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Racemic calcium tartrate tetrahydrate [form (II)] in rat urinary stones

The title compound, $[\text{Ca}(\text{C}_4\text{H}_4\text{O}_6)] \cdot 4\text{H}_2\text{O}$, calcium tartrate tetrahydrate, is a new triclinic centrosymmetric form identified in rat kidney calculus. The crystal structure was determined from powder and single-crystal X-ray diffraction. The four water molecules belong to one square face of the Ca-atom coordination (a square antiprism), the four O atoms of the second square face coming from two tartrate anions, building infinite chains alternating Ca atom polyhedra and tartrate anions along **a**, with the chains cross-linked by a network of hydrogen bonds.

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1. Introduction

Urolithiasis is a rare condition in experimental animals such as rats or mice. Except in specific metabolic conditions induced to investigate some aspects of pathophysiology, most stones formed in such animals are made of struvite, which is magnesium ammonium phosphate hexahydrate (Sallis *et al.*, 1988; Linton *et al.*, 2007). Such stones mainly result from urinary tract infection and do not commonly disrupt the course of the studies, because the stones affect only a small proportion of experimental animals. However, extensive stone formation was reported in two recent studies involving rats fed with a synthetic diet containing DL-choline bitartrate as a choline source (Klurfeld, 2002; Newland *et al.*, 2005). The synthetic DL-tartaric acid, which was substituted in the diet for natural L-tartaric acid was considered to be the cause of the stone formation. Despite a number of analytical methods used for studying the stones, no convincing data were provided as to stone composition. We recently observed stone formation in rats subjected to various experiments and fed with different types of diets, all of which contained L-choline tartrate. The aim of this paper is to identify the stones in the animals and to compare the forms of calcium tartrate tetrahydrate (CaT).

The crystal structure of the previously known form of CaT, denoted form (I), which frequently grows in bottles of wine, has been reported four times (Ambady, 1968; Hawthorne *et al.*, 1982; Boese & Heinemann, 1993; Kaduk, 2007) in the $P2_12_12_1$ space group. A racemic CaT [form (II)] has been mentioned (Mastai *et al.*, 2002), but the crystal structure of this thin-needle-shaped form was not reported, although it was said that Frolow, Zimmerman and Addadi (unpublished) have studied and solved it. The title compound, with a massive parallelepiped crystal shape, could therefore have been a third calcium tartrate tetrahydrate polymorph. A noisy powder diffraction pattern of racemic CaT was published very recently (Menahem & Mastai, 2008), showing however that our title compound corresponds to this racemic form (II).

Table 1
Experimental details.

Crystal data	
Chemical formula	C ₄ H ₁₂ CaO ₁₀
<i>M_r</i>	260.22
Crystal system, space group	Triclinic, <i>P</i> $\bar{1}$
Temperature (K)	293
<i>a</i> , <i>b</i> , <i>c</i> (Å)	6.241 (2), 8.214 (3), 10.411 (5)
α , β , γ (°)	94.92 (4), 106.00 (5), 107.55 (3)
<i>V</i> (Å ³)	480.8 (4)
<i>Z</i>	2
Radiation type	Mo <i>K</i> α
μ (mm ⁻¹)	0.69
Crystal form, size (mm)	Parallelepiped, 0.12 × 0.10 × 0.03
Data collection	
Diffractometer	Siemens AED2
Data collection method	2 θ / ω scans
Absorption correction	None
No. of measured, independent and observed reflections	2222, 2222, 1495
Criterion for observed reflections	<i>I</i> > 2 σ (<i>I</i>)
<i>R</i> _{int}	0.023
θ _{max} (°)	27.6
No. and frequency of standard reflections	3 every 120 min
Intensity decay (%)	15
Refinement	
Refinement on	<i>F</i> ²
<i>R</i> [<i>F</i> ² > 2 σ (<i>F</i> ²)], <i>wR</i> (<i>F</i> ²), <i>S</i>	0.065, 0.187, 1.05
No. of reflections	2144
No. of parameters	174
H-atom treatment	Mixture†
(Δ / σ) _{max}	0.001
$\Delta\rho$ _{max} , $\Delta\rho$ _{min} (e Å ⁻³)	0.97, -0.56

