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*Acta Cryst.* (1972). **B28**, 1533

## The Crystal Structure of Potassium Dihydrogen *trans*-Aconitate

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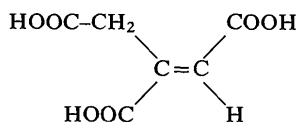
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(Received 25 August 1971)

Potassium dihydrogen *trans*-aconitate,  $\text{KH}_2(\text{C}_6\text{H}_3\text{O}_6)$ , crystallizes in the monoclinic space group  $C2/c$ , with lattice parameters  $a = 11.861 \pm 0.002$ ,  $b = 15.919 \pm 0.002$ ,  $c = 8.416 \pm 0.001$  Å, and  $\beta = 90.90 \pm 0.02^\circ$  at  $20^\circ\text{C}$ . There are eight molecules per unit cell. The calculated and observed densities,  $D_x$  and  $D_m$ , are  $1.78$  and  $1.77$   $\text{g.cm}^{-3}$  respectively. The structure was determined by Patterson methods from data collected with Mo  $K\alpha$  radiation on a four-circle diffractometer. The final  $R$  value for the 2081 observed reflections after refinement by full-matrix least squares of all atoms including the hydrogen atoms is  $0.043$ . The potassium ions are each surrounded by eight oxygen atoms at distances  $2.70$ – $3.32$  Å. Each anion takes part in two hydrogen bonds, one of which is intramolecular ( $2.53$  Å). A seven-membered ring is formed by the bidentate chelation of one potassium ion by the *trans*-aconitate ion.

### Introduction

The crystal structure of potassium *trans*-aconitate was undertaken to supplement a study of the enzyme aconitase and the possible mechanism of its action as proposed by Glusker (1968). *trans*-Aconitate is a weak competitive inhibitor of the action of aconitase (Thomson, Nance, Bush & Szczepanik, 1966). It is an isomer of *cis*-aconitate, a substrate of aconitase, and is found to comprise at least 6% of a normal sample of *cis*-aconitate. The formula of *trans*-aconitic acid is shown below.



The two isomers of aconitic acid may be interconverted by changing the  $\text{pH}$  of the solution (Ambler & Roberts, 1948) or by the action of the enzyme aconitate isomerase (Rao & Altekar, 1971; Klinman & Rose, 1970). The structure of dipotassium *cis*-aconitate has been reported (Glusker, Orehowsky, Casciato & Carrell, 1972).

### Experimental

Crystals of the monopotassium salt of *trans*-aconitic acid were grown by dissolving equimolar quantities of

potassium hydroxide and *trans*-aconitic acid in water. A few drops of dilute hydroquinone were added to prevent the polymerization that occurred with the first batch of crystals that were left in contact with their mother liquor. The solution was allowed to stand, and large rectangular crystals formed. The crystal data are given in Table 1.

Table 1. *Crystal data for potassium dihydrogen trans-aconitate*,  $\text{KH}_2(\text{C}_6\text{H}_3\text{O}_6)$

Formula weight: 212.205

System: monoclinic

$a = 11.861 \pm 0.002$  Å

$b = 15.919 \pm 0.002$

$c = 8.416 \pm 0.001$

$\beta = 90.90 \pm 0.02^\circ$

$\lambda$  (Mo  $K\alpha_1$ ) =  $0.70926$  Å

$Z = 8$

$F(000) = 864$

Systematic absences:  $h0l$ ,  $h$  odd,  $l$  odd;  $0k0$ ,  $k$  odd;  $hkl$ ,  $h+k$  odd.

Space group:  $C2/c$  (from systematic absences and intensity statistics).

$D_x = 1.78$   $\text{g.cm}^{-3}$

$D_m = 1.77$   $\text{g.cm}^{-3}$  determined by flotation in bromoform and carbon tetrachloride.

