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Polymorphism of Thiamine Hydrochloride. II.[1] Crystal Structure of Thiamine Hydrochloride Hemihydrate and Its Stability

Atsushi Watanabe,[2a] Saburo Tasaki,[2b] Yoshikazu Wada, and Hideo Nakamachi[2c]

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University,[2a] Hikari Plant, Takeda Chemical Industries, Ltd.,[2b] and Central Research Division, Takeda Chemical Industries, Ltd.[2c]

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The content of water of crystallization in the phase II modification of thiamine hydrochloride has not previously been definitely determined. This is widely thought to be an anhydrous form, since it does not show any weight change on drying at 105° for 2 hr. The present work describes the crystal structure of the phase II modification of thiamine hydrochloride crystals. It was found to be a hemihydrate and to be stable under normal conditions both in the solid state and in suspension in a saturated solution.

Keywords——thiamine hydrochloride; water of crystallization; phases I and II modifications; X-ray crystal structure analysis; conversion of phase I into phase II; activation energy for dehydration; stability; crystal growth rate

Crystallization of thiamine hydrochloride from water, aqueous methanol or ethanol affords two crystalline modifications, phases I and II, depending on the conditions used. These two phases together with the dehydrated form of phase I are the crystalline modifications most frequently found in marketed thiamine hydrochloride. It seems important, therefore, to clarify the properties of these modifications and their stability under various conditions.

In our previous paper,[1] we described the physicochemical properties of several crystalline modifications of thiamine hydrochloride, including phases I and II, and we assumed that phase I, a monohydrate, was the most stable form at room temperature. However, we recently found that phase I crystals were occasionally transformed into phase II ones during prolonged storage in air and that the transformation was often accompanied by caking of the crystals. A more detailed examination of the phase II crystals showed that this modification is a hemihydrate, and that the water of crystallization cannot be removed even on drying at 105° for 2 hr.[3]

This paper reports the results of single-crystal X-ray analysis performed on phase II, and of a solubility study and thermal analysis of phases I and II made to establish their structural and stability relationships.

Experimental

Instrumentation——Infrared (IR, nujol)[4] spectra were recorded on a Hitachi 215 spectrometer. X-ray powder diffraction patterns were recorded with a Rigakudenki X-ray diffractometer, Rotaflex RU-3. Single

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[1] Part I: A. Watanabe and H. Nakamachi, *Yakugaku Zasshi*, 96, 1236 (1976).

[2] Location: *a) Ikawadai-cho, Tarumi-ku, Kobe 673, Japan*; *b) Mitsui, Hikari 743, Japan*; *c) Jusohon-machi, Yodogawa-ku, Osaka 532, Japan.* To whom communications should be addressed.

[3] These drying conditions are accepted in the United States Pharmacopeia, nineteenth revision and Pharmacopoeia Japonica Editio Nona.

[4] The infrared spectrum of a potassium bromide dispersion of thiamine hydrochloride is the accepted identification method in the U.S. Pharmacopeia. However, the IR spectrum in a nujol mull was used in this paper, because of the tendency of phase I to transform into phase II under pressure. See reference 1.

crystal diffraction intensities were measured with a Rigakudenki AFC type-4 diffractometer (CuKα).⁵⁾ Thermogravimetric analysis was performed with a Perkin Elmer TGS-1 thermobalance under an atmosphere of dry nitrogen. Differential scanning calorimetry thermograms were recorded on a Perkin Elmer DSC-1B differential calorimeter at various scanning rates using a sample pan having holes in its cover.

Preparation of Thiamine Hydrochloride Crystals——a) Phase I: Crystals of phase I were prepared according to the method described previously.¹⁾

b) Phase II: Thiamine hydrochloride (30 g) was dissolved in water (20 ml) at 60° and the solution was cooled to 40° followed by seeding with phase II crystals. The solution was cooled to room temperature and allowed to stand for a few days. The precipitated phase II crystals were collected by filtration and dried in air. *Anal.* Calcd. for $C_{12}H_{17}ClN_4OS \cdot HCl \cdot 1/2H_2O$: C, 41.62; H, 5.53; N, 16.18. Found: C, 41.60; H, 5.55; N, 15.69. Water content, Calcd.: 2.6%. Found: 2.4% (determined by the Karl–Fischer method).

c) Dehydrated phase II: Phase II was dried *in vacuo* (0.05 mmHg) at 135° for 5 hr to obtain dehydrated phase II. *Anal.* Calcd. for $C_{12}H_{17}ClN_4OS \cdot HCl$: C, 42.73; H, 5.38; N, 16.61. Found: C, 42.39; H, 5.44; N, 15.57. Water content, Found: 0.3% (determined by the Karl–Fischer method).