Computer programs used: *STADIA* (Stoe & Cie, 1998a), *XRED32* (Stoe & Cie, 1998b), *ESPOIR* (Le Bail, 2001), *SHELXL97* (Sheldrick, 2008), *DIAMOND* (Brandenburg, 2001), *ENCIFER* (Allen *et al.*, 2004). † Mixture of independent and constrained refinement

2. Experimental

2.1. Animals and stones

Thirty male Wistar rats were fed with customized diets based on the AIN-93 formulation. They were allocated into

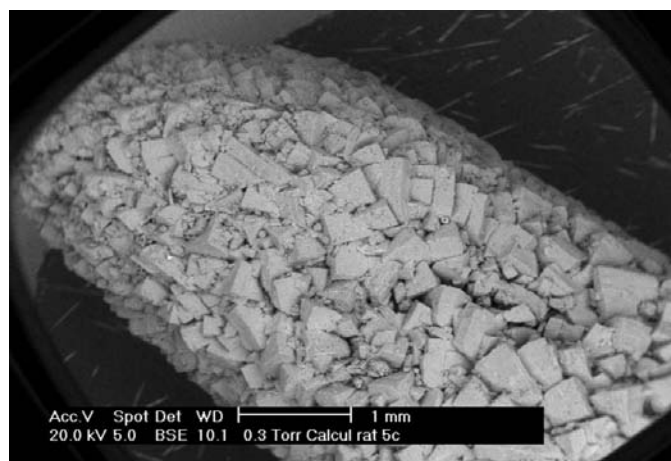


Figure 1
View of a massive block of interpenetrated crystals forming a large calculus completely filling a rat kidney.

three groups: a control group including ten animals (G1), a group of ten animals for studying a model able to induce kidney failure by a dietary load of dihydroxyadenine (G2), and a group of ten animals to develop a model of atherosclerosis (G3). The first case of stone was found in a G3 rat which died after 5 weeks of the experiment. Another rat of this group exhibited urolithiasis. In addition, five G2 rats and four G1 rats had kidney and/or bladder stones, all of which were discovered in sacrificed animals after 5–15 weeks under control or experimental conditions. Stones were analyzed by Fourier transform IR (FTIR) spectroscopy, scanning electron microscopy (SEM), powder and single-crystal X-ray diffraction. Stone analysis based on FTIR spectroscopy revealed an organic compound never found previously in urinary stones, the spectrum of which was similar to that published by Newland *et al.* (2005). The same compound was found in all stones which were composed of large crystals (100–200 microns), as observed by SEM. The crystal structure determination revealed a new form of calcium tartrate tetrahydrate. This is unusual since calcium in human urinary stones is mainly found included in oxalates (70% of the cases) or phosphates (15%; Daudon *et al.*, 1995; Bazin *et al.*, 2007) and rodents do not spontaneously form stones made of calcium salts (Sallis *et al.*, 1988). DSC, TGA and DTA results are available in the supplementary material.¹

2.2. Structure solution and refinement

Kidneys were filled by massive blocks of interpenetrated crystals (Fig. 1), so it was difficult to isolate a real single crystal. Consequently, the first characterization attempts failed due to the presence of extra reflections. Powder diffraction data were therefore indexed using the McMaille software (Le Bail, 2004) and a triclinic cell was obtained. The structure was solved from the powder data by direct space methods using the *ESPOIR* software (Le Bail, 2001) applied to intensities extracted by iterating the Rietveld decomposition formula (Le Bail, 2005). The positions of the calcium ion and water O atoms, and the position and orientation of the tartrate ion were optimized using a Monte Carlo procedure. Based on these convincing results (Fig. 2), additional effort was carried out with the data recorded from the pseudo-single crystal, producing atomic coordinates and displacement coefficients of much higher quality than from the powder data.

