Influence of Crystal Packing on the Solid-State Desolvation of Purine and Pyrimidine Hydrates: Loss of Water of Crystallization from Thymine Monohydrate, Cytosine Monohydrate, 5-Nitrouracil Monohydrate, and 2′-Deoxyadenosine Monohydrate

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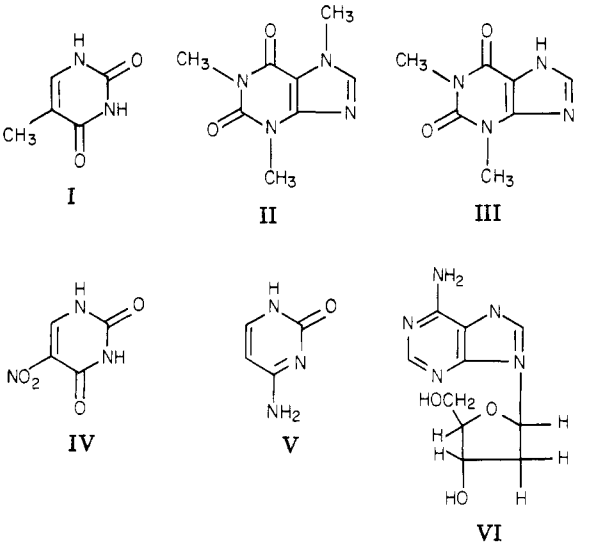
The desolvation of single crystals of thymine monohydrate, cytosine monohydrate, 5-nitrouracil monohydrate, and 2′-deoxyadenosine monohydrate was investigated. It was shown that desolvation reactions of thymine monohydrate, cytosine monohydrate, and 5-nitrouracil monohydrate proceed along a preferential direction corresponding to one of the crystallographic axes. Crystal packing analysis showed that this direction corresponded to the direction of water tunnels through the crystal. The desolvation of 2′-deoxyadenosine did not proceed in a clear front; however, the reaction appeared to be anisotropic with the reaction proceeding preferentially along the crystallographic axis corresponding to the direction of the water tunnel. The temperature required to desolvate these hydrates ranged from 25 to 100 °C. The threshold temperature for desolvation could be correlated with the cross sectional area of the water tunnel and the number and length of hydrogen bonds to the water molecules. This paper illustrates, for the first time, the important effect crystal packing (the tunnel area and hydrogen bonding) can have on desolvation reactions.

Desolvation of crystalline hydrates of organic compounds is an important process, particularly in the pharmaceutical industry,[1] and an understanding of the factors controlling this process is needed. In general, desolvation can form product crystals with the same crystal structure as the hydrate or product crystals with a different crystal structure often identical with the anhydrate. The product crystals with the same crystal structure as the hydrate sometimes have enhanced chemical reactivity and are thus undesirable because of their instability. The product crystals with different crystalline structures have different physical properties and thus different pharmaceutical properties and different bioavailabilites. Thus, it is important to develop a basic understanding of the factors influencing the desolvation reaction so that this reaction can be prevented, if possible.

In preceding papers[2,3] from our laboratory the desolvation of the crystal solvates of organic compounds, including caffeine hydrate, theophylline hydrate, cycloserine hydrate, cyclohexadiene-L-alanine–0.75-water, and bis(salicylaldehyde)(ethylenediimine)cobalt(II)–chloroform, was briefly discussed. These studies indicate that crystal packing plays an important role in these reactions.

Studies of inorganic crystal hydrates show that three additional factors influence the facility of dehydration: surface area, relative humidity, and production and growth of nuclei.[4-10] An increase in surface area, in general, results in an increase in the facility of desolvation.[8] An increase in relative humidity results in a decrease in the facility of desolvation.[8,9] In addition, a study of the dehydration of four inorganic hydrates showed that three of these dehydrated isotropically, with the reaction spreading from a few points within the crystal. the fourth hydrate, $Na_2B_4O_7 \cdot 10H_2O$ dehydrated anisotropically. The direction of dehydration is along the $Na(H_2O)_4$ chains running through the crystal.[10]

Chart I



The approach adopted in this paper is to control, as much as possible, the surface area, relative humidity, and, particularly, the production and growth of nuclei so that the effect of crystal packing and hydrogen bonding can be related to the direction of frontal migration in the crystal and the facility of desolvation. The results of this study indicate that, indeed, crystal packing and hydrogen bonding are important factors which need to be considered to provide a balanced view of desolvation of crystal hydrates.

