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Phase Diagram and Dissolution Studies of Mepyrizole (DA-398)/Water System[1]

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Mepyrizole (DA-398) was found to have the two crystalline forms of anhydride and dihydrate. Aqueous solution which contain more than 5 per cent of mepyrizole separate into two liquid layers at temperatures above 45°. It was found from the results of thermal analysis that crystal of mepyrizole dihydrate is transformed into anhydrous form at about 46°.

Phase diagram of mepyrizole/water system was obtained by summarizing the results of solubility studies, hygroscopic equilibriums and thermal analysis. Such abnormalities in the vicinity of 46° can be explained as based on the transition between mepyrizole anhydride and dihydrate and the mutual solubility of mepyrizole anhydride and water. From the results of calorimetric analysis, the endothermic reaction given after the dissolution reaction is deduced to the hydration reaction of mepyrizole anhydride in solution. Consequently, the dissolution phenomena of mepyrizole anhydride can be explained as follows: mepyrizole anhydride $[MP]_{solid}$ first dissolves quickly in water as anhydride $[MP]_{aqueous}$ and then gradually changes into dihydrate $[MP.2H_2O]_{aqueous}$.

$$[MP]_{solid} \longrightarrow [MP]_{aqueous} \longrightarrow [MP.2H_2O]_{aqueous}$$

Mepyrizole (DA-398)[3] was found to have two crystalline forms, anhydride and dihydrate, from the results of hygroscopic equilibrium and thermal analysis.

The solubility of dihydrate in water increases considerably at temperatures exceeding about 35°. Aqueous solutions which contain over 5 percent of mepyrizole, separate into two liquid layers at elevated temperatures exceeding 45°. From the results of thermal analysis, crystal of mepyrizole dihydrate was found to be transformed into nonsolvated form at about 46°. The liberated water and the anhydride form the liquid phase.

Phase diagram of mepyrizole/water system was given by summarizing the results of solubility studies, hygroscopic equilibriums and thermal analyses. Charonnat[4] has found the separation phenomenon in the aminopyrine/water system. Kiryu, *et al.*[5] investigated the solubility and separation phenomena of aminopyrine/water system and prepared a phase diagram.

The separation phenomenon have occasionally been found in aqueous solutions of amines, and the reason is given as the decrease in the hydrogen bonding energy between amine molecule and water with increase in temperature. These abnormalities in the solubility and in the separation phenomenon found in the mepyrizole/water system have been explained by the transition between anhydrous form and hydrated form in solution, and mutual solubility of anhydrous form and water. Dissolution behavior of organic compounds have been discussed by many workers,[6,7] in relation to the crystalline form. The crystal of anhydrous

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[1] This work was presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.

[2] Location: *Narihira, Sumida-ku, Tokyo.*

[3] Mepyrizole is the general name for 1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-methyl-5-methoxypyrazole, a new analgesic and anti-inflammatory, synthesized by Naito, *et al.* [T. Naito, T. Yoshikawa, S. Kitahara, and N. Aoki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1467 (1969)].

[4] R. Charonnat, *Compt. Rend.*, **185**, 284 (1927).

[5] S. Kiryu and S. Iguchi, *Yakugaku Zasshi*, **89**, 707 (1969).

[6] E. Shefter and T. Higuchi, *J. Pharm. Sci.*, **52**, 781 (1963).

[7] H. Nogami, T. Nagai, and T. Yotsuyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 499 (1969).

form dissolves much more than the hydrated form to give a maximum value. The concentration of these solution decreases gradually and finally gave the equilibrium concentration of the hydrated form.

Mepyrizole anhydride dissolves rapidly to a supersaturated concentration of nearly eight times as much as the solubility of the hydrate. The metastable supersaturated solution is comparatively stable for the crystallization of the hydrate. On the other hand, the crystalline form of mepyrizole hydrate was stable only in the atmospheric condition of comparatively high humidity and at low temperature. Hydration energy of mepyrizole was determined from the results of calorimetric analysis and a low value of 2.6 kcal/mole was obtained.

These results suggest a weak interaction between mepyrizole and water.

Experimental

Materials——Mepyrizole anhydride was recrystallized from water and dried in vacuum (mp 89—90°, specific surface area by BET method 2.90 m^2/g). Mepyrizole hydrate was obtained from the hygroscopic equilibrium of recrystallized mepyrizole and water content was determined by the Karl–Fischer method (specific surface area was 5.16 m^2/g by BET method, outgassed at liquid nitrogen temperature). Propylene glycol was JP grade.

Hygroscopic Equilibrium——Saturated salt solutions were used in order to obtain constant relative humidities at the various temperatures. Changes in weight of anhydrous form and wet crystal were measured periodically. Water content at equilibrium was finally determined by the Karl–Fischer method.

