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Effect of a Minor Component on Thermal Transformation of Crystalline 6-Mercaptopurine

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The influence of 6-thioxanthine (6TX) on the thermal transformation of 6-mercaptopurine (6MP) crystals was investigated using scanning electron microscopy and X-ray diffraction.

The crystal lattice of 6MP and 6TX molecules can include up to 10% of the counterpart molecules to form a solid solution. Monohydrate crystals (form I) of pure 6MP were directly transformed into anhydrate crystals (form II) by heating at 150—220°C, but similar crystals (form I_{TX}) of 6MP containing more than 2% 6TX were converted into another form of anhydrate crystals (form III) *via* an amorphous state by the same treatment. These phase transformations were kinetically studied and there was a clear difference in mechanism under the present experimental conditions. The transformations of form I into form II and form I_{TX} into an amorphous state were assumed to proceed according to the contracting cube equation whereas that of the amorphous state into form III appeared to follow the Prout-Tompkins equation. The reason for the transformation into different crystal forms, from form I into form II and from form I_{TX} into form III, is discussed on the basis of the experimental results.

Keywords——6-mercaptopurine; 6-thioxanthine; solid solution; X-ray powder diffraction; scanning electron microscopy; crystal transformation kinetics study; solid-state phase transformation; minor component effect

A potent antimetabolite of purine, 6-mercaptopurine (6MP), has been used as an effective drug for the treatment of pediatric leukemia and as an immunosuppressant in transplantation surgery. 6MP on the market is generally in the form of the monohydrate (form I) and its low solubility (130 μg/ml) in water is considered to be a cause of incomplete and variable gastrointestinal absorption.[1] Recently Huang and Niazi have reported that on heating at 220°C, form I was dehydrated to afford the anhydrate (form II) which was about twice as soluble as the monohydrate. Form II was thus assumed to have higher bioavailability than form I.[2] Upon checking these results, Kuroda *et al.* found that 6MP monohydrate crystals containing a small amount of 6-thioxanthine (6TX) were converted into another anhydrate (form III)[3] by heating and that the solubility of form III was unexpectedly high (1350 μg/ml); further a high blood level was attained after oral administration to rabbits.[4] In the present study, we investigated the cause of the difference in the transformation in the presence and absence of 6TX using crystallographic as well as thermodynamic techniques and clarified that the 6TX containing monohydrate (form I_{TX}) was transformed into form III through a mechanism different from that of the conversion of form I into form II.

Experimental

Preparation of Form I——About 1 g of 6MP was dissolved in 200 ml of boiling water, and after rapid filtration the solution was kept standing overnight at room temperature. The resulting crystals were collected by filtration and dried in air to give form I. *Anal.* Calcd % for $C_5H_6N_4SO$: C, 35.28; H, 3.55; N, 32.92. Found %: C, 35.09; H, 3.59; N, 32.96. Water content % (Karl Fischer method) Calcd 10.5, Found: 10.8.

Preparation of Form II——In a stream of nitrogen, form I was dried at 200°C for 3 h. *Anal.* Calcd % for $C_5H_4N_4S$: C, 39.46; H, 2.65; N, 36.82. Found %: C, 39.04; H, 2.56; N, 37.14.

Crystallization from a Mixture of 6MP and 6TX——Mixtures of 500 mg of 6MP and 6TX in various proportions were dissolved in 100 ml of boiling water and the solutions were kept standing overnight at room temperature. The contents of 6MP and 6TX in the crystals thus obtained were determined by high performance liquid chromatography under the condition described in a later section.

Preparation of Form I_{TX}——By the method described above, monohydrate crystals of 6MP containing 2% 6TX were obtained from 470 mg of 6MP and 30 mg of 6TX.

Preparation of Form III——After form I_{TX} had been heated at 100°C for 30 min under reduced pressure (0.05 mmHg), the temperature was raised to 200°C in 1 h, and maintained at that level for 2 h to afford form III.

Preparation of Amorphous 6MP (Amorph)——Similarly, after form I_{TX} had been heated at 100°C for 30 min under reduced pressure, the temperature was raised to 140°C in 1 h and kept there for 2 h.

High Performance Liquid Chromatography (HPLC)——The following conditions were used: column, packed with Nucleosil 5C-18 (4 × 150 mm); solvent, mixture of 1/3 M phosphate buffer (pH 3.5), water and methanol, (4:56:3); pump, a glass mini-micropump SQ-1 (Takahashi Giken); detector, Shimadzu UV-254. About 1 mg of the sample was weighed and dissolved in a mixture, 10 ml of water and methanol (1:1). Of the solution, 5 µl was injected onto the column using a loop injector (Kyowa Seimitsu).

X-Ray Analysis——A Rigaku Denki RU-3 diffractometer was used with CuKα radiation to measure powder diffraction patterns. To determine the cell constants, a Rigaku AFC-5 diffractometer was employed.