The purines and pyrimidines, thymine (I), caffeine (II), theophylline (III), 5-nitrouracil (IV), cytosine (V), and 2′deoxyadenosine (VI) (Chart I) crystallize from water in the hydrate form. In addition, the crystal sizes are similar. These hydrates are, thus, expected to be good candidates for the study of the effect of crystal packing and hydrogen

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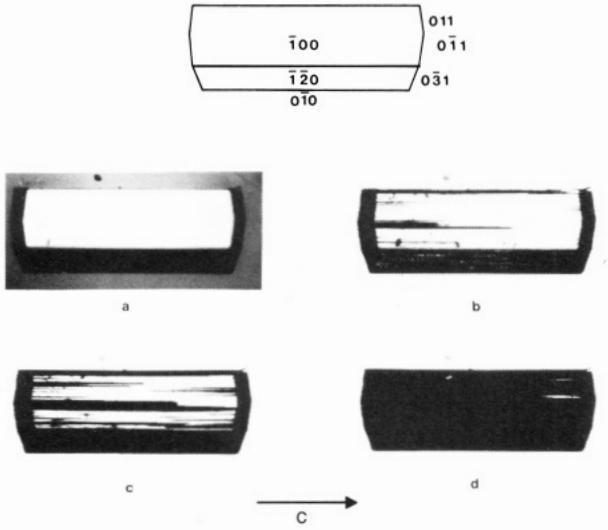

Figure 1. Desolvation of a single crystal of thymine hydrate at 40 °C: (a) at start, (b) after 49 min, (c) after 76 min, (d) after 115 min.

bonding on the dehydration of crystal hydrates.

Experimental Section

Reactants. The compounds investigated were caffeine, theophylline, thymine, cytosine, 5-nitrouracil, and 2′-deoxyadenosine. Single-crystal hydrates were obtained by recrystallization of commercialy available compounds either from a chilled solution in the refrigerator or by evaporating a saturated solution at room temperature over concentrated (95%) sulfuric acid in a closed space.

Solid-State Decomposition Reactions. Single crystals of each compound were placed on a Zeiss microscope equipped with a camera and a Mettler FP5 or FP52 hot stage. The reaction was followed by photographing the crystal with time.

Optical Goniometry. The interfacial angles were measured on a Huber optical goniometer and compared to the calculated angles by use of a computer program. The assigned faces were confirmed by using X-ray precession photographs.

Tunnel Cross-Sectional Area. For each compound, the molecules inside the unit cell were projected on the plane perpendicular to the dehydration direction by using the published crystallographic coordinates.[12-17] Circles centered on the atom positions with their corresponding van der Walls radii were then used to delimit the perimeter of the tunnels. Cross-sectional areas were then measured with a planimeter and transformed in appropriate units.

Dehydration temperature thresholds have been measured by means of a Du Pont 951 thermogravimetric analyzer. A 6 °C/min temperature gradient was applied to the sample (10–20 mg) by means of a Colman UP 55 temperature programmer. The threshold temperature was defined as the temperature at which 2% of the solvent is lost. The estimated error in these measurements is ±1.0°.

Powder Diffraction of Desolvated Crystals. The powder diffraction patterns of desolvated single crystals of cytosine and 5-nitrouracil were measured on a Debye–Scherrer powder camera by using Cu Kα radiation. In addition, the powder patterns of ground cytosine hydrate, 5-nitrouracil hydrate, and 2′deoxyadenosine hydrate and of ground samples of dehydrated cytosine hydrate, 5-nitrouracil hydrate, and 2′-deoxyadenosine hydrate were measured.