Thermogravimetric Analysis——Extension value of silica spiral spring was 85.383 mm/g. Temperature of the atmosphere was maintained constant with circulated water. 1.5—2.0 g of sample was used.

Thermal Analysis——Thermal analysis was carried out using differential scanning calorimeter (Perkin Elmer model DSC-1B). Samples were prepared by mixing mepyrizole anhydride, mepyrizole hydrate or water. Water content was determined by the Karl–Fischer method just before thermal analysis. Samples were sealed in an aluminium pan cell for volatile sample to prevent loss of water from the system. Indicated temperature was corrected with water, ammonium nitrate and indium.

Solubility——Circulating thermostat (Taiyo thermo unit, type OH-100) was used (±0.1°). Mixtures were stirred by a fluorocarboncovered magnetic stirring bar for more than 3 hours . The sample from the dissolution system was withdrawn directly with cotton filter attached pipette. The solutions were analysed spectrophotometrically at 250 mμ in 0.1N hydrochloric acid.[8]

Phase Separation Temperature——In the weight fraction range from 0.15 to 0.50 of mepyrizole, the phase separation phenomenon and the melting of dihydrate particles overlapped. In these cases, the mixed system was heated to about 50° to make phase separation completely, and then the temperature was decreased at a rate of less than 0.3°/min, under stirring. The phase separation temperature was defined as the temperature at which the milky emulsion state disappears. After the emulsion state, a translucent supersaturated state was observed. This phase was differentiated as the opalescent state. In the other fraction range, the separated solutions were stirred for not less than 30 minutes at a constant temperature and then stood to be separated. Both layers were analysed spectrophotometrically.

Rate of Dissolution——A weighed sample was added rapidly to exactly 100 ml of water while stirring at 6000 rpm with a homogenizer (Polytron PT-2000). A small sample was withdrawn from the system at fixed time intervals with a cotton-filter attached pipette. The concentration was determined spectrophotometrically.

Heat of Dissolution——A twin-type conduction calorimeter (Oyodenki Kenkyujo model CM-204) was used. The measurements were carried out at a constant temperature of 37° on 0.1—0.3 g of mepyrizole in 30 ml of water. The apparatus constant of this calorimeter was calculated according to Koishi,[9] and the following equation was obtained,

$$Q = 35.65\Delta\theta + 2.90 \times 10^{-2}\int \theta dt$$

where Q is the liberated heat in the reaction cell. θ is the temperature difference between the reaction cell and the cell of the reference calorimeter, and $\Delta\theta$ is the temperature difference at t minute. Full scale of the recorder was 500 μv.

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[8] M. Sano, I. Itoh, Y. Nakai, and T. Naito, *Chem. Pharm. Bull.* (Tokyo), **17**, 1485 (1969).

[9] M. Koishi, *Bull. Chem. Soc. Jap.*, **39**, 2406 (1966).

Result and Discussion

Determination of the Hydrated Form

Hygroscopic equilibrium curves of mepyrizole anhydride and wet product containing about 30% of water are given in Fig. 1, at a relative humidity of 96% at 37°. Although the water vapour pressure was relatively high, the hygroscopic equilibrium was attained in a long period of more than 12 days. Almost the same relationship was observed in the other atmospheric conditions.

As shown in Table I, the crystalline form of mepyrizole can be classified into two groups. At a relatively low humidity, water content becomes zero (corresponds to anhydrous form). Under a high humidity condition, the water content become 13.3—14.0%, which agree with the theoretical water content of dihydrate (13.33 %).

Thermogravimetric analysis was also carried out as shown in Fig. 2.

From the results of hygroscopic equilibrium and thermogravimetric analysis, it was found that there are two crystalline forms of anhydride and dihydrate.

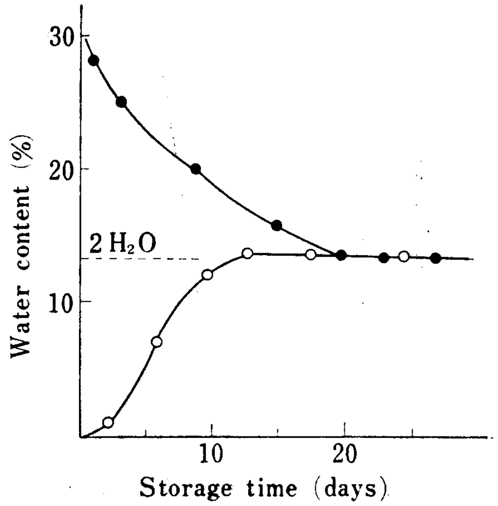

Fig. 1. Hygroscopic Rate of Mepyrizole in RH 98% at 37°

—○—: anhydride; —●—: 30% water contained

Table I. Saturated Salt Solutions for Constant Relative Humidity and Equilibrium Water Content in Mepyrizole