$\mu = 6.6$   $\text{cm}^{-1}$

Intensity data were collected from a crystal of dimensions  $0.45 \times 0.3 \times 0.3$  mm, on a Syntex *PT* automatic diffractometer with  $\text{Mo } K\alpha$  radiation, using a highly oriented graphite monochromator and a scintillation counter. The *c* axis of the crystal was set parallel to the  $\varphi$  axis of the diffractometer, and reflections were measured out to  $2\theta = 70^\circ$ . The data were corrected for Lorentz and polarization factors, and an absorption correction was applied assuming an ellipsoid of revolution (Johnson, 1963). Values of  $A^*$  for maximum and minimum transmittance path are 1.15 and 1.24 respec-

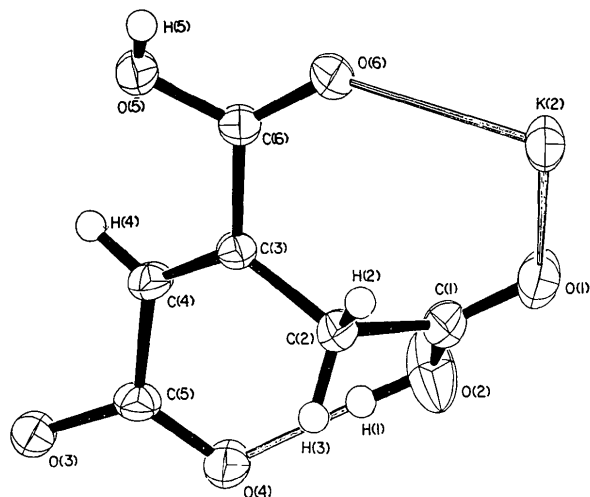


Fig. 1. Thermal ellipsoids (at 50% probability) for the *trans*-aconitate ion, illustrated by *ORTEP*. The chelation to the potassium ion and the intramolecular hydrogen bond are shown.

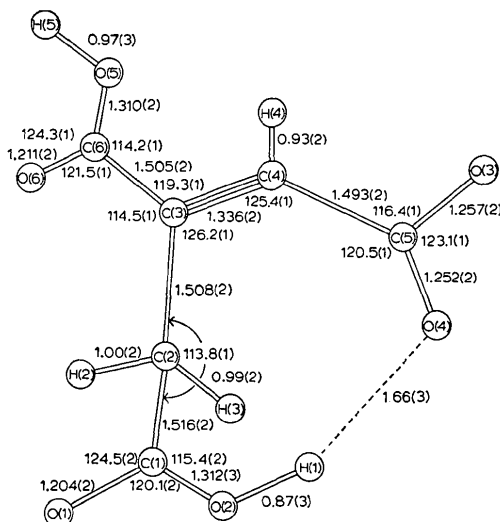


Fig. 2. Bond lengths and interbond angles in the *trans*-aconitate ion. The e.s.d.'s are indicated, in parentheses, with respect to the least significant digit of any measurement.

tively. Values of  $\sigma|F_{\text{obs}}|$  were calculated from counting statistics and instrumental uncertainties. Unobserved reflections are defined as those reflections with intensities less than  $2.33 \sigma(I)$ . Of a total of 3172 unique reflections scanned, 2081 were considered by this criterion to be above the threshold of measurement.

### Structure determination and refinement

The solution of the structure presented difficulties because the heavy atom was situated on a twofold axis

Table 2. Atomic parameters and their e.s.d.'s

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  $\text{Å}^2$ . 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.