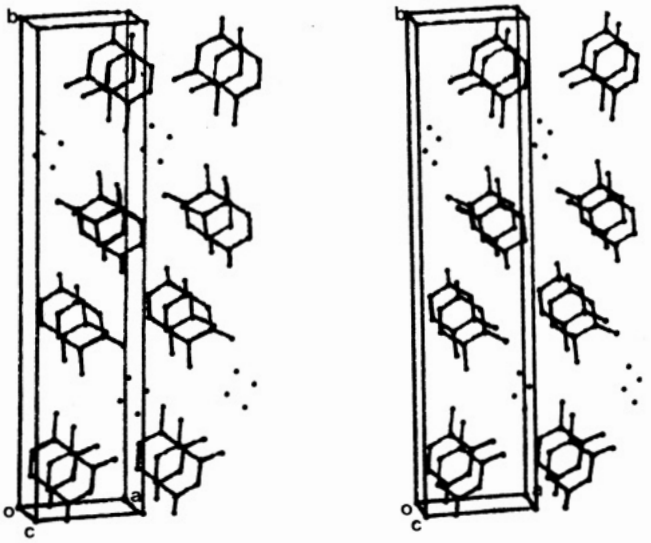

Figure 2. Crystal packing of thymine monohydrate. The axial directions, with respect to the origin at the bottom left hand corner, are as follows: *a*, across; *b*, vertical; *c*, out of the plane of the paper.

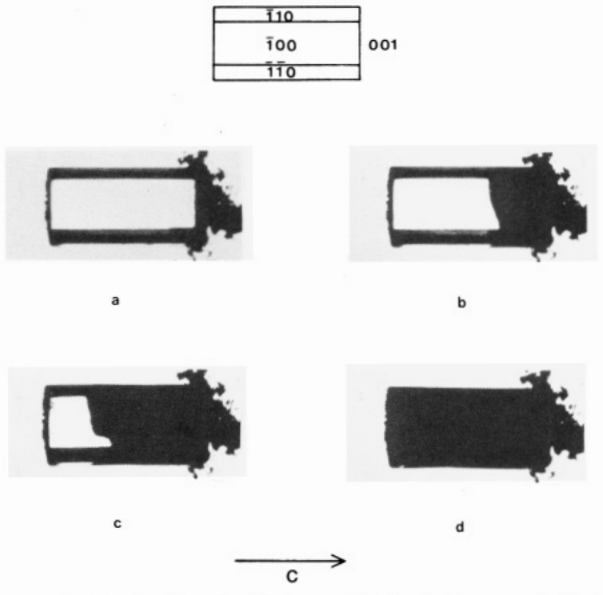

Figure 3. Desolvation of a single crystal of cytosine monohydrate at 40 °C: (a) at start, (b) after 54 min, (c) after 97 min, (d) after 115 h with the 001 face activated by the powdered anhydrous compound.

Initiation of Desolvation of Hydrate Crystals. Attempts were made to initiate the desolvation of the hydrate crystals by (1) removing the ends with a scalpel, (2) dusting the ends with powdered desolvated crystals, and (3) both 1 and 2.

Compactness of the crystal has been calculated by using a computer program which performs the calculation of the molecular volume according to Kitaigorodskii's formula and then calculates the ratio of molecular volume to the unit cell volume.[18]

Results and Discussion

As previously demonstrated[2,3] growing opaque zones inside a single crystal are due to the loss of solvent. Desolvation of a typical crystal of thymine monohydrate is shown in Figure 1. When the crystal is allowed to stand, dehydration occurs from the end faces, (011), ($0\bar{1}1$) and