Determination of the Phase Transformation Rate——A Perkin-Elmer DSC 1B differential scanning calorimeter was used. In an atmosphere of nitrogen, about 40 mg of crystals in the size range of 30 to 500 µm heated for a suitable period on the DSC furnace which was kept at a suitable temperature. Then they were rapidly cooled to room temperature and the X-ray powder diffraction pattern was measured to determine the content of each crystal form. The following strong peaks (2θ,°) which were characteristic of each crystal form and located at similar 2θ values, were employed as key diffractions: form I, 27.3; form II, 28.3; form III, 27.0; Amorph, 26.2. The intensity of each diffraction was determined by the Alexander-Klug[5] method to eliminate the background. Calibration curves for two-component mixtures of form I and form II, form I_{TX} and Amorph, and form III and Amorph, were drawn in advance and used for component analysis of the heated sample.

Scanning Electron Microscopy——A JEOL JSU-3 electron microscope was used with an accelerating voltage of 15 kV The sample was coated with gold.

Results and Discussion

1) Solid Solution of 6MP and 6TX

Analytical results (HPLC) for the solid solutions or crystal mixture obtained from solutions containing 6MP and 6TX in various ratios are shown in Fig. 1. The *d*-values read from the X-ray powder diffraction patterns of the crystals are given in Fig. 2 with the composition of the crystals on the abscissa. When crystals were grown from a solution containing less than 25% 6TX, the 6TX content in the form I_{TX} crystals obtained was about half the 6TX content in the solution (Fig. 1). Some of the *d*-values of the crystals tended to increase with increase of the 6TX content (Fig. 2). Similarly, if crystallization was pursued from a solution consisting of more than 75% 6TX (less than 25% 6MP), the crystals contained 6MP equivalent to about half of the level in the solution (Fig. 1). Some *d*-values decreased as the content of 6MP in the crystals increased (Fig. 2). From the solutions containing 25—75% 6TX, mixtures of crystals of 6MP and 6TX were obtained as judged from the X-ray powder diffraction patterns, and the contents of 6MP and 6TX in the crystals were nearly the same as those in the solution. Clearly, from the X-ray patterns shown in Fig. 3, the crystal form of form I (6MP) resembles that of 6TX, and since the molecular structures of 6MP and 6TX were also similar, they were assumed to form a solid solution, which means that some 6TX molecules can replace 6MP molecules in the form I crystal lattice and *vice versa*. According to the results in Figs. 1 and 2, the limiting amounts of 6TX contained in form I crystals and of 6MP contained in 6TX crystals are within 10%. Fig. 4 shows a phase-contrast photograph of form I_{TX} containing 5% 6TX. The crystal surface shows irregular crystal growth indicating distortion or dislocation in the crystal.

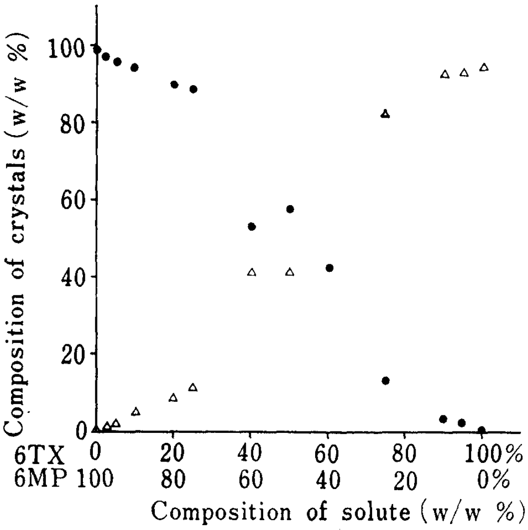

Fig. 1. Composition of 6TX and 6MP in Crystals and Solutions from Which the Crystals were obtained

●: 6MP, △: 6TX.