	<i>x</i>	<i>y</i>	<i>z</i>	<i>b</i> <sup>11</sup>	<i>b</i> <sup>22</sup>	<i>b</i> <sup>33</sup>	<i>b</i> <sup>12</sup>	<i>b</i> <sup>13</sup>	<i>b</i> <sup>23</sup>
K(1)	0.0000 (0)	0.13826 (4)	0.25000 (0)	87 (1)	18 (1)	98 (1)	0 (0)	-42 (1)	0 (0)
K(2)	0.0000 (0)	-0.42609 (3)	0.25000 (0)	44 (1)	16 (1)	122 (1)	0 (0)	-12 (1)	0 (0)
O(1)	0.2368 (1)	0.5702 (1)	0.2954 (2)	63 (1)	19 (1)	149 (3)	0 (1)	-28 (3)	20 (2)
O(2)	0.3886 (1)	0.4950 (1)	0.3330 (3)	39 (1)	26 (1)	285 (4)	-11 (1)	-6 (3)	49 (3)
O(3)	0.4266 (1)	0.2135 (1)	0.4073 (2)	47 (1)	22 (1)	122 (2)	18 (1)	-76 (2)	-16 (2)
O(4)	0.4353 (1)	0.3507 (1)	0.4420 (2)	52 (1)	23 (1)	145 (2)	6 (1)	-94 (2)	-12 (2)
O(5)	0.0945 (1)	0.2830 (1)	0.0941 (2)	55 (1)	18 (1)	130 (2)	-6 (1)	-96 (3)	1 (2)
O(6)	0.0799 (1)	0.4225 (1)	0.1049 (2)	47 (1)	21 (1)	99 (2)	12 (1)	-47 (2)	2 (2)
C(1)	0.2787 (2)	0.5044 (1)	0.3351 (2)	42 (1)	20 (1)	88 (2)	-8 (1)	-17 (3)	-3 (2)
C(2)	0.2128 (1)	0.4281 (1)	0.3883 (2)	35 (1)	18 (1)	67 (2)	0 (1)	-10 (2)	-5 (2)
C(3)	0.2157 (1)	0.3559 (1)	0.2724 (2)	29 (1)	17 (1)	65 (2)	-4 (1)	-18 (2)	3 (2)
C(4)	0.2924 (1)	0.2945 (1)	0.2698 (2)	37 (1)	21 (1)	75 (2)	5 (1)	-39 (3)	-14 (2)
C(5)	0.3913 (1)	0.2867 (1)	0.3808 (2)	33 (1)	24 (1)	69 (2)	8 (1)	-27 (2)	-4 (2)
C(6)	0.1232 (1)	0.3573 (1)	0.1482 (2)	28 (1)	19 (1)	69 (2)	0 (1)	-14 (2)	0 (2)
H(1)	0.407 (3)	0.445 (2)	0.367 (4)	6.4 (8)					
H(2)	0.132 (2)	0.444 (1)	0.399 (2)	2.6 (4)					
H(3)	0.246 (2)	0.411 (1)	0.492 (3)	2.7 (4)					
H(4)	0.285 (2)	0.252 (1)	0.195 (2)	2.6 (4)					
H(5)	0.028 (3)	0.287 (2)	0.026 (3)	8.1 (9)					