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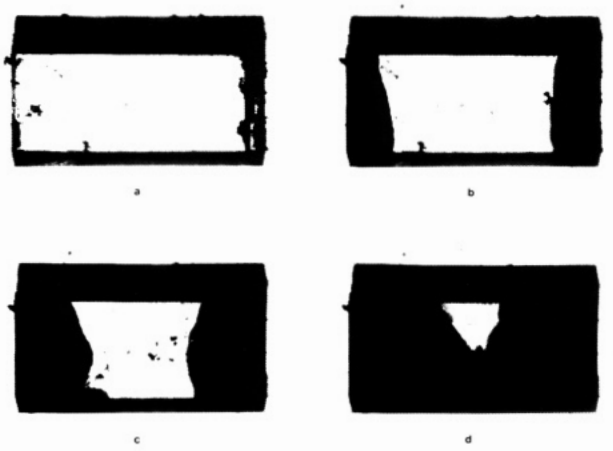

Figure 4. Desolvation of a single crystal of cytosine monohydrate at 60 °C: (a) at start, (b) after 8 min, (c) after 15 min, (d) after 24 min with both (001) faces activated by scratching with the anhydrous compound.

Figure 5. Crystal packing of cytosine monohydrate. Two unit cells are shown along the *c* axis (adjacent molecules to the *b* axis have been added). The axis direction is as follows: *a*, across; *b*, vertical; and *c*, out the plane of the paper.

$(0\bar{3}1)$ and $(0\bar{1}\bar{1})$, $(01\bar{1})$, and $(03\bar{1})$, toward the center of the crystal in a direction parallel to the *c* axis. Figure 2 shows the crystal packing of thymine, and the water molecules (dots on the drawing) are arranged in a zigzag chain along the *c* axis direction, so that this anisotropic behavior can be explained by the preferential escape of the water along these chains, as in a pipe or tunnel. No obvious tunnel can be seen in other directions.

Figure 3 shows the dehydration of cystosine monohydrate; in this case the (001) face has been activated by pressing the powder of the anhydrous compound against this face, so that the first step of the reaction corresponding to the production of the nuclei is considerably shortened. Dehydration can take place in an anisotropic fashion, proceeding from the activated end toward the center of the crystal in a well-defined front. Figure 4 shows the front advance from both of the end faces, (001) and $(00\bar{1})$, which have been activated by scratching with the anhydrous compound. The crystal packing of cytosine monohydrate is shown in Figure 5, where tunnels can be seen parallel to the *c* axis. The preferential dehydration direction of cytosine is thus explained as in the case of thymine, by escape of water along the tunnel direction. Unactivated crystals have also been studied and have shown dark lines along the *c* axis randomly distributed from both the (001) and $(00\bar{1})$ faces or the (102) and $(\bar{1}0\bar{2})$ faces in the same manner as for thymidine hydrate. At 60 °C dehydration of cytosine monohydrate also proceeds in a direction perpendicular to the *a*,*c* plane; this process occurs mainly at the end of the overall dehydration reaction and may be again explained by looking at the crystal packing: the intermolecular distance between sheets of cytosine is 3.78 Å which corresponds to an average free passage of 0.2 Å and might allow the escape of water between these sheets.

Figure 6 shows the behavior of a crystal of 5-nitrouracil monohydrate viewed perpendicular to the 010 face. The

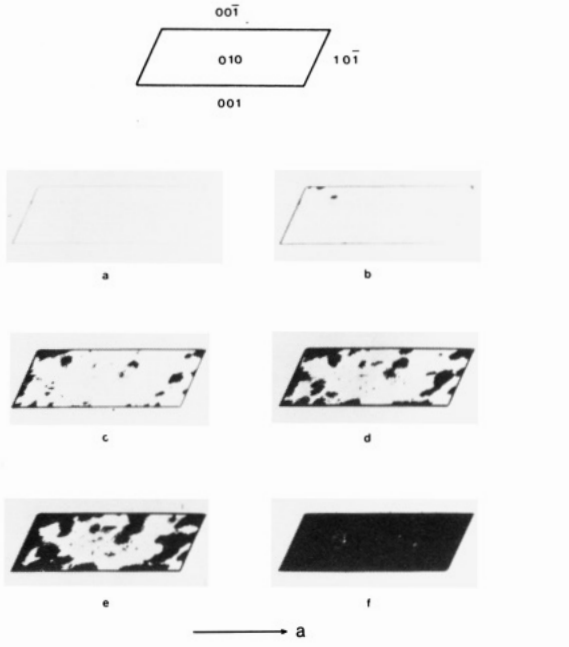

Figure 6. Desolvation of a single crystal of 5-nitrouracil monohydrate at room temperature over anhydrous $CaSO_4$: (a) at start, (b) after 14 h, (c) after 25 h, (d) after 32 h, (f) after 48 h. The orientation of the crystal axes is as follows: *a*, across; *b*, out of the plane of the paper; *c*, down.