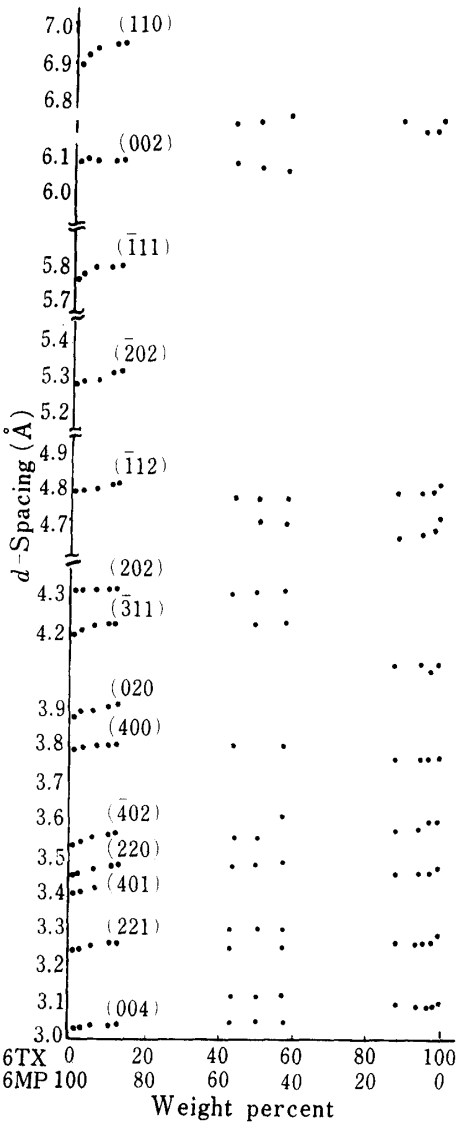

Fig. 2. Composition Dependence of Some *d*-Values of Form I

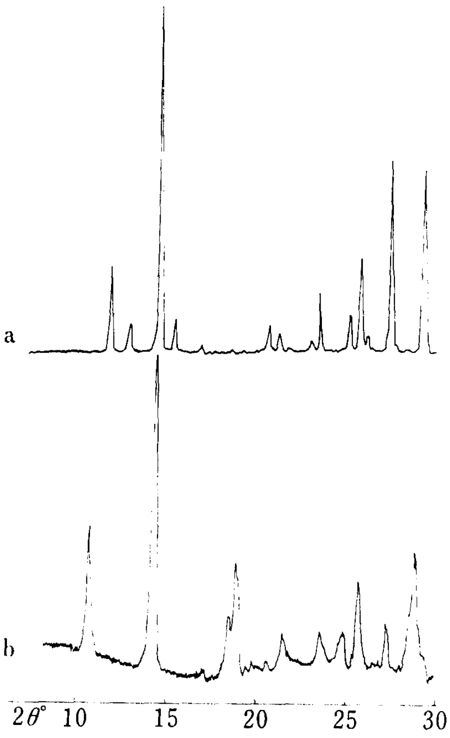

Fig. 3. X-Ray Powder Diffraction Patterns of (a) 6MP Hydrate (Form I) and (b) 6TX

Fig. 4. Phase-contrast Photograph of Form I_{TX} (5% 6TX in 6MP)

2) Crystallographic Properties of Form I

The lattice constants of form I were determined to be a=15.3, b=7.76, c=12.46Å and =101.9°. Systematic absences observed in the Weissenberg photographs about the b-axis indicated that the crystal belongs to a monoclinic system with the space group of C2/c or Cc. On the assumption that eight molecules of 6MP were contained in a unit cell, the density was calculated to be 1.39 g cm^{-3}, which is reasonable for this kind of crystal. The lattice constants of the form I_{TX} crystals containing about 2% 6TX were also determined. The results were indistinguishable from those of form I, within the experimental errors. The crystal habits

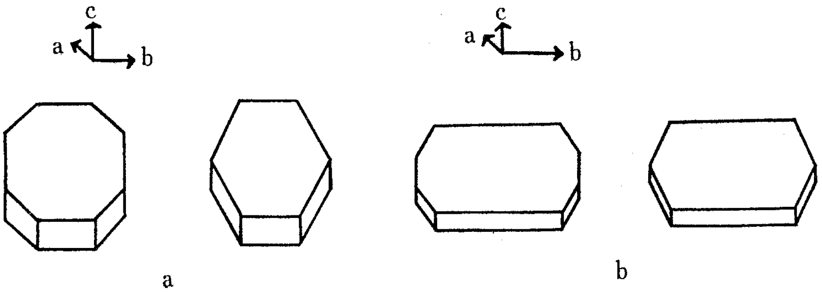

Fig. 5. Crystal Habits of 6MP; (a) Form I, (b) Form I_{TX} Orientations were determined from X-Ray Photographs

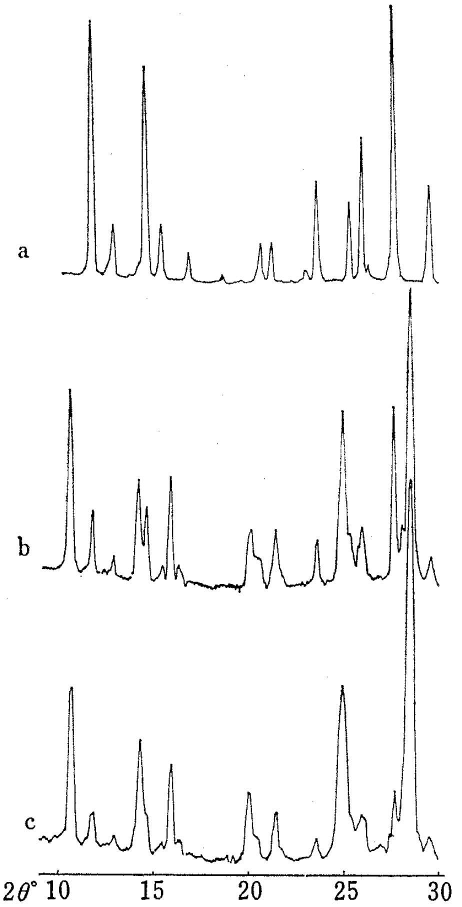

Fig. 6. X-Ray Powder Diffraction Pattern Changes of 6MP; (a) Form I before heating, (b) Form I after 10 min at 160°C, and (c) Form II formed after 1 h at 160°C