Structure Analysis of Phase II——The crystals of phase II ($0.2 \times 0.1 \times 1.0$ mm) are monoclinic with cell dimensions of $a=30.13$, $b=6.175$, $c=22.06$ Å and $\beta=128.7°$. Systematic absences showed that the space group is either C2/c or Cc. The calculated density, assuming eight and four molecules of thiamine hydrochloride and water, respectively, 8 $[C_{12}H_{17}ClN_4OS \cdot HCl \cdot 1/2H_2O]$ per unit cell, was 1.40 g cm^{-3}. Intensity data were collected on a four-circle diffractometer using the ω-2θ scan technique. These data were corrected for Lorentz and polarization factors, and a total of 2330 independent structure factors were derived. Structure elucidation was attempted for both space groups, C2/c and Cc, by the direct method with the MULTAN program.⁶⁾ Based on the partial structure obtained from an E-map calculated for the set with the highest ABS. FOM. for space group C2/c, the positions of the remaining non-hydrogen atoms in the unit cell were found by the usual procedure. Positional and thermal parameters were refined by the block-diagonal least-squares method to an R factor of 0.0925. Atomic coordinates and temperature factors are listed in Table I.

Table I. Atomic Coordinates and Temperature Factors with Their Standard Deviations in Parentheses

Atom	$x/a(\times 10^4)$	$y/b(\times 10^4)$	$z/c(\times 10^4)$	B
S (1)	0317(01)	7550(04)	5602(01)	2.64(04)
C (2)	0778(03)	7002(14)	5420(05)	2.54(15)
N (3)	1089(03)	5294(10)	5815(03)	2.05(12)
C (4)	0970(03)	4296(13)	6263(04)	2.34(15)
C (5)	0546(03)	5326(13)	6216(04)	2.44(15)
C (6)	1288(04)	2305(16)	6721(05)	3.57(18)
C (7)	0320(04)	4831(15)	6626(05)	3.33(18)
C (8)	0640(04)	6037(17)	7393(06)	4.07(20)
O (9)	1233(03)	5306(11)	7928(04)	3.88(13)
C (10)	1549(03)	4449(14)	5797(05)	2.71(16)
N (11)	1562(03)	6633(12)	4241(04)	3.12(14)
C (12)	1843(03)	8572(14)	4552(05)	2.77(16)
N (13)	2047(03)	9187(11)	5245(04)	2.68(13)
C (14)	1955(03)	7943(13)	5659(04)	2.44(15)
C (15)	1649(03)	5918(13)	5354(04)	2.41(15)
C (16)	1467(03)	5371(14)	4634(05)	2.76(16)
C (17)	1891(04)	9980(17)	4038(06)	4.27(21)
N (18)	2157(03)	8662(12)	6363(04)	3.13(14)
O (19)	0000(00)	8412(24)	2500(00)	7.56(38)
Cl (20)	2014(01)	7598(04)	7660(01)	2.70(04)
Cl (21)	0627(01)	0678(04)	4177(01)	3.62(04)

Estimation of the Activation Energy for Dehydration of Phases I and II——Differential thermograms were obtained at scanning rates of 0.625°, 1.25°, 2.5°, 5°, and 10° per minute. The activation energy was estimated by the Kissinger method⁷⁾ using the following equation.

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5) The diffractometer in the Faculty of Engineering of Osaka University was used.
6) G. Germain, P. Main, and M.M. Woolfson, *Acta Cryst.*, **A27**, 368 (1971).
7) H.E. Kissinger, *Anal. Chem.*, **29**, 1702 (1957).

$$-E = R\left(\ln\frac{\phi}{T_m^2}\right)\Big/1/T$$

E: activation energy, T_m: peak temperature
ϕ: scanning rate, R: gas constant

Solubility Measurement——Thiamine hydrochloride (phase I, 40—50 g) was added to water (20 ml). The mixture was stirred at a regulated temperature of 30°, 40° or 60°. At appropriate intervals, a small amount of sample was withdrawn from the mixture and filtered to remove the solid portion. The filtrate was precisely weighed and dried to remove water. The residual matter was precisely weighed again to determine the concentration of thiamine hydrochloride in the solution. In parallel with this experiment, the nature of the crystalline phase of the solid portion was checked by X-ray powder diffraction.

Measurement of the Conversion Rate of Phase I into Phase II——The intensity ratio of the two peaks at $2\theta = 8.4°$ and $8.0°$ in the X-ray powder diffraction pattern was used to estimate the proportions of phases I and II in a mixture. The calibration curve was prepared in advance using mixtures with known proportions of phases I and II.

