Polymorphism

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Single-Crystal X-ray Diffraction Structure of the Stable Enol Tautomer Polymorph of Barbituric Acid at 224 and 95 K

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Abstract: *The thermodynamically stable enol crystal form of barbituric acid, previously prepared as powder by grinding or slurry methods, has been obtained as single crystals by slow cooling from methanol solution. The selection of the enol crystal was facilitated by a density-gradient method. The structure at 224 and 95 K confirms the enol inferred on the basis of powder data. The enol has bond lengths that are consistent with the expected bond order and with DFT calculations that include treatment of hydrogen bonding. In isolation, the enol is higher in energy than the tri-keto form by 50 kJmol^{-1} which must be more than compensated by enhanced hydrogen bonding. Both crystal forms have four normal H-bonds; the enol has two additional H-bonds with O–O distances of 2.49 Å. Conversion into the enol form occurs spontaneously in the solid state upon prolonged storage of the commercial tri-keto material. Slurry conversion of tri-one to enol in ethanol is reversed in direction in ethanol-D$_1$.*

The enol tautomer of barbituric acid (Figure 1) is an example of tautomeric polymorphism. Tautomeric polymorphism is defined[1,2] as a pair of crystals each with a distinct tautomer. A 2011 systematic survey of the CSD showed that as of that date there were only 16 cases of this phenomenon.[1] There are many more cases of two tautomeric forms appearing in the same crystal. The related case of 2-thiobarbituric acid adds to this tally with the same keto/enol tautomeric pair.[3]

The reported single-crystal structures of barbituric acid, including its hydrates, are all in the tri-keto tautomer (**1**).[4–7] Ball-mill grinding experiments showed that a tautomeric form can be prepared in this fashion.[8] The resulting material was investigated by solid-state NMR and vibrational spectroscopy. An obvious feature of the Raman spectrum of the new polymorphic form was the relative absence of the characteristic C=O stretch vibrations. On this basis, it was concluded that the most likely possibility was the tri-hydroxy aromatic tautomer[8] as noted in Ref. [2]. It was, however, inferred from X-ray and neutron powder diffraction data that this tautomeric form is the enol structure (**2**; Figure 1).[9] Slurry experiments in methanol, ethanol, and butanol showed that this alternative tautomeric polymorph is the thermodynamically stable form.[9]

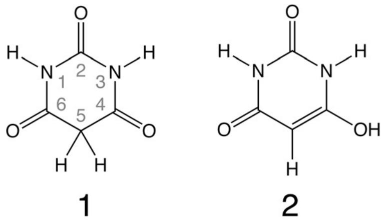

Figure 1. Relevant tautomeric forms of barbituric acid, keto (**1**) and enol (**2**).

In that work cooling and evaporation methods of crystal growth and several solvents, initial temperatures, initial concentrations, and cooling rates were employed. The density of the enol tautomer from the X-ray powder data is 1.669 gcm^{-3}.[9] The density of the tri-keto tautomer at room temperature[4] is 1.589 gcm^{-3}. This distinct density difference permits easy detection of candidate tautomers using density gradient analysis.[10] A mixture of tetrabromoethane and tetrachloroethylene was used for the high-density bottom of the gradient and a mixture of tetrachloroethylene and benzene as the low-density upper limit. A standard gradient maker consisting of two cylindrical glass containers connected via a stopcock at the bottom with one containing a stir bar and an effluent line via a second stopcock produces a linear gradient.[10] The resolution of a density gradient can be expanded by using a shallow gradient in a long column.

A solution of 1 g of our sample of commercial barbituric acid (see below) in 175 mL of methanol was heated to 60 °C and then cooled to 20 °C over four days and placed in a refrigerator at 4 °C. This is 3–4-fold below saturation at 60 °C. Crystals of this material had only one location in the density gradient indicating that only one polymorphic form was present with a density close to the value of the enol form.[9] Crystal growth from ethanol solution using a similar procedure resulted in crystals with the same density as those from methanol. They co-located in the same density gradient.

The single-crystal diffraction structure at 224 K and at 95 K were determined and found to be the enol.[9,11,12] The 224 K structure $P2_1/n$, $Z = 4$ has a density of 1.661 gcm^{-3} and a unit cell volume of 512.19 Å^3 in agreement with that reported for the X-ray powder data with a density of 1.659 gcm^{-3}. The bond lengths for the room temperature data are shown along with those of the two powder data sets in Table 1. The numbering system follows that of Ref. [8] and Figure 1.