Temperature (°C)	Relative humidity (%)	Salt	Equilibrium water content[a] (%)
20	66	$NaNO_2$	0
	76	$C_2H_2O_4$	14.4 (14.5)
	95	Na_2HPO_4	14.0 (13.8)
25	81	$(NH_4)_2SO_4$	0
	87	Na_2CO_3	14.2 (14.2)
	97	K_2SO_4	14.4 (14.8)
30	84	KCl	0
	97	K_2SO_4	14.3 (14.3)
37	83	KCl	0
	96	K_2SO_4	13.5 (13.4)
40	82	KCl	0
	96	K_2SO_4	13.3 (13.5)

a) Water content was determined from weight variation. Parenthesized value was determined by Karl-Fischer method. Theoretical water content for dihydrate is 13.33%.

Thermal Analysis

From the results of the usual thermal analysis, mepyrizole dihydrate does not indicate a distinct thermal change corresponding to the liberation of the hydrated water because of the rapid transformation to the anhydrous form during increase in temperature. Finally, a peak corresponding to the melting point of anhydrous form (about 89°) was indicated.

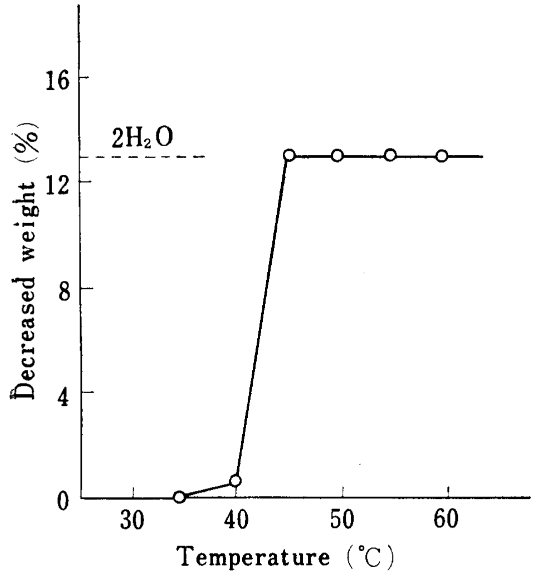

Fig. 2. Thermogravimetric Analysis of Hydrated Form of Mepyrizole (Water content: 13.4%)

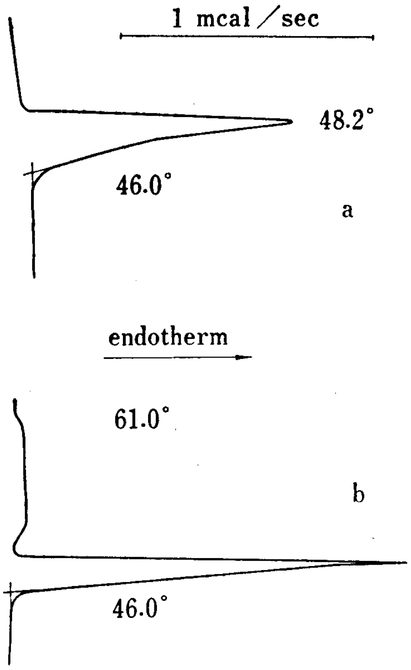

Fig. 3. DSC Chart of Mepyrizole/Water System (Differential Scanning Calorimeter Type 1-B)

a : weight fraction of DA-398: 0.833
sample weight: 2.22 mg
scan speed: 1°/min
chart speed: 1/4 inch/min
closed pan cell
b : weight fraction of DA-398: 0.923
sample weight: 2.36 mg
scan speed: 2°/min

Sealed aluminium pan cell for volatile sample was used to prevent the loss of hydrated water at below the transition temperature. As shown in Fig. 3, the thermogram of the mixed systems indicates clear endothermic changes at about 46°. The first endothermic reaction was observed at a constant temperature for samples with water content below 25 per cent and it is believed that this corresponds to transition from the dihydrate to the anhydrous form. The continued endothermic reaction, especially shown in (b), can be explained as the dissolution of anhydride into the liberated water.

When the glass capillary tube is sealed and observed with the naked eye, solid particles have disappeared and becomes a liquid monolayer when the temperature is over the end point of the second endothermic reaction.

Phase Diagram of Mepyrizole/Water System

The crystalline form of mepyrizole dihydrate is stable only at a low temperature not exceeding 46° and in a high relative humidity atmosphere. The solid state of the dihydrate is indicated as S_2 in Fig. 4. The melting point of the anhydrous form is about 89°. The solid phase of anhydride is also indicated as S_1 in Fig. 4.