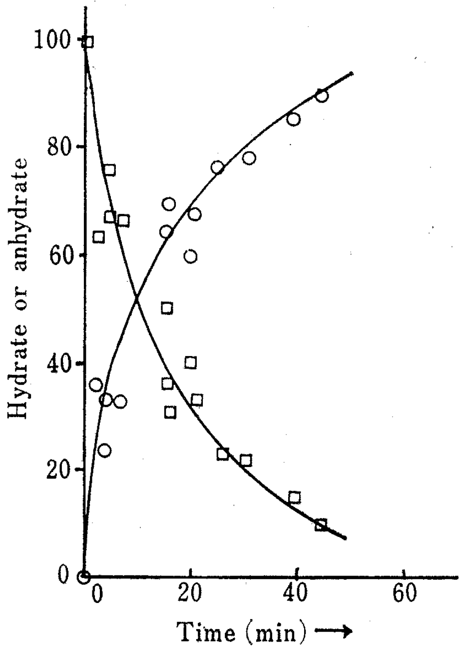

Fig. 7. Time-dependent Phase Transformation from Form I (Hydrate, □) into Form II (Anhydrate, ○) followed by X-Ray powder Diffraction Measurements

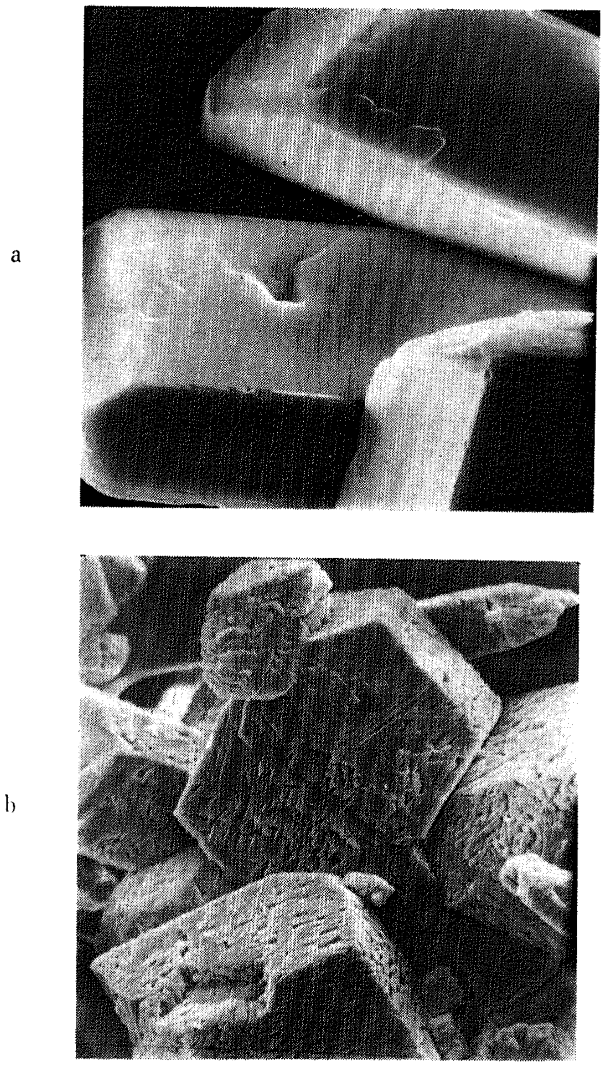

Fig. 8. Scanning Electron Micrographs of 6MP Crystals; (a) Form I (Hydrate), (b) Form II (Anhydrate) obtained from Form I on heating at 220°C for 30 min

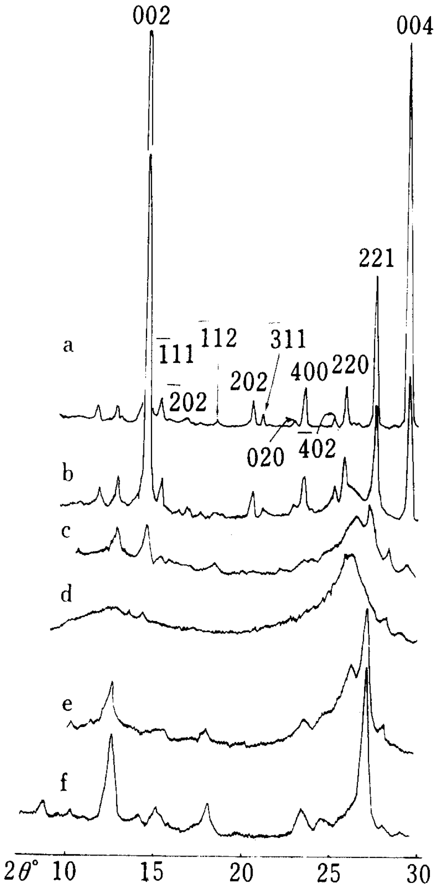

Fig. 9. X-Ray Powder Diffraction Pattern Changes of 6MP containing 6TX on Heating

(a) form I before heating, (b) after 10 min, at 160°C, (c) after 30 min at 160°C, (d) after 3 h at 160°C, (e) after 8 h at 160°C, and (f) Form III formed after 19h at 160°C.

of those form I and I_{TX} crystals were observed with a microscope in relation to the X-ray diffractions of single crystals. As shown in Fig. 5, form I crystals were thick octahedral or decahedral plates. In the case form I_{TX}, crystals were a little elongated along the b-axis and the plates were relatively thin.