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Table 1: Enol bond lengths from data obtained near room temperature.

Bond	SCX-ray[a]	XPD[b]	NPD[c]
N1-C2	1.362	1.376	1.385
C2=O	1.228	1.209	1.185
C2-N3	1.364	1.398	1.398
N3-C4	1.366	1.389	1.330
C4-O	1.311	1.332	1.271
C4=C5	1.366	1.325	1.397
C5-C6	1.402	1.443	1.423
C6=O	1.264	1.269	1.287
C6-N1	1.376	1.369	1.369

[a] Single-crystal X-ray diffraction. [b] X-ray powder diffraction. [c] Neutron powder diffraction.

The esd values for the 224 K single-crystal X-ray data of Table 1 are 0.0016 ± 0.00015 Å. (Table S5) The corresponding values for the powder-diffraction studies are reported Figure S5 the Supporting Information of Ref. [9] as "*The statistically calculated standard deviations of all bond lengths are 0.001 Å, but the actual accuracy can be estimated to be in the order of 0.01 to 0.02 Å only.*" This estimate reflects appreciation of the effect of systematic error which is evident in the last two columns of Table 1 that show the X-ray and neutron results. The bond angle esd's are similarly estimated to be 10–20 times the statistical values (Supporting Information of Ref [9]).

The bond lengths involving the heavy atoms, obtained from the 95 K data set, are given in Table S11 and are shown in Figure 2 where they are shown along with the results of two DFT calculations both with the B3LYP functional and the 6-31G(2d, 2p) basis set. The experimental results from the 95 K diffraction data are in black in Figure 2. The esd values for these numbers are 0.0014 Å. The calculations in red in Figure 2 include neighboring hydrogen-bonding groups- as shown in Figure S1 of the Supporting Information. The values in blue in Figure 2 are for the same computation without added hydrogen-bonding groups that are optimized with the barbituric acid structure.

Table 2: Hydrogen-bonding heavy atom distances [Å] in barbituric acid crystals. Note the difference in temperature.

Enol	Tri-keto		
this work	this work	Bolton[4]	Lewis[5]
95 K	95 K	RT	150 K
2.861	2.871	2.903	2.856
2.74	2.855	2.795	2.8
2.497			

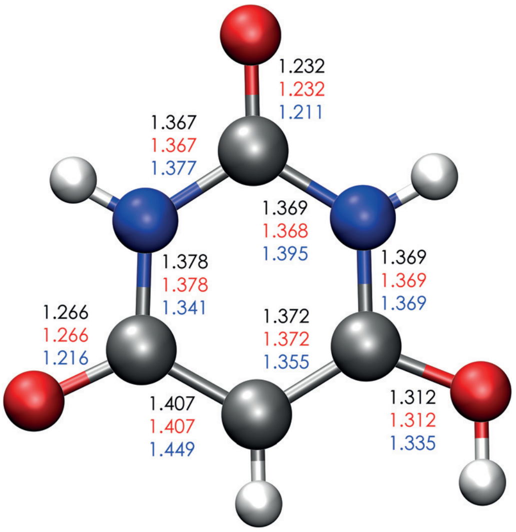

Figure 2. Heavy-atom bond lengths of the enol form of barbituric acid: black: observed at 95 K. Red: computed from DFT B3LYP-31G(2d,2p) for a hydrogen bonded model (see Supporting Information). Blue: as computed for an isolated enol tautomer. Atoms: C gray, O red, N blue, H white.

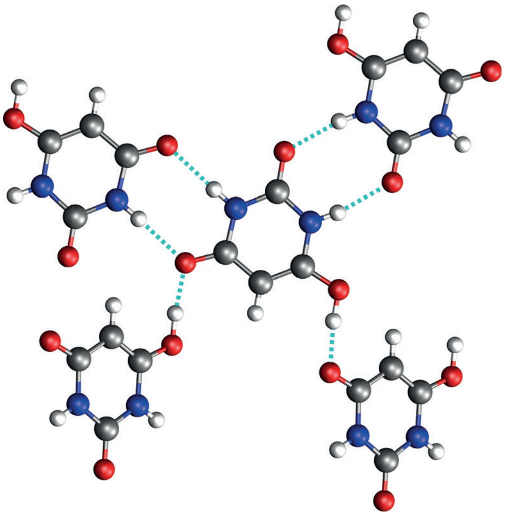

Figure 3. Pentamer hydrogen-bonding (broken lines) pattern for barbituric acid in its enol structure. The top two units are coplanar with the central unit; the bottom two are out of plane with a dihedral angle of 134.54°.