To measure the conversion rate of phase I into phase II in the suspension, phase I was suspended in a saturated solution of thiamine hydrochloride and at appropriate times, the solid portion was separated by filtration for X-ray powder diffraction analysis. In the case of conversion in the solid state, phase I was stored under controlled relative humidity (RH) and temperature, and sampled at appropriate intervals. The ratio of phases I and II was determined using the aforementioned calibration curve based on X-ray powder diffraction data of these specimens.

Measurement of Crystal Growth Rate——Thiamine hydrochloride solutions of various concentrations in water were prepared at 60°, then carefully cooled to 50±0.2° or 45±0.2° to achieve a supersaturated state and maintained at that temperature. A small crystal of phase I or II, which was mounted on a glass fiber of 0.2 mm diameter in a selected orientation, was put into the solution. The growth rate along the long axis (phase I: *a*-axis, phase II: *b*-axis) was observed through a travelling microscope.

Results and Discussion

Crystal Structure and Physicochemical Properties of the Phase II Crystals of Thiamine Hydrochloride

The content of water of crystallization in phase II of thiamine hydrochloride has not been definitely determined. It is widely thought to be an anhydrous form, as no weight loss has been observed even on rather drastic drying at 105° for 2 hr. However, elemental analysis and Karl–Fischer analysis of the water content suggested that it might contain a half mole equivalent of water per mole thiamine hydrochloride in the crystal lattice. Thermogravimetric analysis of the phase II crystals (Fig. 1) supports this view, exhibiting a weight loss corresponding to 0.5 mol of water at about 125°. In order to obtain further information on this, X-ray crystal structure analysis was attempted. Crystals grown slowly from an aqueous solution of thiamine hydrochloride at room temperature showed the same X-ray powder diffraction pattern (Fig. 2a) and infrared spectrum (Fig. 3a) as those of the phase II modification in the previous report. Structure analysis of a single crystal obtained by careful selection from these crystals revealed that a C2/*c* unit cell contains eight molecules of thiamine hydrochloride and four water molecules; the oxygen atoms of the latter are situated at special positions on the two-fold axis (Fig. 4). Since the molar ratio of thiamine hydrochloride to water was 2:1, the phase II crystal was assigned as a hemihydrate.

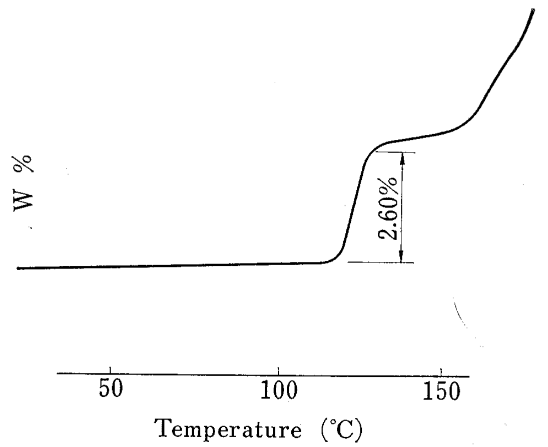

Fig. 1. Thermogravimetric Change of Phase II of Thiamine Hydrochloride

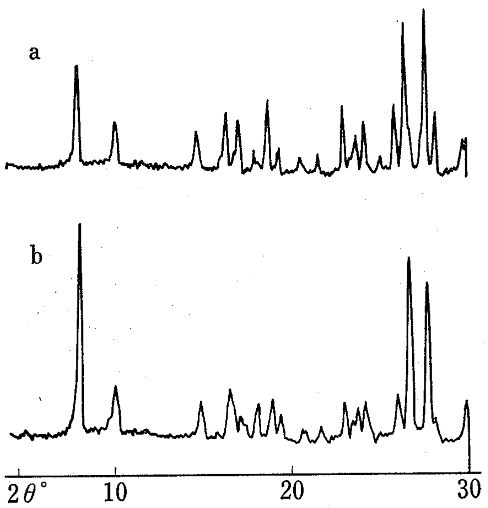

Fig. 2. X-Ray Powder Diffraction Patterns of Thiamine Hydrochloride

a: phase II.
b: dehydrated sample of phase II.