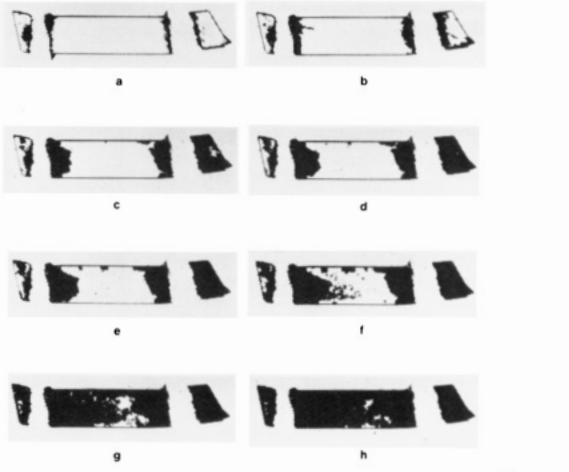

Figure 7. Desolvation of a single crystal of 5-nitrouracil monohydrate at room temperature over anhydrous $CaSO_4$: (a) at start, (b) after 13 h, (c) after 24 h, (d) after 28 h, (e) after 32 h, (f) after 38 h, (g) after 50 h, (h) after 56 h. Both ends of the crystal have been cut to create artificial (100) faces. The orientation of the crystals is a, across; b, out of the plane of the paper; and c, vertical.

crystal shape is a parallelogram where the (001) and $(00\bar{1})$ faces correspond to the longer edges and the $(10\bar{1})$ and $(\bar{1}01)$ to the shorter. Dehydration occurs mainly by a nucleation process randomly dispersed on the (010) and $(0\bar{1}0)$ faces. From each nuclei the propagation of dehydration reaction proceeds along two directions, the *a* axis and the *c* axis, with nearly the same velocity. Figure 7 shows the behavior of a similar crystal in which artificial (100) and $(\bar{1}00)$ faces have been created by cutting the crystal with a scalpel. Dehydration now proceeds by front advance along the long morphological axis (the *a* axis) as seen previously for the other hydrates.

The crystal packing of 5-nitrouracil monohydrate is shown in Figure 8. Water tunnels can be seen along the *a* and *c* axes, and these tunnels explain the anisotropic behavior of the dehydration reaction. In this case, the

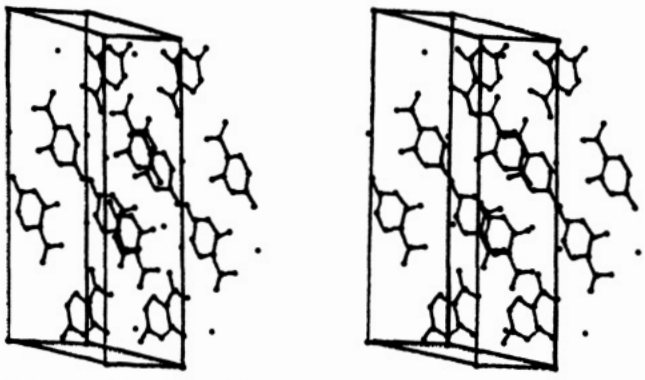

Figure 8. Crystal packing of 5-nitrouracil monohydrate where the axial directions are as follows: *b*, vertical; *c*, approximately across; *a*, approximately out of the paper plane.