The hydrogen bonding of the enol tautomer of barbituric acid in Figure 3 shows that each molecule forms four NH:O hydrogen bonds and one hydrogen bond with a central carbonyl group acting as a double acceptor. These are **NH**-O=C 2.861 and 2.740 Å, **C=O**-HN 2.740 and **COH**-O=C 2.497 Å and the **C=O** to HN (2.861 Å) and HO-C (2.497 Å) where bold denotes central molecule groups. In contrast the original tricarbonyl structure of Bolton[4] BARBAC, has only four H-bonds **NH**-CO of 2.795 and 2.903 Å (Table 2) and **C=O**-HN of the same lengths[4] The BARBAC01 of Lewis et al. has two H-bonds of length 2.856 Å and two of 2.800 Å[5] If we compare the H-bond lengths, it is clear that four of the H-bonds are about the same in each case or slightly shorter and thus stronger in the enol form and there is an additional hydrogen bond in the enol form that is considerably shorter, 2.497 Å. Based on its O–O distance, this O-H-O hydrogen bond, is "strong", as classified in standard Reviews.[13,14]

The structure determined at 95 K (Figure 2) differs significantly from that obtained by optimizing the structure in isolation. The major difference is the longer C=O bond and the shorter C–N bond in the crystal compared to that for the optimized structure without hydrogen bonding. This situation is well established from previous work on peptides.[15]

A normal mode calculation for the $Z=4$ unit cell shows that the C=O stretch vibrations in this structure are strongly admixed over all four units and that the C=O stretch is also admixed with other of in-plane motions and is distributed over a range of 150 cm^{-1}. This is also the case if we use the pentamer hydrogen-bonded structure of Figure 3. This arrangement explains the absence of the usual characteristic C=O stretch activity in the vibrational spectra that formed the basis of the original proposal as to the nature of this material.

The enol form of barbituric acid in isolation is less stable than the tri-keto form. This energy difference is on the order of 45–55 $\mathrm{kJ\,mol^{-1}}$ (see Supporting Information and Ref. [9]). This case is thus an unusual situation, especially for the lowest energy polymorphic form. The dipole moment of the tri-one form is 0.25 D while that for the enol is 5 D so this energy difference will be decreased by high dielectric solvents. For the case of a 6-31G(3d,3p) B3LYP calculation where the enol/tri-keto difference is 44 $\mathrm{kJ\,mol^{-1}}$ in vacuuo, this value drops to 37 $\mathrm{kJ\,mol^{-1}}$ for a solvation computation simulating water. This energy difference must be at least compensated by the strength of the hydrogen bonds.

The survey of tautomeric polymorphism in the CSD[1] used the energy difference between two reported tautomeric forms as a criterion for detailed examination of the structures involved. In this case a simplified DFT method (B3LYP/6-311++G**/PCM $\varepsilon=3$) was necessarily used. Using this method the majority of the 16 pairs designated as tautomeric polymorphs have energy differences less than 12 $\mathrm{kJ\,mol^{-1}}$. The statistics reported for the combined sample of structures including both tautomeric polymorphs and the much more numerous mixed tautomer structures shows that in this more general sense the energy difference as computed by the above method, extends to slightly over 30 $\mathrm{kJ\,mol^{-1}}$. The value computed by this above method for the current enol/keto pair is 36 $\mathrm{kJ\,mol^{-1}}$ and is thus just at the limit of other known cases for this larger group of tautomeric materials but well beyond any other tautomeric polymorph. The corresponding values for the thiobarbituric acid keto/enol pair are about 10% smaller than for the barbituric acid pair.

A key point in the validation of the conclusions of the powder diffraction studies of Ref. [9] was the inclusion in that report of the results of a calculation indicating that based on the lattice energy of the enol structure was 58.5 $\mathrm{kJ\,mol^{-1}}$ more stable than that for the tri-keto form. This is larger than a 53.7 $\mathrm{kJ\,mol^{-1}}$ value of the energy difference using CCSD-T/cc-pvtz[9] of the two tautomers by 4.8 $\mathrm{kJ\,mol^{-1}}$ and thus in agreement with and supporting of the finding that the enol forms the most stable crystal. The computational method used is specified only as a periodic boundary dispersion corrected DFT. Neither the functional or convergence criteria were specified. The cited references[16] are not much help. The optimized structure obtained from this calculation was not reported, so it cannot be compared to the results of our structure determination. Further, the use of a highly correlated gas-phase reference is debatable.