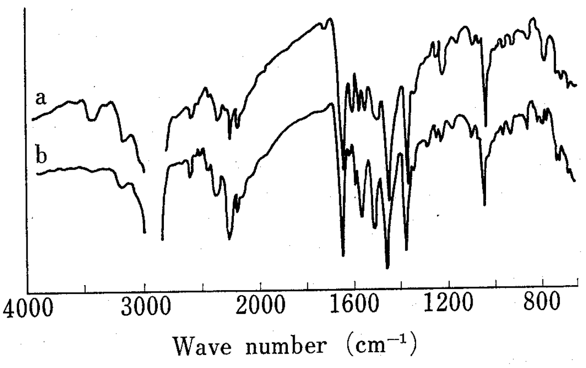

Fig. 3. Infrared Spectra of Thiamine Hydrochloride

a: phase II.
b: dehydrated sample of phase II.

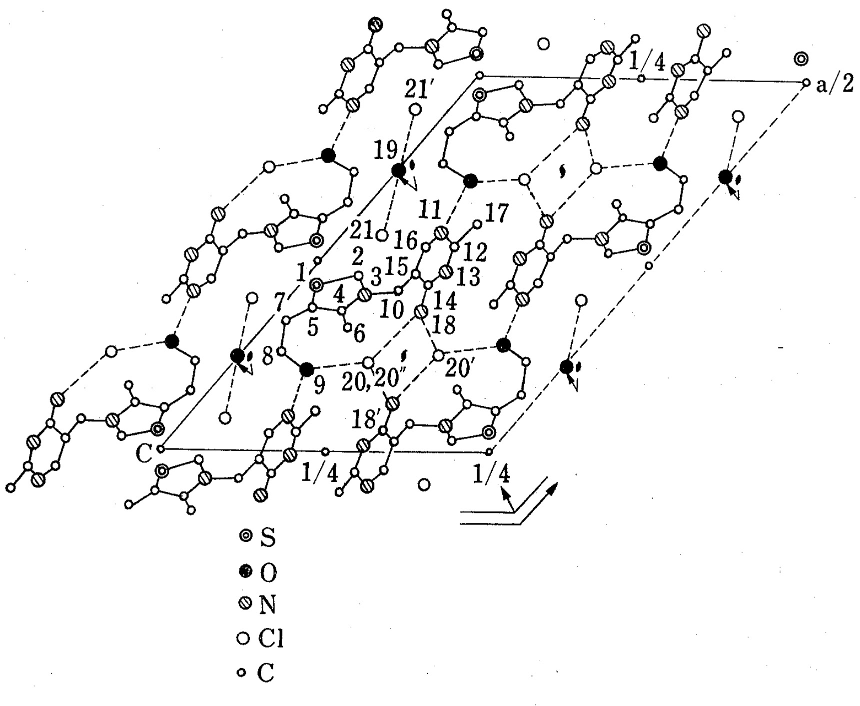

Fig. 4. Molecular Arrangement and Hydrogen Bonding in Crystalline Thiamine Hydrochloride Phase II

A comparison of the conformation of the thiamine molecule in the phase II crystals with that in phase I analyzed by Kraut *et al.*[8] showed that the intramolecular spatial relationship between the pyrimidine and thiazole rings are very similar (Fig. 5). The angle between the planes containing the pyrimidine and the thiazole ring is 76°; N (11) in the pyrimidine is protonated and thus has a positive charge in both cases. This suggests that such a structure is the most stable for thiamine hydrochloride, independent of the intermolecular association, which is rather different in phases I and II.

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8) J. Kraut and H.J. Reed, *Acta Cryst.*, **15**, 747 (1962).

In phase II, the water of crystallization is located between the chloride anions, Cl^- (21) and Cl^- (21′) strongly linked by hydrogen bonds. These chloride anions are bonded to the positively charged pyrimidine ring by ionic interactions. The stability of the water in phase II with respect to drying is well explained by this environment of water in the solid structure.

Dehydration

On heating over 125°, the phase II crystals lost weight corresponding to their water of crystallization, and the X-ray diffraction pattern showed a slight but distinct change (Fig. 2b). In the infrared spectrum, the absorption at 3500 cm^{-1} attributable to the O–H stretching vibration of the water is lost, but the remainder of the spectrum is unaltered. These changes can be reasonably explained in terms of the crystal structure of phase II described above, since the water molecule is hydrogen bonded only with chloride and does not interact directly with the thiamine molecule.

Activation energies for the dehydration of phases I and II were calculated by Kissinger's method.⁷⁾ The reciprocals of dehydration temperatures (T_m) obtained at various scanning rates (ϕ°/min) were plotted against log ϕ/T_m^2 and a linear relationship was obtained (Fig. 6). From the slope, the activation energies were calculated to be 14 and 50 kcal/mol for phases I and II, respectively.