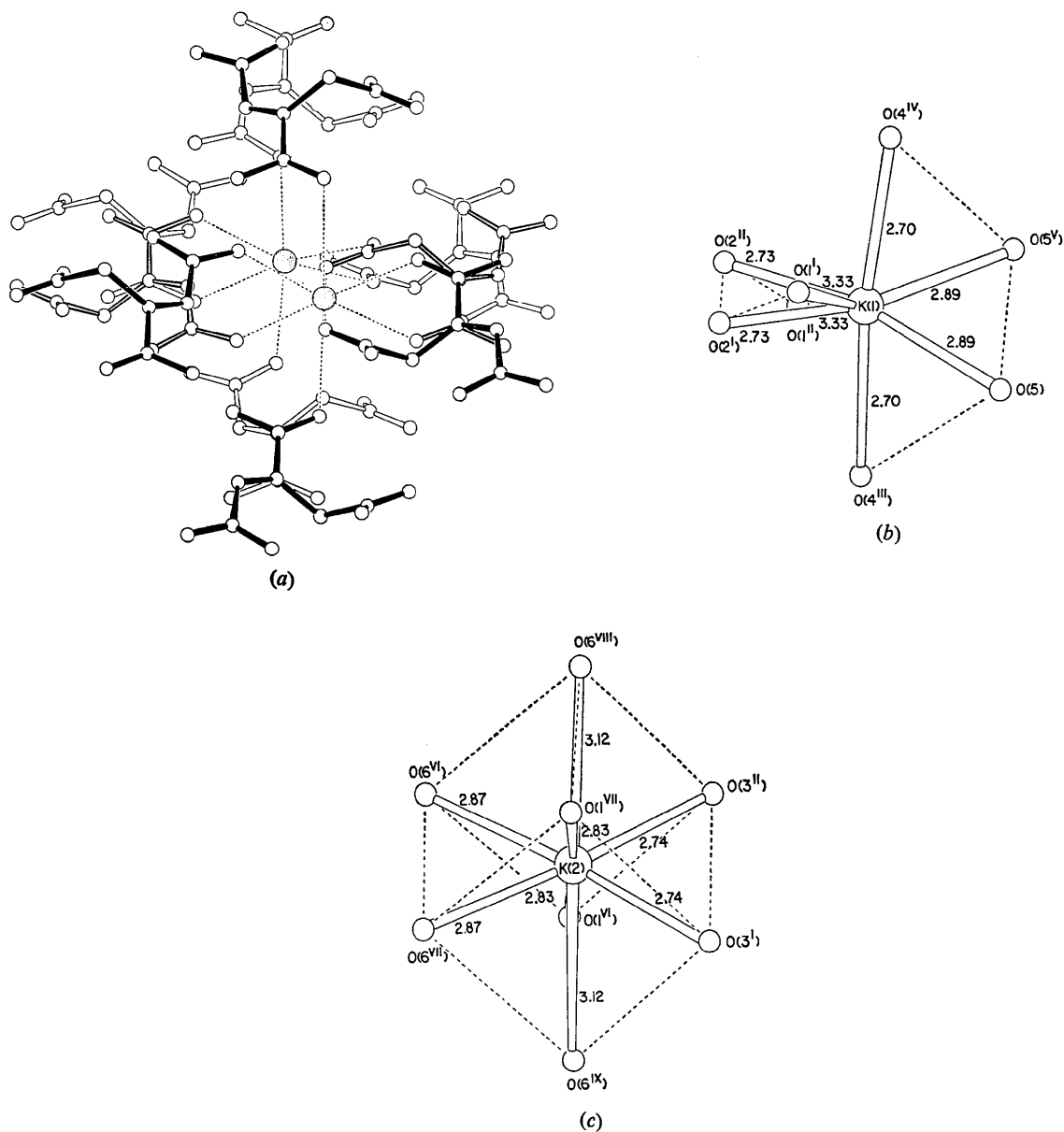


Fig. 3. (a) Surroundings of potassium ions in *trans*-aconitate. The height of the molecule is shown by the color of the bonds. Black, white, and striped designate highest, middle, and lowest levels respectively. (b) Distances of oxygen atoms surrounding K(1). (c) Distances of oxygen atoms surrounding K(2). Roman numerals refer to the following symmetry code.

	$x$	$y$	$z$
I	$\frac{1}{2}-x$	$y-\frac{1}{2}$	$\frac{1}{2}-z$
II	$x-\frac{1}{2}$	$y-\frac{1}{2}$	$z$
III	$x-\frac{1}{2}$	$\frac{1}{2}-y$	$z-\frac{1}{2}$
IV	$\frac{1}{2}-x$	$\frac{1}{2}-y$	$1-z$
V	$-x$	$y$	$\frac{1}{2}-z$
VI	$-x$	$y-1$	$\frac{1}{2}-z$
VII	$x$	$y-1$	$z$
VIII	$x$	$-y$	$\frac{1}{2}+z$
IX	$-x$	$-y$	$-z$

at 0.00,  $y$ , 0.25. The structure was solved by a Patterson vector superposition on an arbitrarily chosen peak. One potassium position was located from this map, along with part of the *trans*-aconitate ion. The other potassium position was found directly from the Patterson map. The rest of the atoms were located from peaks on a Fourier synthesis phased on the known atoms. The  $R$  value decreased from 0.55 for the first trial structure, derived from the superposition map, to 0.24 after all atoms were located.

After two cycles of isotropic full-matrix least-squares refinement, the  $R$  value dropped to 0.185. Corrections were made for anomalous dispersion, and two cycles of anisotropic least-squares refinement resulted in an

$R$  value of 0.096. At this point, a difference map was calculated and the five hydrogen atoms were located.

Several cycles of full-matrix least-squares anisotropic refinement of the heavier atoms, and isotropic refinement of the hydrogen atoms, resulted in a final  $R$  value of 0.043 for observed reflections. The weighted  $R$  value was also 0.043. The maximum shift in the final cycle was less than half the estimated standard deviation of that parameter and most shifts were less than a tenth of the e.s.d. No extinction correction was applied. The final parameters of the atoms are listed in Table 2. The structure factors are given in Table 3.

### Computations

The full-matrix least-squares program (*UCLALS 4*; Gantzel, Sparks, Long & Trueblood, 1969; modified by HLC) was run on a CDC 6600 computer at New York University, *via* a UNIVAC DCT 2000 terminal. All other computations were done on a UNIVAC 1108 computer *via* a DCT 2000 terminal. The program to compute distances and angles was written by H. L. Carrell and A. Caron. The weights in the least-squares calculations were  $[1/(\sigma(F))^2]$  and the quantity minimized was  $\sum w(|F_o| - |F_c|)^2$ . Diagrams were plotted with the program *ORTEP* (Johnson, 1965).