3) Phase Transformation Mechanism

At a temperature over 120°C, form I loses water of crystallization and transforms into form II. The change of the X-ray powder diffraction pattern through this process is shown in Fig. 6, and agrees well with the patterns reported by Huang and Niazi. The transformation proceeds through mixture states of forms I and II as shown in Fig. 6b.

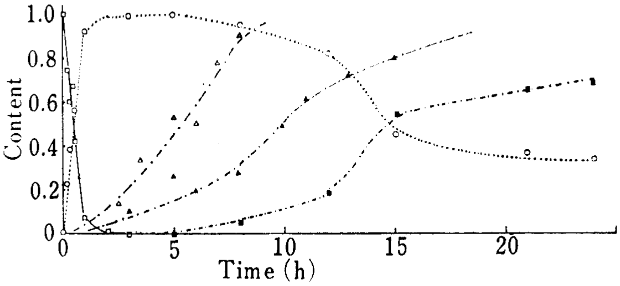

Fig. 10. Transformation from Form I into Form III *via* an Amorphous State (Amorph) followed by X-Ray Powder Diffraction Measurements

—□——□—: form I } 160°C.
—○——○—: Amorph
—■—·—■—: form III
—·▲—·—▲—: form III 165°C.
—·△—·—△—: form III 170°C.

Decrease in the height of the peak at $2\theta=27.3°$ due to form I was compensated for nearly quantitatively by increase in the height of the peak at $2\theta=28.3°$ due to form II, suggesting that no other definite state exists between forms I and II (Fig. 7). Observation of form I and II crystals with a scanning electron microscope (Fig. 8) shows that even after complete transformation of form I into form II the external shape of form I was retained and form II microcrystals could be seen where form I crystals had been . This phenomenon suggests that the transformation from form I into form II was accompanied by a three-dimensional rearrangement within the crystal. In contrast to this, tranfsormation from I_{TX} into form III proceeded through the following three stages (Fig. 9).

(1) At the beginning, the intensity of the diffraction peaks due to form I_{TX} decreased slowly (Fig. 9b) and diffuse diffractions at $2\theta=12.5°$ and $26.2°$ appeared in their place (Fig. 9c).

(2) In a little while, peaks due to form I_{TX} disappeared with two diffuse diffractions