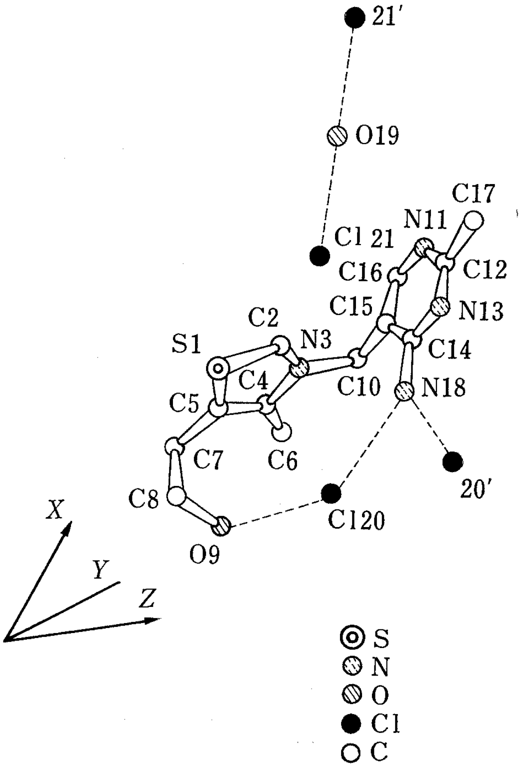

Fig. 5. Perspective View of Thiamine Hydrochloride in Phase II seen along the *b*-Axis

Conversion of Phase I into Phase II

Time-dependent changes in the solubility of thiamine hydrochloride when an excess of phase I crystals was added to a nearly saturated solution are shown in Fig. 7. The same figure also shows the change of crystal structure that takes place in the crystals suspended in the solution. The concentration of thiamine hydrochloride rapidly reached a maximum, and the crystalline phase of the suspended crystals remained unchanged. After an induction period, conversion into phase II took place in the solid portion and the concentration of the solution decreased. Finally the concentration reached an equilibrium; by this time almost all the suspended crystals had been transformed into phase II. The conversion rate decreased at low temperature, but even at 5°, a large part of the suspended phase I was transformed into phase II after storage for 3 months.

Assuming the maximum and equilibrium concentration in Fig. 7 to be the saturated concentrations of phases I and II, respectively, a van't Hoff plot of the solubility was drawn for both crystalline phases (Fig. 8). Within the temperature range investigated in the present work, the solubility of phase II was clearly lower than that of phase I. This implies that phase II, rather than phase I, is the stable form of thiamine hydrochloride in an aqueous suspension in the temperature range from at least 5° to 60°. The free energy difference between the two crystalline phase was calculated from their solubility difference to be −33 cal/mol at 30°.

Phase II is the stable form, not only in aqueous suspension, but also in air under relatively high RH conditions. Conversion of phase I into phase II at 40° and 60° was observed at

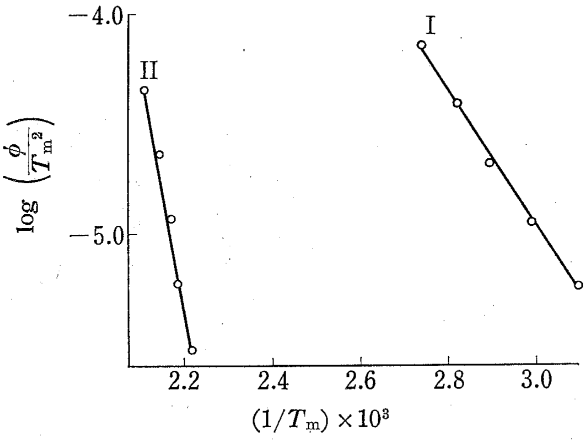

Fig. 6. Kissinger Plot for the Dehydration of Thiamine Hydrochloride

I: phase I, II: phase II.
T_m: temperature of peak (°K).
ϕ: scanning rate (°K/min).

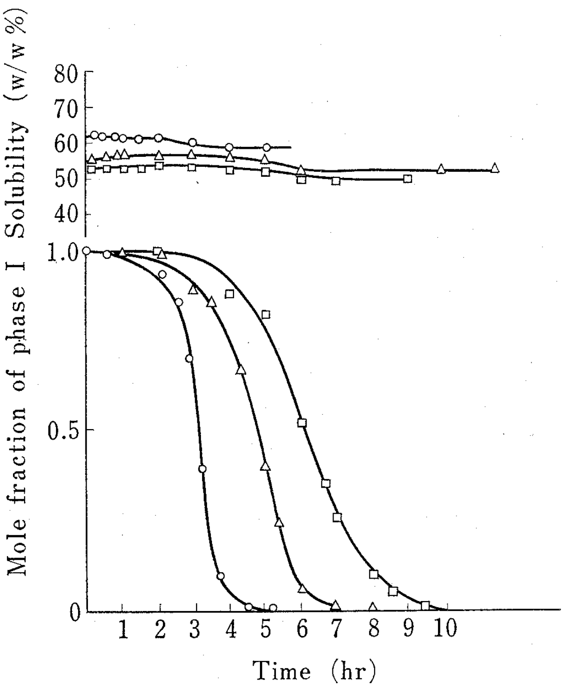

Fig. 7. Comparison of the Solubility Changes starting from Phase I together with Conversion Profiles of Phase I into Phase II of Thiamine Hydrochloride in Suspension at Various Temperatures