Scattering factors were taken from *International Tables for X-ray Crystallography* (1962), except those for hydrogen, for which the values of Stewart, Davidson & Simpson (1965) were used. The scattering factors of potassium ( $K^+$ ) were corrected for the real component of anomalous dispersion ( $\Delta f' = +0.2$ ).

### Discussion of the structure

The labelling of the atoms in *trans*-aconitate, and the thermal ellipsoids, are shown in Fig. 1. In Fig. 2 the bond lengths and interbond angles are illustrated. The geometry of the  $HOOC-C=C-COO^-$  group is similar to that of the fumarate ion, as reported by Gupta & Sahu (1970*a,b*) in the structures of sodium hydrogen fumarate and dipotassium fumarate dihydrate. The distances also agree with those determined for the *cis*-aconitate ion in this laboratory (Glusker *et al.*, 1972).

The  $C(5)-C(4)=C(3) \begin{matrix} \nearrow C(6) \\ \searrow C(2) \end{matrix}$  part of the anion is approximately planar with maximum deviations from the least-squares plane of  $\pm 0.02$  Å.

Since the potassium ion lies on a twofold axis, there are two unique potassium positions,  $K(1)$  and  $K(2)$ . Eight oxygen atoms from six *trans*-aconitate ions surround each potassium ion, with  $K^+-O$  distances ranging from 2.70 to 3.32 Å (Fig. 3). The coordination of  $K(2)$  may be described as a distorted cube, while that of  $K(1)$  does not seem to conform to any standard geometry. The *trans*-aconitate ion forms a bidentate chelate with one potassium ion [ $K(2)$ ], to give a seven-membered ring:  $K(2)O(1)C(1)C(2)C(3)C(6)O(6)$  (Fig. 1.)

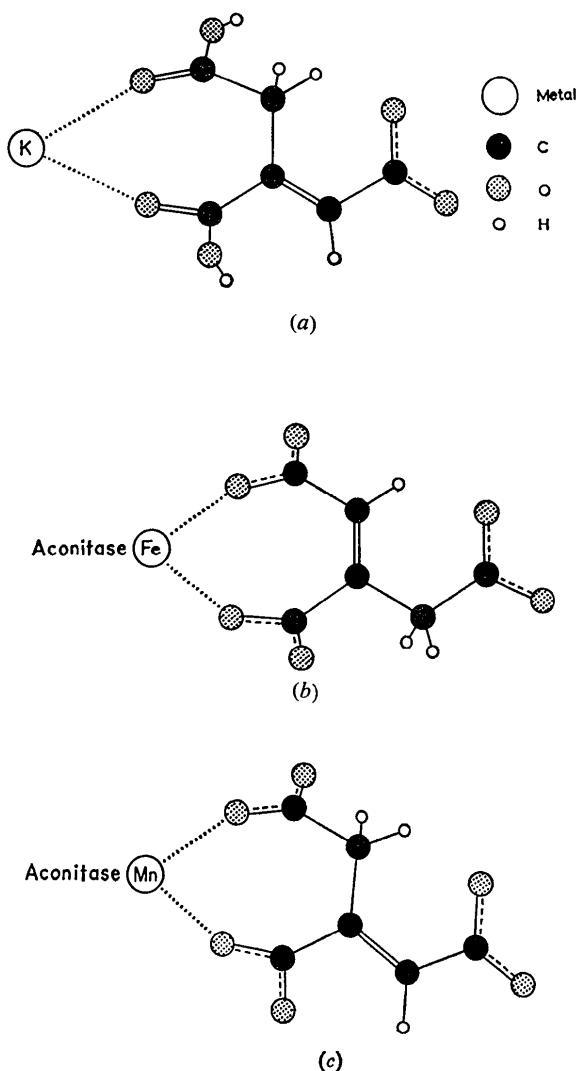


Fig. 4. (a) Chelation of the *trans*-aconitate ion to the potassium ion, as found in this study. (b) The proposed binding of the *cis*-aconitate ion in the active site of aconitase. (c) The aconitase-manganous-*trans*-aconitate complex detected by n.m.r. studies. Note the position of the double bond in these three diagrams.