Refinement applied full-matrix least-squares methods (Sheldrick, 2008). H atoms were taken from a difference-Fourier map and refined isotropically with geometrical constraints and two common isotropic displacement factors (0.074 Å² for water-H and 0.036 Å² for the others). The relatively high *R* and *Rw* factors are probably due to the fact that the crystal was not really single. A few (35) reflections severely affected by diffraction peaks from other crystals were omitted. Experimental details are given in Table 1. Selected interatomic distances and angles are given in Table 2 and 3.

¹ Supplementary data for this paper are available from the IUCr electronic archives (Reference: KD5029). Services for accessing these data are described at the back of the journal.

Table 2
Selected geometrical parameters (Å).

Ca polyhedron		Tartrate	
Ca1—O1	2.401 (4)	O1—C2	1.245 (6)
Ca1—O2 ⁱ	2.403 (4)	O1—O6	2.236 (5)
Ca1—OW1	2.424 (4)	O2—C1	1.254 (6)
Ca1—OW3	2.439 (4)	O2—O5	2.213 (5)
Ca1—OW2	2.447 (4)	O3—C3	1.407 (6)
Ca1—OW4	2.472 (4)	O4—C4	1.426 (6)
Ca1—O3 ⁱ	2.479 (4)	O5—C1	1.245 (6)
Ca1—O4	2.522 (3)	O6—C2	1.265 (6)
		C1—C3	1.533 (6)
		C2—C4	1.527 (6)
		C3—C4	1.542 (6)

Symmetry codes: (i) $x - 1, y, z$.

The powder pattern was distributed as sample 1 of the SDPDRR3 (third round robin on structure determination by powder diffractometry; Cranswick & Le Bail, 2008). The structure could be determined by eight participants using various computer programs.

3. Results and discussion

The calcium environment in CaT form (II) is an almost perfect square antiprism (Fig. 3) in which the four water molecules form one of the square faces. The other four O atoms belong to two tartrate anions. Each tartrate ion is coordinated to two Ca ions so infinite chains are formed which are parallel to the *a*

Table 3
Hydrogen-bond geometry (Å, °).

<i>D</i> —H··· <i>A</i>	<i>D</i> —H	H··· <i>A</i>	<i>D</i> ··· <i>A</i>	<i>D</i> —H··· <i>A</i>
OW1—H1W1···OW2 ⁱ	0.89 (4)	1.84 (5)	2.729 (6)	178
OW1—H2W1···O4	0.89 (8)	2.26 (8)	2.919 (5)	131
OW2—H1W2···O5 ⁱⁱ	0.90 (7)	1.86 (6)	2.728 (6)	163
OW2—H2W2···OW3 ⁱⁱⁱ	0.89 (6)	2.11 (6)	2.933 (5)	152
OW3—H1W3···O6 ^{iv}	0.89 (5)	1.91 (5)	2.798 (6)	173
OW3—H2W3···O5 ^v	0.91 (4)	1.87 (5)	2.738 (6)	157
OW4—H1W4···O6 ^{vi}	0.90 (5)	1.96 (6)	2.853 (5)	170
OW4—H2W4···O6 ^v	0.90 (5)	2.10 (5)	2.947 (5)	155
O4—HO4···O2 ^{vii}	0.88 (3)	1.94 (3)	2.783 (5)	160
O3—HO3···O1 ^{iv}	0.87 (3)	1.82 (3)	2.683 (5)	169

Symmetry codes: (i) $-x + 1, -y + 1, -z + 1$; (ii) $-x + 1, -y, -z + 1$; (iii) $x - 1, y, z$; (iv) $-x + 1, -y, -z$; (v) $x, y + 1, z$; (vi) $-x, -y, -z$; (vii) $-x + 2, -y, -z + 1$.