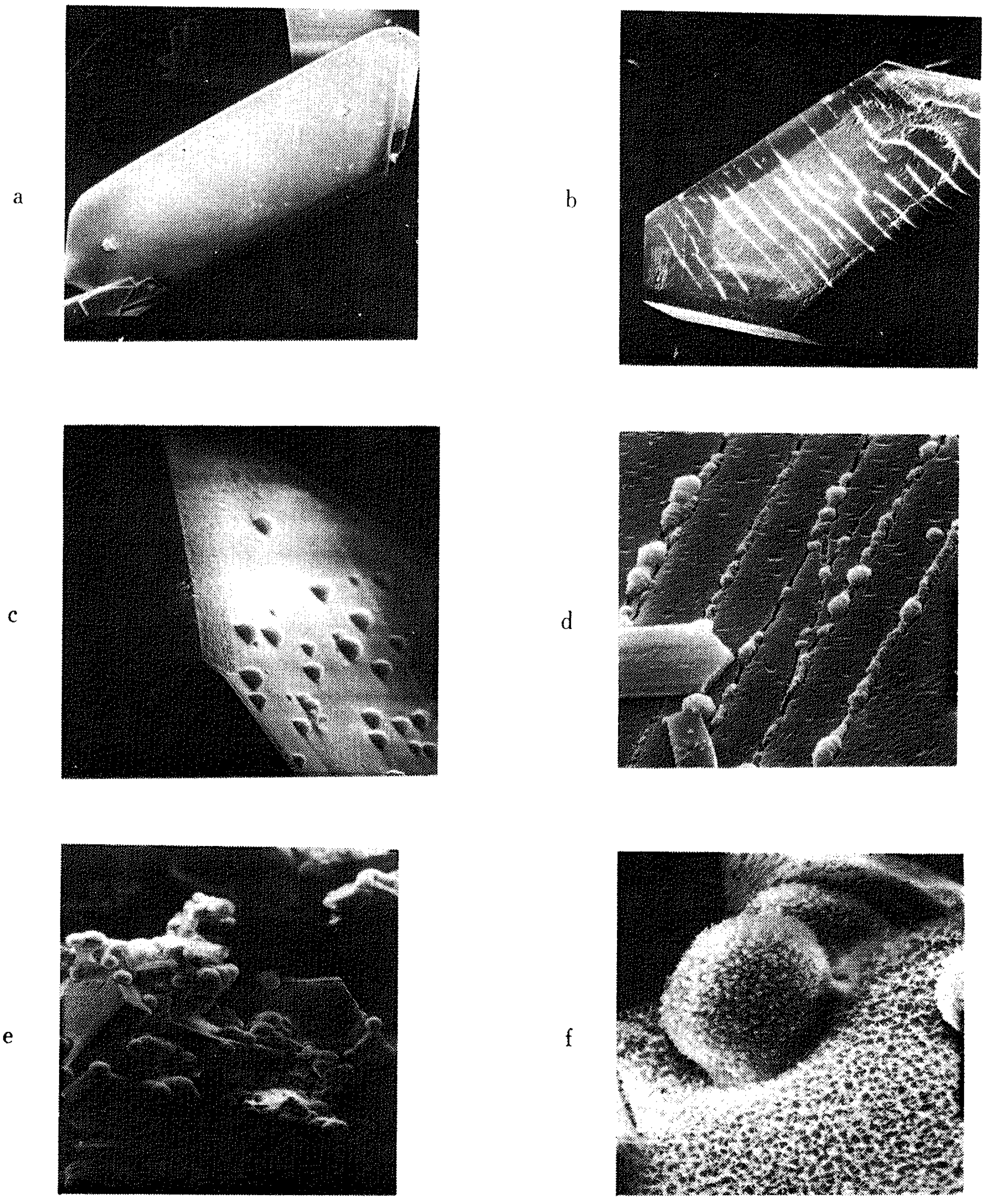

Fig. 11. Scanning Electron Micrographs of 6MP Crystals containing 6TX at Various Stages of Transformation from Form I_{TX} to Form III on heating at 160°C

(a) form I_{TX} before heating (×150), (b) after 10 min (×150), (c) after 8 h (×100), (d) after 8 h (×1500), (e) after 15 h (×300), (f) after 15 h (×1500).

remaining (Fig. 9d). Observation of the sample at this stage with a microscope using polarized light showed birefringence, suggesting that crystallinity still remained. However, for convenience, we described this stage as amorphous (Amorph).

(3) On further heating, the intensity of the diffuse diffractions decreased and instead, relatively sharp diffractions due to form III became evident (Fig. 9e and f).

Fig. 10 graphically shows the change of form I_{TX} to form III *via* Amorph in a quantitative manner using the key diffraction peaks described in the experimental section. Scanning electron microphotographs of the samples in various stages also suggest a different pathway from form I_{TX} to form III compared to that from form I to form II. The untreated form I_{TX} crystal is elongated along the b-axis, the (001) surface being extensively developed (Fig. 11a) as mentioned before. At stage (1), cracks ran along (0k0) (Fig. 11b) and at stage (2) or the amorphous stage, small newly born crystal nuclei were seen on the external surface (Fig. 11c) as well as on the surfaces generated by cracks (Fig. 11d). Finally, at stage (3), the shape of the form I_{TX} crystal disappeared and lumps of microcrystals were seen (Fig. 11e and f). The direction of these phase transformations was affected by the treatment conditions. For example, when form I_{TX} was rapidly heated to 200°C, form II grew in place of form III. In contrast, slow heating of form I under reduced pressure produced amorph followed by crystal-