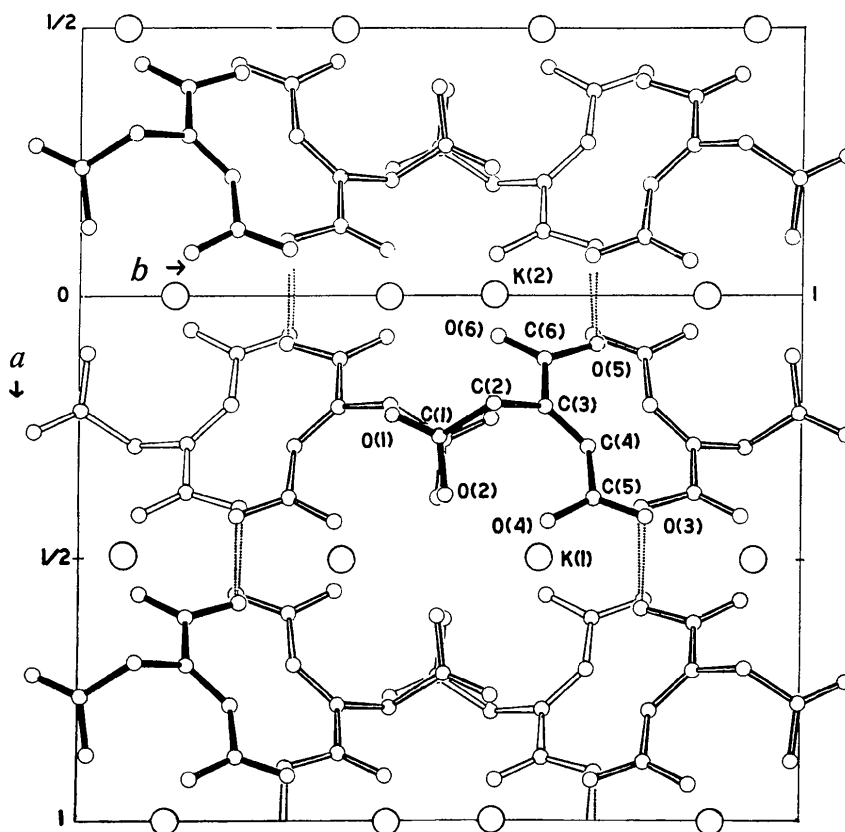


Fig. 5. Packing of the *trans*-aconitate molecule as viewed down the *c* axis. Hydrogen bonds are designated by dotted lines. The black molecule is on top, the striped in the center and the white on the bottom.

In the mechanism of the action of aconitase, proposed by Glusker (1968), the *cis*-aconitate ion is assumed to form a bidentate chelate with the enzyme-bound ferrous ion, resulting in a seven-membered ring as shown in Fig. 4(b). Since the *trans*-aconitate ion is a competitive inhibitor of aconitase (Thomson, *et al.*, 1966), it seems reasonable to assume that it would also bind at the active site of the enzyme. In the *trans*-aconitate ion the two carboxyl groups adjacent to the double bond are *trans* to each other. Therefore, in order to chelate to the ferrous ion in the active site of the enzyme the *trans*-aconitate ion must bind to the enzyme with the double bond further from the ferrous ion than that of the methylene grouping as shown in Fig. 4(a). This is opposite to the manner in which the double bond of the *cis*-aconitate ion is related to the metal ion [Fig. 4(b)]. It is assumed that the tri-ionized *trans*-aconitate ion chelates to the enzyme-bound ferrous ion in the same way as the mono-ionized ion has been found, by this crystallographic study, to bind to the potassium ion [Fig. 4(a)]. An aconitase-manganous-*trans*-aconitate complex has been detected by nuclear magnetic resonance studies (Villafranca & Mildvan, 1972), with the manganese-methylene hydrogen distance ( $\sim 5.0$  Å) greater than the manganese-methylene hydrogen distance ( $\sim 4.4$  Å). This result is consistent with the

crystallographic analysis reported in this paper and with the above remarks.