—□—: 30°, —△—: 40°, —○—: 60°.

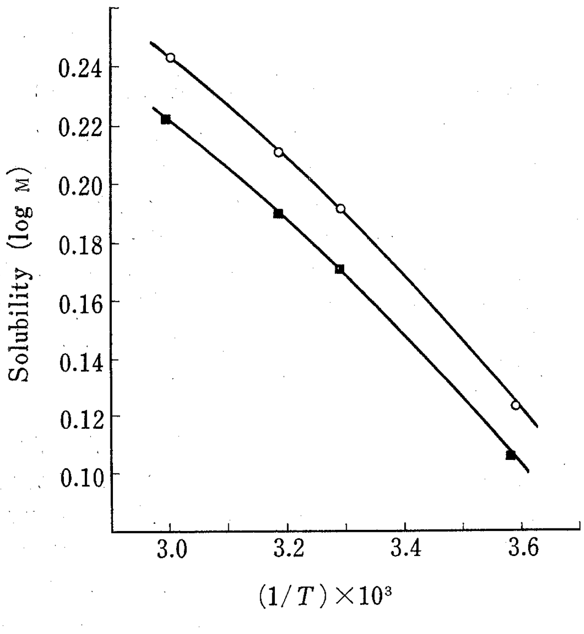

Fig. 8. Van't Hoff Plot of the Solubilities of Phases I and II of Thiamine Hydrochloride

—○—: phase I, —■—: phase II.
T: Temperature (°K).

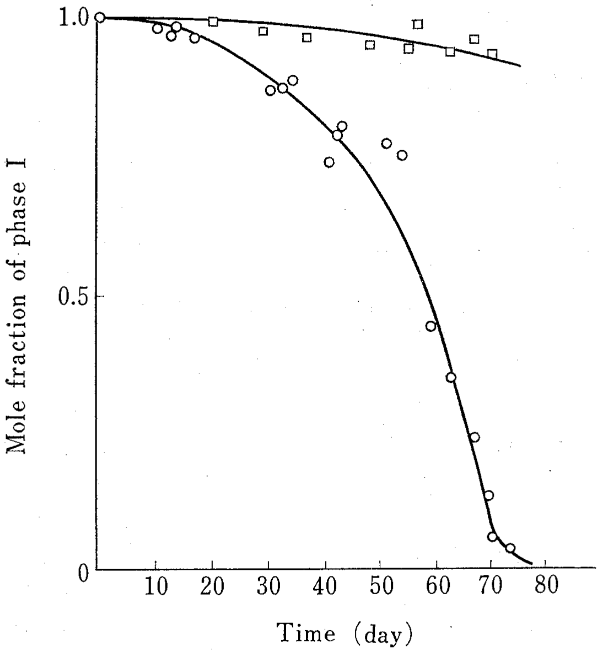

Fig. 9. Conversion Profile of Phase I of Thiamine Hydrochloride into Phase II in Air

—□—: 40°, RH 75%.
—○—: 60°, RH 75%.

RH 75% (Fig. 9). In this case, S-shaped curves were obtained similar to those seen with suspensions (Fig. 7). The curves could be expressed by the rate equation (Eq. 1) proposed by Prout and

$$\log\left(\frac{x}{1-x}\right) = Kt + C \qquad \text{Eq. 1}$$

x: mol fraction of phase I t: time
K: rate constant C: constant

Tompkins. The plots of log (x/1-x) against time (t) shown in Fig. 10 gave a linear relationship in every case. This relationship suggests that, at the early stage of the conversion, formation of phase II nuclei is very slow but is accelerated by the formed nuclei themselves, while both nucleus formation and the crystal growth rate of phase II are stimulated progressively at the middle stage. At the final stage, the conversion into phase II is controlled by the amount of remaining phase I, which means it slows down again. The activation energy and frequency factor were estimated from Arrhenius plots (Fig. 11) with the logarithm of the rate constant (K) obtained from the slopes in Fig. 10 on the ordinate and the reciprocal of the absolute temperature on the abscissa. The results obtained in both experiments, that is, in the solid state and in aqueous suspension, are listed in Table II. The activation energies were similar, whereas the frequency factors differed greatly. This suggests that the mechanism of conversion of phase I into phase II is similar in both cases, though in the suspension, the presence of the medium increases the conversion rate by two orders of magnitude. Seeding was effective in accelerating the conversion in the absence of the medium. On addition of about 1% phase II crystals to the initial phase I, the conversion was completed