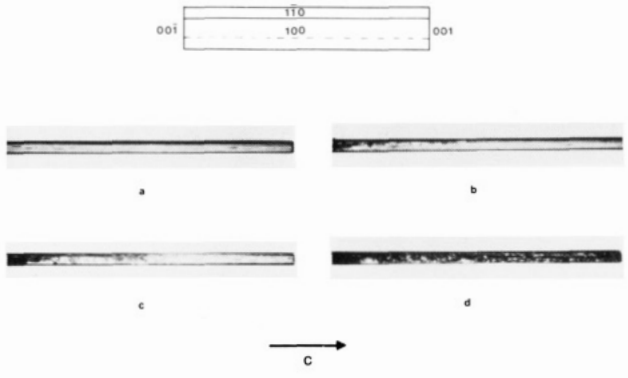

Figure 9. Desolvation of 2′-deoxyadenosine monohydrate at 100 °C: (a) at start, (b) after 16 min, (c) after 87 min, (d) after 170 min. The direction of the axes are as follows: *c*, across; *a*, out of the plane of the paper; *b*, down.

activation of the crystal appears to be more important than for the preceeding compounds, perhaps because the habit does not give a regular (100) face.

2′-Deoxyadenosine monohydrate is a quite stable compound at room temperature; no dehydration reaction is observed even at 80 °C for 24 h. Figure 9 shows the dehydration reaction of a single crystal at 100 °C; in this case no regular front advance nor dark lines can be seen, even when the faces are activated. However, dehydration proceeds along *c* axis with a "tarnishing phenomena" occurring at the same time as shown in Figure 9. The crystal packing of 2′-deoxyadenosine is shown in Figure 10 where dehydration tunnels can be seen along the *c* axis and which come out of the plane of the paper.

At this point, it is appropriate to summarize the behavior of the crystal hydrates and the factors controlling their desolvation. The anisotropic desolvation of crystals under a microscope can be explained by crystal packing. The crystals lose their solvent of crystallization along particular crystallographic directions which, in general, correspond to the direction of solvent tunnels in the crystal. This is probably because an escaping water molecule encounters the fewest nonpolar functionalities during escape along the tunnel direction.

The tunnel direction in these five examples is the needle axis of the crystals. In addition, the tunnel direction is nearly perpendicular to the rings of cytosine, thymine, and 5-nitrouracil. For crystals of caffeine hydrate, the tunnel direction is also nearly perpendicular to the heterocyclic aromatic ring.[2] The needle axis is the direction of fastest growth of these crystals because the ring–ring interactions favor growth along this axis. Thus, the direction along which desolvation occurs is also the direction of fastest growth of the crystal.

Powder diffraction showed that the crystal structure of the dehydrated products of cytosine hydrate, 5-nitrouracil hydrate, 2′-deoxyadenosine hydrate, theophylline,[10] and caffeine[18] were different from the starting hydrates. Powder diffraction showed that dehydrated thymine has the same crystal structure as thymine hydrate. Thus the reaction in these cases (except for thymine) involves loss of water and transformation to a new crystal form. Powder photographs of desolvated single crystals of cytosine and 5-nitrouracil gave the same powder pattern as ground dehydrated products, showing that an amorphous form was not produced. The photograph of the dehydrated cytosine single crystal showed some arc-shaped spots, indicating the crystalites of product were fairly large.

Experiments aimed at initiating the desolvation by cutting crystals with a scalpel or dusting the ends with dehydrated powder showed that the desolvation of cytosine hydrate and 5-nitrouracil hydrate was initiated by this treatment, but the desolvation of thymine hydrate and 2′-deoxyadenosine was unaffected. No experiments could be performed on theophylline and caffeine because it was impossible to dust the ends of the very thin elongated plates with dehydrated powder.

A more in-depth consideration of the desolvation process indicates that other crystal packing factors may also play a role in hindering the escape of water molecules out of the crystal. They are as follows.

(a) Tunnel Size. For example, it would be easier for solvent to exit a large tunnel than a small tunnel. Thus this parameter may be approximated by measuring the cross-sectional area of the tunnel. Figure 11 shows the dramatic difference in tunnel size for 5-nitrouracil hydrate and thymine hydrate.