axis. The two remaining O atoms (O5 and O6) of the tartrate anion are involved in inter-chain hydrogen bonding in the *ac* and *ab* planes together with the water molecules (Fig. 4). The two crystallographically characterized CaT forms are quite different. In the $P2_12_12_1$ form (I), the Ca atom is in a distorted Siamese dodecahedron built with two water molecules and six O atoms from four tartrate anions; the two remaining water molecules are interstitial and participate only in inter-molecular linkages; the six O atoms of a tartrate anion are shared with four different Ca atoms. The new form (II) is 2.4% less dense (1.797 g cm^{-3}) than the $P2_12_12_1$ form, and only four of the six O atoms of a tartrate anion participate in coordination to two different Ca atoms. One would have expected

form (II) to be denser than form (I), because of the absence of solvate water molecules, however, the consequence is also that the Ca atoms are less directly tied to the tartrate anions, forming chains instead of a three-dimensional network. The two carboxylate groups are differently oriented along the central C—C bond, almost staggered in form (I) and almost eclipsed in form (II) (Fig. 5). In spite of the coordination differences, the sum of the Ca—O bond valences (Brown & Altermatt, 1985) are similar, 2.22 and 2.19 in forms (I) (Kaduk, 2007) and (II), showing that the Ca is ‘crowded’. As stated by Kaduk (2007), the large amount of energy gained by the formation of the hydrogen bonds in both forms helps explain the high Ca bond valence and the relatively high density of these compounds.

Thermodiffractometry would be needed to confirm if the multiple events seen on the DSC, TGA and DTA of CaT forms (I) and (II)

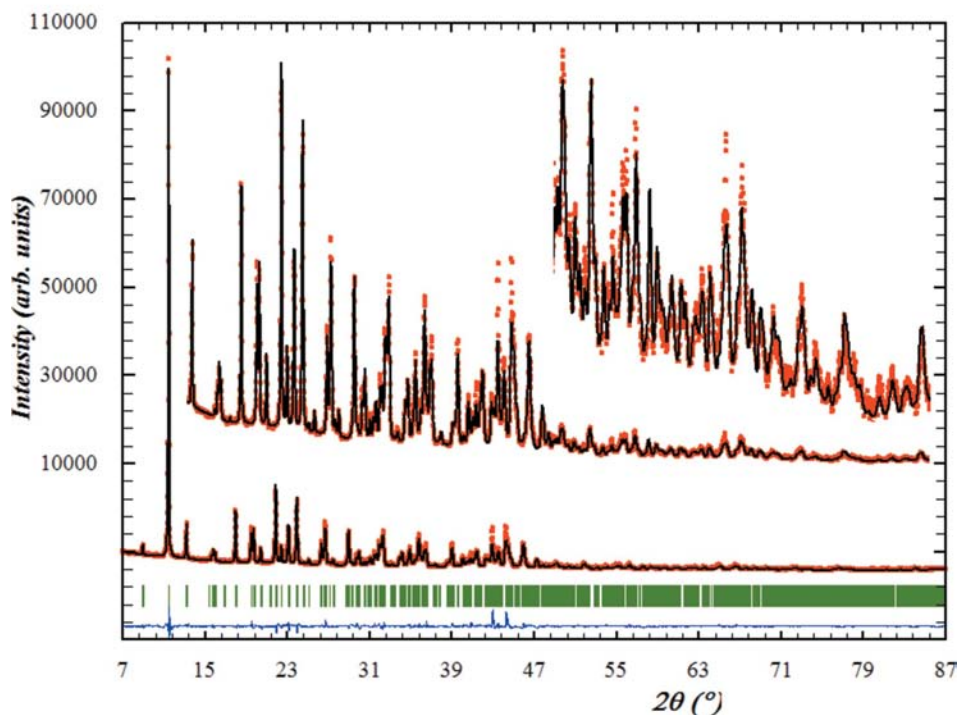


Figure 2
FULLPROF (Rodriguez-Carvajal, 1993) Rietveld plot obtained after the *ab initio* structure solution and refinement from powder data. Some preferred orientation was uncorrected at this stage and the H atoms were not located.

correspond to the existence of one or two intermediate hydrated crystalline phases. The existence of two loosely bounded interstitial water molecules in CaT form (I) is consistent with a hypothesis that with heating they are lost first at 388 K on the DSC by Menahem & Mastai (2008), or at 393 K on the DTA by Sahaya Shajan & Mahadevan (2005). It seems that two water molecules are first lost by form (II) at a higher temperature: 422 K on the DSC by Menahem & Mastai