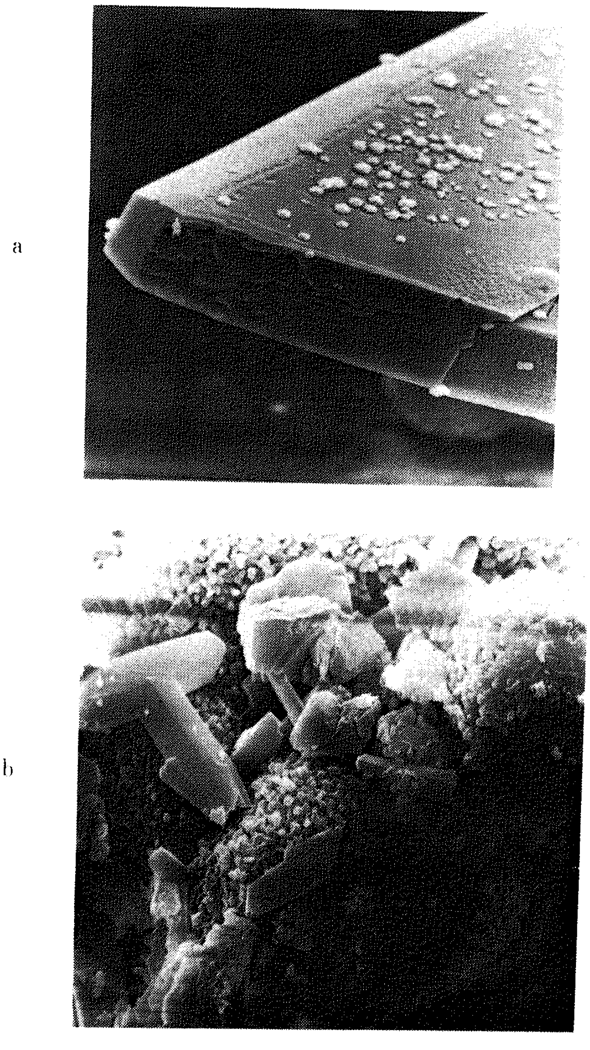

Fig. 12. Scanning Electron Micrographs of 6MP Crystals containing 6TX

(a) form II obtained from form I on heating, (b) a mixture of forms II and III obtained from form I on slow heating *in vacuo*.

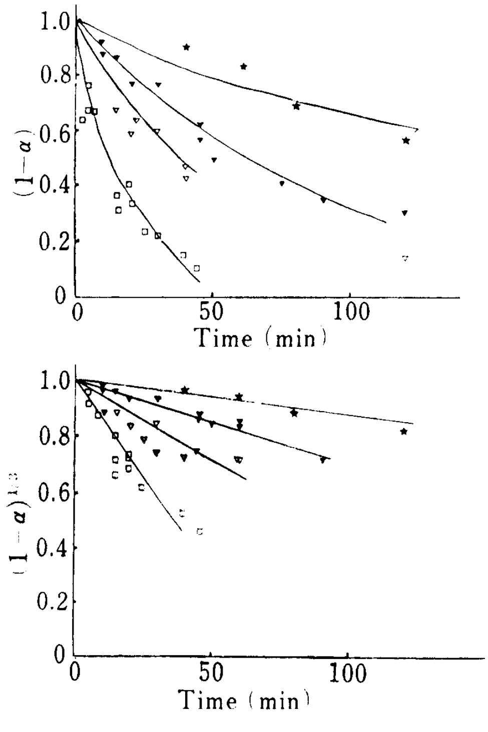

Fig. 13. Residual amount of Form I (Hydrate) in the Transformation to Form III (Anhydrate) on heating

(a) zero-order model plot, (b) diminishing sphere model plot.

★ 120°C. ▼ 125°C, ▽ 130°C, □ 135°C.

lization into a mixture of forms II and III. Microphotographs of these unusual samples are shown in Fig. 12. The former case shows that if sufficient thermal vibration takes place before cracking of the crystal lattice, transformation into form II will become the predominant process, whereas the latter suggests that if water of crystallization is removed before providing thermal energy to 6MP molecules, the crystal changes to the amorphous state followed by the growth of form III.

4) Kinetic Study of Phase Transformation

The remaining amount of form I, 1-α, on transformation to form II was plotted against treatment time as shown in Fig. 13a. The decrease of form I slowed down with time. If the transformation proceeds through dehydration in the homogeneous phase, the rate of formation of form II will be expressed by a pseudo-first-order equation. However, if we consider heterogeneous phase process and suppose that the transformation occurs first on the surface of the crystals and progresses to the inner part at a definit rate, then the formation of form II, α, may be represented by equation 1 which is called the contracting cube equation.[6]

$$(1-\alpha)^{1/3} = -kt + 1 \qquad \text{(eq. 1)}$$

Plotting $(1-\alpha)^{1/3}$ against t on the abscissa gave a straight line as shown in Fig. 13b, which supports the above-mentioned mechanism for the transformation. The mechanism of the transformation from form I_{TX} into form III is distinctly different from this one. The outbreak of form III nuclei seems to be so late that, before it starts, form I_{TX} has been almost completely converted into Amorph. Conversion of form I_{TX} into Amorph also follows equation 1, as the plot of $(1-\alpha)^{1/3}$ against time resulted in a straight line (Fig. 14). The time course of the formation of form III from Amorph is a sigmoid curve (Fig. 10). This type of curve may be explained in the following way. The slow rise of the curve at the initial stage may represent the induction time of the outbreak of nuclei. As nuclei formation is accelerated, the content of form III increases sharply and the rate of the formation becomes maximum at $\alpha=0.5$. The later part of the curve may be controlled by the amount of Amorph remaining. In such a case, the time course can be expressed by the Prout-Tompkins equation (equation 2).[6]

$$\log\left(\frac{1-\alpha}{\alpha}\right) = -kt + C \qquad \text{(eq. 2)}$$

Plots according to this equation straight lines, as shown in Fig. 15. Arrhenius plots of the rate constants determined for the changes from form I to form II, from form I_{TX} to Amorph and from Amorph to form III resulted in straight lines (Fig. 16). The activation energies and frequency factors estimated from these plots are listed in Table I.

Niazi has determined the activation energies and frequency factors in the dehydration process of form I to be 45.75—63.04 kcal/mol and 10^{23}—10^{25} min^{-1}, respectively, from his differential scanning calorimetry study on the dehydration rate.[7]

Although his methods of measuring the conversion rate and of calculating thermodynamic parameters are different from ours, the values coincided well, suggesting the reliability of the values.

