structure of the nonreducing trisaccharide planteose dihydrate, *O*-α-D-galactopyranosyl-(1→6)-O-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside ($C_{18}H_{32}O_{16}.2H_2O$), has been determined using a combination of Patterson superposition techniques and tangent-phase refinement. Three-dimensional intensities were measured on a Picker FACS-I diffractometer using Ni-filtered Cu $K\alpha$ radiation. The crystals are orthorhombic with space group $P2_12_12_1$. Unit-cell constants are $a = 32{\cdot}43$ (1), $b = 8{\cdot}152$ (2), and $c = 8{\cdot}711$ (2) Å. Measured density, $D_m = 1{\cdot}547$ g.cm^{-3}, is consistent with the calculated density, $D_x = 1{\cdot}531$ g.cm^{-3}, for a unit cell containing four sugar molecules and eight water molecules. The final R is 3·6% for 2197 reflections. The molecule has a circular conformation with O(6) of the glucose and O(6′) of the galactose, at the opposite end, both hydrogen bonded to the same hydroxyl, O(2)H, of an adjacent molecule. Both pyranosyl rings have the expected $^4C_1$ ($C1$) conformation. The fructofuranosyl ring has a twisted $_3T^4$ conformation, which places the O(3′)H hydroxyl in the equatorial plane of the ring. Conformational similarities of the (1 → 2) linkage in the sucrose moieties of planteose and raffinose are more easily recognized using the pseudotorsional angles between C(1) and C(2′), defined herein, than using the torsional angles involving O(1). The largest difference in the pseudotorsional angles between the two molecules is only 12° compared to differences as large as 36° in the torsional angles involving O(1). The (1 → 6) linkage between the fructose and galactose portions has antiperiplanar conformation similar to the other two known crystal structures containing this type of linkage. Most of the hydroxyls both donate and accept a hydrogen bond with the fructofuranosyl ring oxygen atom also acting as a hydrogen-bond acceptor. One water oxygen atom both accepts and donates two hydrogen bonds, while the other water oxygen atom only accepts and donates one hydrogen bond.

Introduction

Planteose, *O*-α-D-galactopyranosyl-(1 → 6)-*O*-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside ($C_{18}H_{32}O_{16}$), is a nonreducing trisaccharide first isolated from the seeds of *Plantago major* and *Plantago ovata* (Wattiez & Hans, 1943). It is a member of the raffinose family of oligosaccharides, which occur in a variety of plants and are found primarily in seeds, roots, and underground stems, where they probably act as reserve carbohydrates (French, 1954). As shown in (I), the configuration of planteose can be subdivided into two disaccharide portions: sucrose and planteobiose. Raffinose and planteose are structural isomers having the α-D-galactopyranosyl portion linked to C(6′) of the fructose in planteose, seen in (I) below, whereas in raffinose, it is linked to O(6) of the glucose portion. The structure of sucrose has been accurately determined, crystallographically, as sucrose itself (Brown & Levy, 1963), in raffinose pentahydrate (Berman, 1970), in 1-kestose (Jeffrey & Park, 1971), and now in planteose dihydrate. No previous crystallographic study relating to the planteobiose part of the molecule has been made.



(I)