Table II. Activation Energy for Conversion of Phase I into Phase II of Thiamine Hydrochloride

Condition	Activation energy (kcal/mol)	Frequency factor (min^{-1})
Suspension	9.5	32000
Solid state (RH 75%)	9.8	140

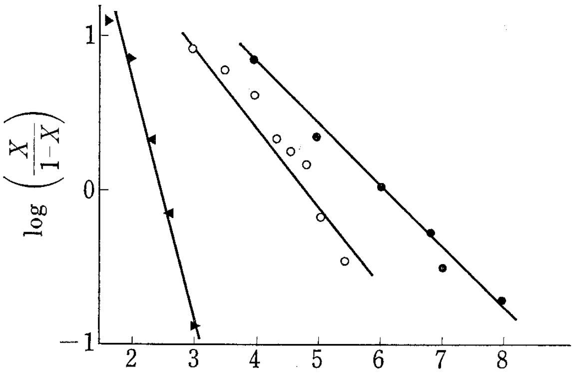

Fig. 10. Prout and Tompkins Plot for the Conversion of Phase I into Phase II of Thiamine Hydrochloride in a Suspension

—●—: 30°, —○—: 40°, —▲—: 60°.

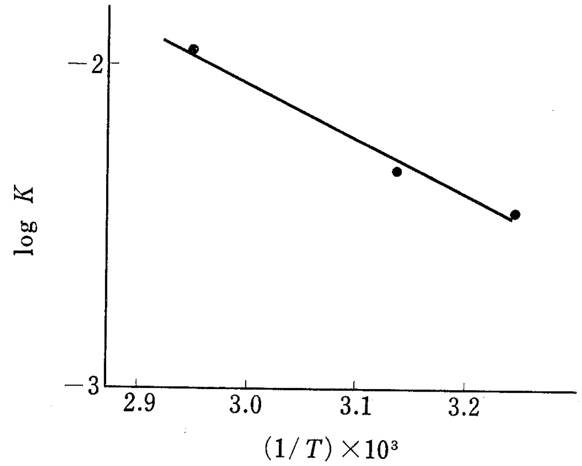

Fig. 11. Arrhenius Plot for the Conversion of Phase I into Phase II of Thiamine Hydrochloride suspended in a Saturated Solution

K: rate constant (min^{-1}).
T: temperature (°K).