There are two hydrogen bonds present in crystalline potassium *trans*-aconitate. One is an intermolecular hydrogen bond of 2.52 Å which serves as a link between the respective ions to form chains which make an angle of 30° with the *xy* plane (Fig. 5). In this hydrogen bond the distance H(5)···O(3) is 1.55 Å, indicating that this is a strong interaction. The hydrogen bonds are formed between the hydroxyl group of one carboxyl group and an oxygen atom of the ionized carboxyl group in an adjacent anion. Similar hydrogen bonding has been reported in others structures, *e.g.* between adjacent anions of fumarate (Gupta & Sahu, 1970*a,b*). The intramolecular hydrogen bond is between the hydroxyl group of the terminal carboxyl group adjacent to the methylene group and the oxygen atom of the ionized carboxyl group that does not participate in the intermolecular hydrogen bond. The O—O distance is 2.53 Å and the O(4)···H(1) distance is 1.66 Å (Fig. 1). Thus both oxygen atoms of the ionized carboxyl group act as proton acceptors by participating in hydrogen bonding, each with the hydroxyl group of a different carboxyl group.

In the structure, the carbon-carbon double bonds lie over each other, but they are too far apart ( $\sim 5.6$  Å)

for it to be possible for the transition to a polymer to occur spontaneously within the solid. Thus, it seems more likely that the polymerization is a surface phenomenon, involving free radicals, and that it slowly affects the entire crystal.

We wish to thank Dr D. E. Zacharias for the use of the diffractometer at Smith, Kline and French Laboratories, Philadelphia, for a preliminary data collection, and Miss Anne Chomyn, Miss Carol Ann Casciato, and Mr Walter Orehowsky Jr for technical assistance. This research was supported by grants CA-10925, CA-06927, and RR-05539 from the National Institutes of Health, U.S. Public Health Service, and by an appropriation from the Commonwealth of Pennsylvania.

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*Acta Cryst.* (1972). **B28**, 1539

## A Barbiturate Without Hydrogen Bonding: The Crystal Structure of Sodium 1-Methyl-5,5-diethylbarbiturate (Sodium Metharbital)

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(Received 1 September 1971)

The crystal structure of sodium 1-methyl-5,5-diethylbarbiturate  $\text{Na}_2\text{C}_9\text{N}_2\text{O}_3\text{H}_{13}$  (also called sodium metharbital or sodium gemonil) has been determined from X-ray data measured on an automatic diffractometer. The crystal data are: space group  $Pna2_1$ ,  $a = 12.670$  (3),  $b = 10.796$  (2),  $c = 7.802$  (2) Å,  $Z = 4$ ,  $D_m = 1.369$  g.cm<sup>-3</sup>. The final  $R$  is 0.023. The conformation of the metharbital ion is similar to the conformations of the barbital ion and the barbital molecule. Methylation of the pyrimidine ring at N(1) and deprotonation at N(3) decrease the internal bond angles at N(1) and N(3) by approximately 4–5° each. In the absence of hydrogen bonding, the dominant role in crystal packing is played by ionic coordination interactions. The sodium ion is fivefold coordinated by four oxygen atoms and the deprotonated nitrogen atom. The extremely short Na–N distance of 2.42 Å suggests that this nitrogen atom carries a great part of the formal negative charge of the metharbital anion.

### Introduction

In the crystal structures of barbiturates determined so far, hydrogen bonding seems to play the dominant role in packing the barbiturate molecules or ions. Depending on the type of compound (free barbituric acid, salt, or complex), other intermolecular interactions may also be important: the requirements of effective packing of the alkyl groups (Gartland & Craven, 1971), C=O...C=O dipole-dipole interactions (Prout & Wallwork, 1966), and coordination of cations (Berking & Craven, 1971; Berking, 1971a,b).

In those barbiturates in which the hydrogen atoms at C(5) are substituted, the imino nitrogen atoms N(1) and N(3) in the pyrimidine ring serve as donors (or if deprotonated, as acceptors), and the exocyclic oxygen atoms serve as acceptors for hydrogen-bonding in a variety of ways.

The crystal structure of sodium 1-methyl-5,5-diethylbarbiturate (also called sodium metharbital or sodium gemonil) was determined because here, N(1) being methylated and N(3) being deprotonated, hydrogen-bonding is *a priori* impossible. Therefore the other binding types mentioned above are dominant. The re-