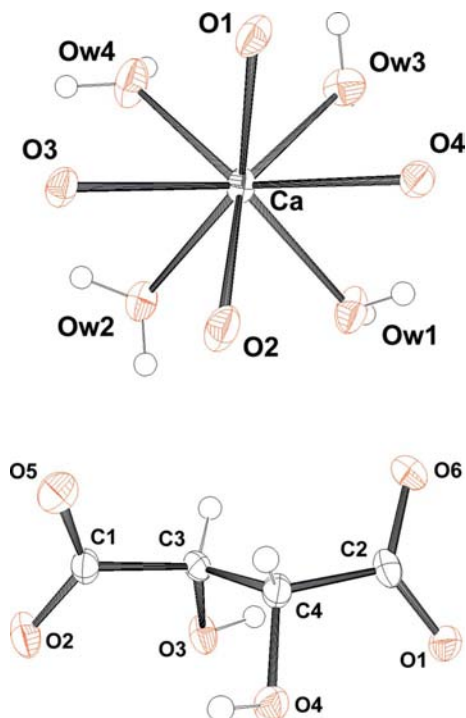


Figure 3
ORTEP (Farrugia, 1997) views of the calcium square antiprism and tartrate anion [(*R,R*) configuration].

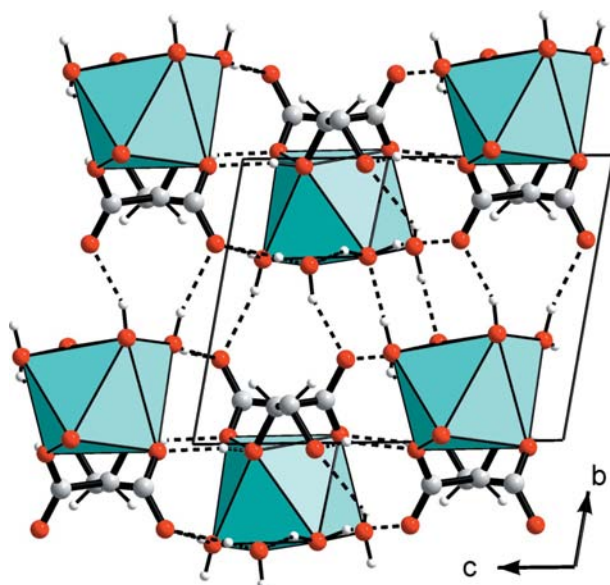


Figure 4
DIAMOND (Brandenburg, 2001) view of the structure with hydrogen bonds.

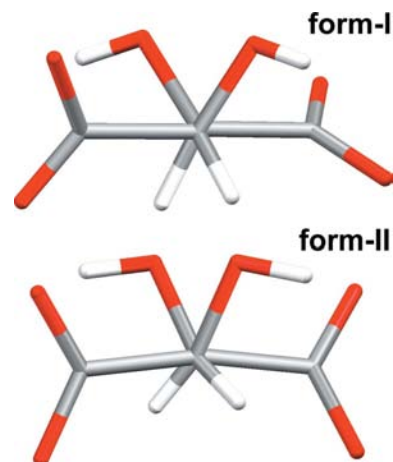


Figure 5
Newman projections along C–C bond: staggered and eclipsed conformations of the tartrate anion in forms (I) and (II).

(2008), this event being seen as two peaks at 415 and 430 K in this work (see supplementary material). Both forms would lose the remaining two molecules then, at ~ 438 K for form (I) and ~ 463 K for form (II).

The question why a previous structure determination (as mentioned in Mastai *et al.*, 2002) remained unpublished cannot have a clear answer here; it may be supposed that the needles were too thin and/or presented some interpenetration/twinning as encountered during this work.

4. Conclusions

The crystal structure of racemic CaT is established from poor quality ‘single crystal’ biomineralized in rat kidneys. The finding of a new form so long after the discovery of the first (which is very common in bottles of wine) is a bit surprising. A complementary use of powder and single-crystal X-ray diffraction was necessary in order to solve and refine the crystal structure. Both form (I) and (II) appear to be closely related to some important human life aspects, wine and urolithiasis, although the occurrence of the latter in human bodies has not yet been confirmed.

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