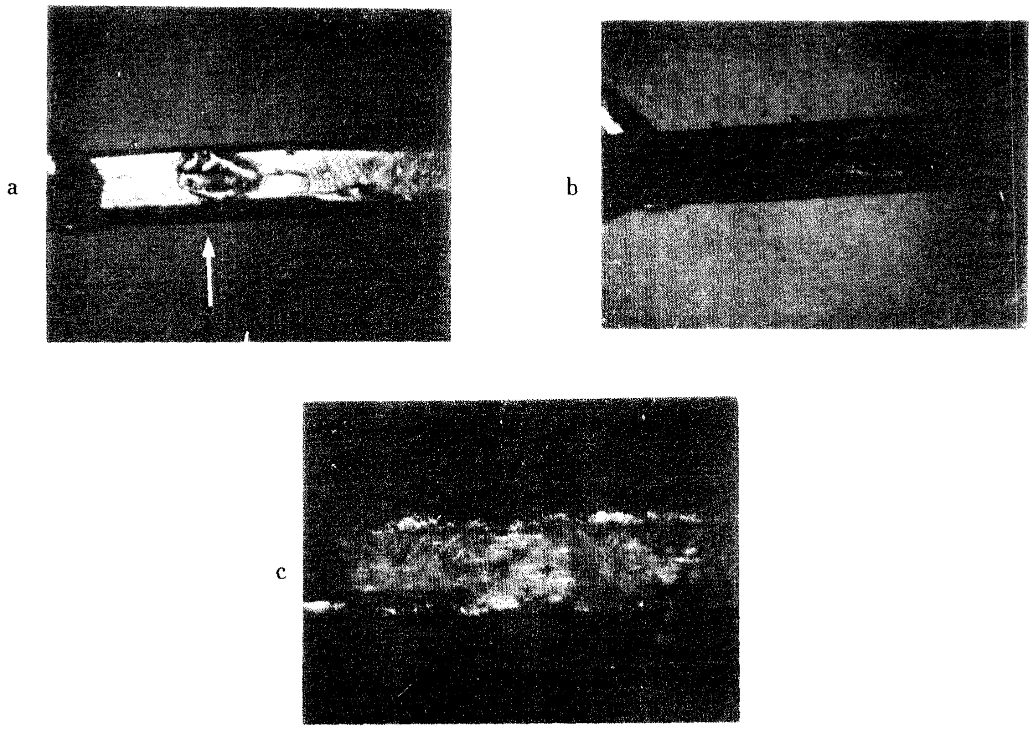

Fig. 12. Micrographs of Thiamine Hydrochloride Crystals showing the Conversion of Phase I into Phase II

a) Initial phase I single crystal on which a small crystal of phase II was fixed (shown by the arrow) (×20).
b) After storage for 3 days at RH 75% and 60° (×20).
c) After storage for 30 days at RH 75% and 60° (×20).

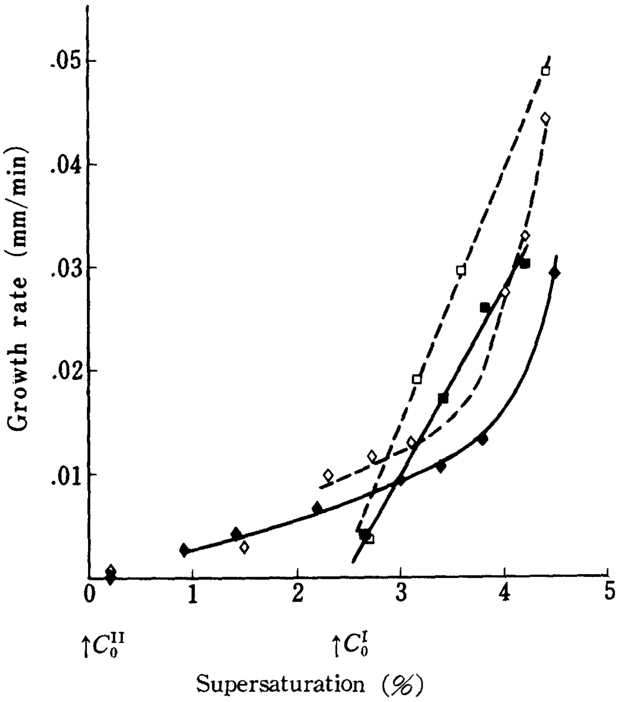

Fig. 13. Crystal Growth Rate of Thiamine Hydrochloride in a Supersaturated Solution

□: phase I at 50°, ◇: phase I at 45°.
■: phase II at 50°, ◆: phase II at 45°.
C_0^{I}: solubility of phase I.
C_0^{II}: solubility of phase II.

in 25 days at 50°, in contrast to the case without seeding where the 25 th day was still within the induction period. Another seeding effect was observed microscopically using single crystals of phase I. When a small crystal of phase II was fixed onto a phase I single crystal, the transformation began at the interface and gradually spread all over the crystal (Fig. 12).

It seems clear that phase II is more stable than phase I under generally used crystallization conditions. In practice, however, crystals grown by the usual methods are much more often of phase I than Phase II. For this reason, we investigated in more detail the difference of crystal growth behavior in these two phases. As an index, we chose the growth rate along the elongated axis of the crystals in a supersaturated solution of thiamine hydrochloride. As shown in Fig. 13, phase I crystals grew at a rate proportional to the degree of supersaturation

($n=1$ in Eq. 2), while the longitudinal growth rate of phase II crystals could be approximated by the square of the supersaturation ($n=2$ in Eq. 2). A report on a similar case in which the growth rate increased with the square of the supersaturation suggests that the rate-determining step in this type of crystal growth is the dehydration of the ions at the kinks.[9] A difference was also observed in the appearance of the growing surfaces of the two modifications.

$$R = k(C-C_0)^n \qquad \text{Eq. 2}$$

R: growth rate, k: growth coefficient,
$C-C_0$: supersaturation

The growing surface of phase I was uniformly flat, whereas that of phase II showed an irregular shape. These observations suggest that different mechanisms govern the crystal growth in the two crystal phases. The easier growth of phase I under the usual crystallization conditions can be partly explained by its different growth mechanism, but it may be mainly attributable to the easy generation of crystal nuclei in phase I.

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[9] Von R. Reich and M. Kahlweit, *Ber. Bunsenges. Phys. Chem.*, **72**, 66 (1968).