(b) Compactness of the Crystal Packing. The more closely packed a crystal the harder it will be for solvent molecules to escape. Thus the compactness of the packing, which is the ratio of the volume of the molecules in the unit cell to the volume of the unit cell, might be expected to pay a role in hindering the escape of solvent molecules particularly in cases where some of the solvent molecules do not escape out the tunnel direction. We have calculated

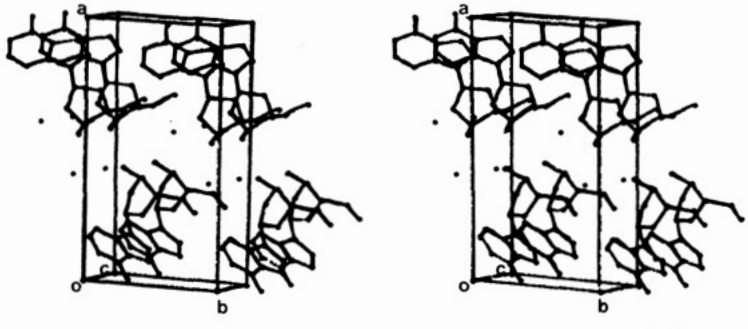

Figure 10. Crystal packing of 2′-deoxyadenosine where the axial directions are *a*, vertical; *b*, across; *c*, out of the paper plane.

Figure 11. Projection of the crystal packing on a perpendicular plane to the dehydration direction for tymine[12] (a) and 5-nitrouracil[16] (b). The water molecules are not shown, but the water tunnel is outlined in the heavy line and was determined by using the van der Walls radii of the atoms lining the tunnel.

the compactness of crystals using a computer program developed in our laboratory and based on the molecular volume elements suggest by Kitaigorodskii.[19]

(c) Other Crystal Packing Factors. Several other crystal packing factors may also influence solid-state de-

(19) Kitaigorodskii, A. I. "Organic Chemical Crystallography"; Consultants Bureau: New York, 1961.

Table I. Tabulation of Important Crystal Packing Parameters which Influence Dehydration

compd	type of water chain and direction	direction of anisotropic dehydration	hydrogen bond[a] (X–H···Y)				no. and type of bond	space group, cell parameters[d]	cross sectional tunnel area, Å^2	compactness[e]	threshold temp for dehydration, °C
			X	proton assigned	Y	dist, Å					
thymine monohydrate[12]	zigzag, parallel to *c* axis	along *c* axis	O_w	–H···	O=C	2.84	2, W–W	$P2_1/c$; a = 6.08, b = 27.86, c = 3.82; β = 94°19′, Z = 4	12.48	0.56	41.6°
			O_w	–H···	O_w	2.76	1, W–M				
			O_w	···H–	O_w	2.70					
caffeine monohydrate[13]	zigzag, parallel to *c* axis	along *c* axis	O_w	–H···	N	2.85	2, W–W	$P2_1/a$; a = 14.8, b = 15.3, c = 4.5; β = 99.5°, Z = 4	10.95	0.64	44.0
			O_w	–H···	O_w		1, W–M				
			O_w	···H–	O_w						
theophylline monohydrate[14]	zigzag, parallel to *c* axis	along *c* axis	O_w	–H···	O_w	2.89	2, W–W	$P2_1/a$; a = 13.3, b = 16.7, c = 3.97; β = 97.0°, Z = 4	9.42	0.60	47.3
			O_w	–H···	O_w	2.70	1, W–M				
			O_w	···H–	O_w	2.76					
5-nitrouracil monohydrate[16]	straight, parallel to *a* axis	along *a* axis	O_w	···H–	N	2.84		$P2_1/c$; a = 5.14, b = 21.96, c = 9.58; β = 143°30′, Z = 4	1.84	0.61	54.9
			O_w	–H···	O[b]	3.07	3, W–M				
					O[b]	3.00					
cytosine monohydrate[15]	nearly straight, parallel to *c* axis	along *c* axis	O_w	–H···	O=C	2.94		$P2_1/c$; a = 7.78, b = 9.83, c = 7.67; β = 99°34′, Z = 4	2.49	0.58	59.4
			O_w	–H···	O=C	2.85					
			O_w	···H–	O=C	2.78	3, W–M				
			O_w	···H–	N	2.97					
2′-deoxyadenosine monohydrate[17]	straight, parallel to *c* axis	tarnishing and along *c* axis	O_w	–H···	O	2.73		$P2_1$; a = 16.07, b = 7.87, c = 4.70; β = 96°4′, Z = 2	2.48	0.64	88.7
			O_w	–H···	O	2.76	3, W–M				
			O_w	···H–	O	2.65					