The fact that forms I and I_{TX}, having substantially the same crystal forms, progressed on different pathways of transformation might be interpreted as follows: in the case of form I, molecules in the lattice may be in suitable positions to rearrange into form II after dehydration, whereas in the case of form I_{TX}, the space interval is enlarged owing to the coexistence of 6TX, and crossing the energy barrier of the phase transformation may be difficult. As a consequence, the direct rearrangement from form I_{TX} to form II is inhibited and the lattice collapses to Amorph. Formation of form III nuclei from Amorph seems to be easier than that of form II nuclei and the whole mass is converted into form III on further heating. This view is supported by the experimental results shown in Fig. 12. Dehydration of form I under reduced pressure may not supply enough energy for rearrangement into form II, resulting in

Table I. Activation Energy for Solid-Solid Transformation of 6-MP Crystals

Reaction	Activation energy (kcal/mol)	Frequency factor (min^{-1})
Form I → form II+H_2O	46.5	1.8×10^{23}
Form I_{TX} → Amorph+H_2O	23.3	2.2×10^{10}
Amorph → form III	22.8	7.4×10^{8}

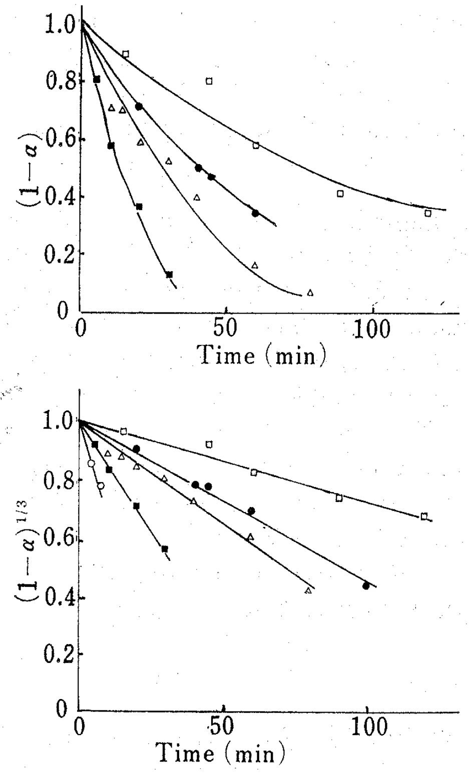

Fig. 14. Residual Amount of Form I_{TX} in the Transformation to Amorph on heating

(a) zero-order model plot, (b) diminishing sphere model plot.

□ 135°C, ● 145°C, △ 150°C, ■ 160°C, ○ 165°C.

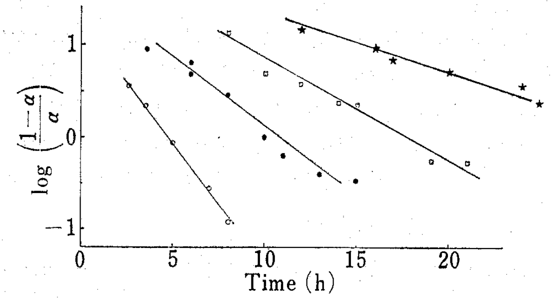

Fig. 15. Prout-Tompkins Plots for Conversion of 6MP containing 2% 6TX from Amorph into Form III

★ 155°C, □ 160°C, ● 165°C, ○ 170°C.

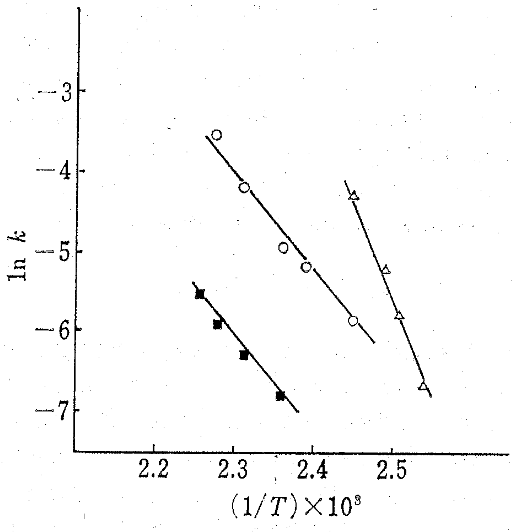

Fig. 16. Arrhenius Plots for Solid-solid Transformation of 6MP Crystals

△, form I → form II+H_2O.
○, form I_{TX} → Amorph+H_2O.
■, Amorph → form III.

formation of Amorph. On the other hand, dehydration of form I_{TX} at a higher temperature may activate the molecules sufficiently for direct transformation to form II.

Very little work has been reported on change of the heat transformation of the phase caused by the existence of some impurity in organic crystals, although the influence of impurity on crystallization from solution has been extensively studied. Further study on phenomena related to those described in the present paper will be necessary to achieve a clear understanding of the processes involved.

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