One of the authors of Ref. [9] has published a paper on the subject of the relative stability of the tautomeric forms of four ammonio benzene sulfonates.[11] The method used is the plane-wave VASP code with the PBE functional using a cited empirical dispersion correction and specified convergence criteria. It is not specified if the lattice parameters are optimized. These four materials crystallize in four different crystal structures all in the zwitterionic tautomer form. In two cases, the correct structure is not predicted to be the lowest by amounts of 2.44 and 2.62 $\mathrm{kJ\,mol^{-1}}$. If the same error limits apply to the barbituric acid case, then the lattice energy difference is uncertain to 50%. The actual uncertainty limits must have added to the above, the difference in energy that favors the correct structure, that is, a zero energy difference is not predictive. There is, in addition, the systematic error in comparison of the energy difference between two molecules with a correlated method with the difference of two solid forms with a DFT method.

The conversion of barbituric acid from the tri-keto into the enol tautomeric crystal polymorph is spontaneous and occurs slowly in the solid state. A commercial sample[17] that is now 7 years old was found by Raman microscopy to be 80% converted into the enol. This conversion is greatly facilitated by grinding and slurry.[8,9] Slurry conversion does not occur if the sample has its four exchangeable hydrogen atoms replaced by deuterium atoms. This result suggests a role for the zero-point level in the thermodynamics as has previously been shown for polymorphic forms of glycine[18] and for other cases.[19] More specifically, our commercial sample that is predominantly the enol tautomer, if slurried in ethanol-$\mathrm{D_1}$ for several days, shows conversion into the tri-keto form as indicated by the reappearance of the C=O stretch intensity in the Raman spectrum. This conversion indicates that there is a significant zero-point energy and or vibrational enthalpy contribution to the energetic difference between the keto and enol forms.

Our work herein provides a reliable basis for full lattice computations and harmonic dynamics. Specifically, following the procedure used for the α- and γ-polymorphs of glycine.[18] The Γ-point computation of the harmonic lattice dynamics will be used to simulate the inelastic neutron scattering spectrum. Comparison of the observed and computed INS spectra provides a full assignment of the vibrations as well as a check on the structure used. The zero-point and thermal energy difference between the tri-keto and enol forms and the effect of H/D replacement can then be included in the tabulation of the enthalpy. In this regard, the enthalpy difference between these two forms as obtained from heats of dissolution would be of considerable interest in providing a test of the energy computation as has been done for two polymorphic forms of glycine.[18]

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[12] $C_4H_4N_2O_3$, FW 128.09, $0.6\times0.05\times0.05$ mm^3, monoclinic, $P2_1/n$, $Z=4$, 0.71073 Å X-ray. At 224(2) K: $a=4.8497(3)$, $b=8.9134(6)$, $c=11.8920(8)$ Å, $\alpha=90$, $\beta=94.892(2)$, $\gamma=90°$, $V=512.19(6)$ Å^3, 1.661 Mg m^{-3}, $\mu=0.145$ mm^{-1}, $\theta_{max}=27.221°$, 11272 reflections (1139 independent), $R_{int}=0.0316$, final R indices $[I>2\text{sigma}(I)]$, $R_1=0.0335$, $wR_2=0.0933$, R indices (all data), $R_1=0.0399$, $wR_2=0.0991$, largest diff. peak and hole, 0.206 and -0.239 e Å^{-3}, full-matrix least-squares refinement on F^2, data/restraints/parameters 1139/0/85, goodness-of-fit on $F^2=1.048$. CCDC 1418712 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre. At 95 K: $a=4.7799(4)$, $b=8.9363(8)$, $c=11.8023(11)$ Å, $\alpha=90$, $\beta=96.133(3)$, $\gamma=90°$, $V=501.25(8)$ Å^3, $V=1.697$ Mg m^{-3}, $\mu=0.148$ mm^{-1}, $\theta_{max}=27.397°$, 11533 reflections collected (1130 independent), $R_{int}=0.0208$, final R indices $[I>2\text{sigma}(I)]$, $R_1=0.0306$, $wR_2=0.0801$, R indices (all data), $R_1=0.0344$, $wR_2=0.0832$, largest diff. peak and hole, 0.273 and -0.255 e Å^{-3}, full-matrix least-squares refinement on F^2, data/restraints/parameters 1130/0/85, goodness-of-fit on $F^2=1.079$. CCDC 1418693 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

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