[a] O_w is water oxygen. Three dots indicate the direction of the hydrogen bond to X or Y. [b] Bifurcated. [c] W = water, M = molecule. [d] Values of *a*–*c* are given in angstroms. [e] Molecular volume/unit cell volume.

solvation reactions. These include the direction of the water chain relative to the main plane of the molecules, the straightness of the water chain, the coplanarity of the water molecules with the molecules of the host compound, and the shape of the water tunnel. All of these factors are to an extent reflected in the tunnel cross-sectional area; however, in certain cases they may need to be specifically taken into account. For example, if the tunnel is zigzag the cross-sectional area measured by projection on the plane perpendicular to the dehydration direction will be too small and will not accurately reflect the size of the tunnel. The coplanarity of the water molecules with the host molecules will also be reflected in the tunnel cross-sectional area, but if there are constrictions in the tunnel due to the fact that the host molecules exist in planes running through the crystal with the water molecules between these planes then escape of water out the tunnels would be hindered and may occur along other directions. Examination of Figures 2, 5, 8, and 10 indicates that these other crystal packing factors are not of major importance for the compounds discussed in this paper.

(d) Hydrogen bonding of water to the host organic molecules in the crystal lattice may also play a important role in the dehydration of organic hydrates. In general, it would be expected that the stronger the hydrogen bond (or the shorter the hydrogen bond distance) the higher the threshold temperature for desolvation.

In order to discover the extent of influence of factors a–d on the desolvation process and in order to explain the common observation that some crystal hydrates can be desolvated at room temperature by air drying while others require vacuum drying and elevated temperatures, we have measured the threshold temperature for desolvation (temperature at which 2% of the solvent is lost) and related it to these factors. The threshold temperature was measured on several crystals by using thermal gravimetric analysis and is shown in Table I along with several other parameters.

The hydrates in Table I are arranged in order of threshold temperature with the hydrate with the highest threshold temperature at the bottom of the table. The crystal compactness seems to be nearly constant for the six compounds listed, and thus this parameter does not explain the threshold temperature. The tunnel area varies by a factor of 6 and is qualitatively related to the threshold temperature. The hydrates with the higher threshold temperature have the smaller tunnels. The lengths of the hydrogen bonds from water to the host molecule vary from 2.89 to 2.65 Å and parallel the threshold temperature with the hydrates, with the shorter and thus stronger hydrogen bonds having the higher threshold temperature. The number of hydrogen bonds from the host to the solvent parallels the threshold temperature with the three hydrates with the greatest number of these hydrogen bonds having the highest threshold temperature. It is also interesting to note that thymine hydrate which has the lowest threshold temperature is the only hydrate which does not change crystal structure upon dehydration.

These studies appear to indicate that in this series of compounds crystal packing and hydrogen bonding are factors which influence their reactivity.

In conclusion, the study reported here marks an important starting point for further research into the mechanism of solid-state reactions and describes for the first time a relationship between the crystal packing of organic hydrates and their desolvation.

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Registry No. Thymine monohydrate, 28171-19-9; caffeine monohydrate, 5743-12-4; theophylline monohydrate, 5967-84-0; 5-nitrouracil monohydrate, 18549-28-5; cytosine monohydrate, 6020-40-2; 2′-deoxyadenosine monohydrate, 16373-93-6.