In a temperature range, not exceeding about 46°, a liquid phase (L_1) of dihydrate and a solid phase (S_2) of dihydrate coexist. The solubility of mepyrizole dihydrate in water increases largely when the temperature exceeds about 35°. The solubility curve crosses with the separation curve of the anhydride at a temperature of about 45.5°.

A mixed system containing not less than 5 per cent of mepyrizole separates into two layers, L_1 (upper layer) and L_2 (lower layer) in a temperature range exceeding about 45°. L_1 is a liquid phase of mepyrizole dissolved in water, and L_2 is a liquid phase of water dis-

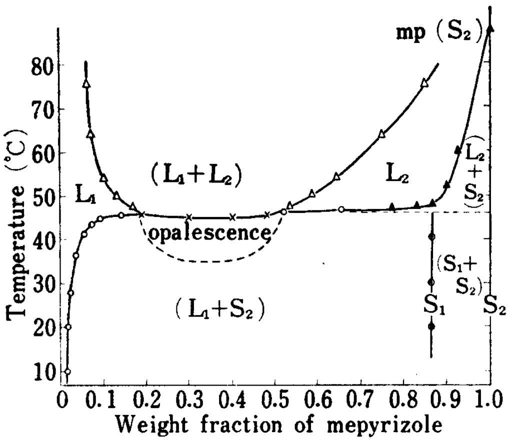

Fig. 4. Phase Diagram of Mepyrizole/ Water System

○: measured by solubility method
⊙: measured by hygroscopic equilibrium
×: measured by cooling or elevating temperature
△: measured by analysing the concentration of each liquid layer
▲: measured by DSC in sealed pan cell

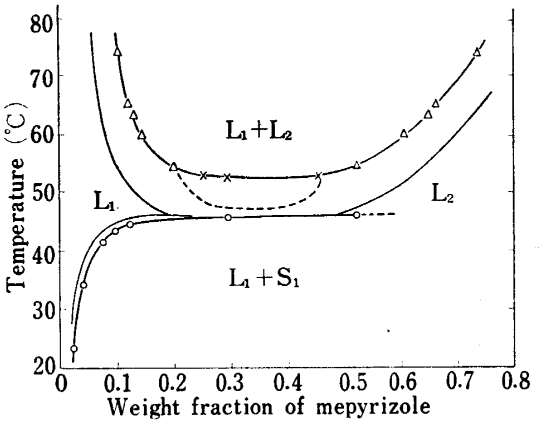

Fig. 5. Effect of Propylene Glycol on the Phase Diagram of Mepyrizole/ Water System (Weight fraction of PG: 0.035)

solved in mepyrizole. The phase separation phenomenon is reversible by increasing or decreasing the temperature.

A translucent supersaturated solution is obtained, when the temperature of mixed systems of the fraction range from 0.15 to 0.50 of mepyrizole is decreased below 45°. This state disappears at temperatures below about 35°.

Figure 5 shows the effect of propylene glycol (PG) on the solubility and separation phenomena of mepyrizole/water system. The effect of the addition of PG on the solubility of mepyrizole dihydrate is not very large, but the effect on the separation temperature and on the opalescent state can be clearly seen. These effects can be explained by the higher solubility of mepyrizole anhydrate in PG than the solubility of the dihydrate.

Thus the phase separation phenomenon and the abnormality of the solubility, found in the mepyrizole/water system at around 46°, can be explained on the basis of transition between anhydride and dihydrate in solution, and the mutual solubility of mepyrizole anhydride and water.

The transition temperature from the dihydrated form to the anhydrous form can also be considered from the results of hygroscopic equilibrium. As presented in Table I, the relative hymidities over which the mepyrizole crystal changes from the anhydrous form to the dihydrated form, increases with increase in temperature.

The relationship between the equilibrium water vapour pressure and temperature, when mepyrizole crystals change from the anhydrous form to the dihydrated form, is given in Fig. 6, as plots between log P and $1/T$. The temperature at the intersection with the straight line of saturated water vapour pressure is correspond to a temperature between 44° and 47° and this temperature range is in good agreement with the transition temperature of 46°, obtained by thermal analysis.

Hydration Energy of Mepyrizole in Solution

Heats of dissolution of mepyrizole anhydride and dihydrate were measured with a calorimeter and they were found to be proportional to the sample weight, as shown in Fig. 7. The calculated heat of dissolution was 1.5 kcal/mole for mepyrizole anhydride and 4.1 kcal/mole for the dihydrate.

On the other hand, it was found from the thermodiagram of anhydrous form that an endothermic reaction took place even after the maximum point of the thermodiagram, as

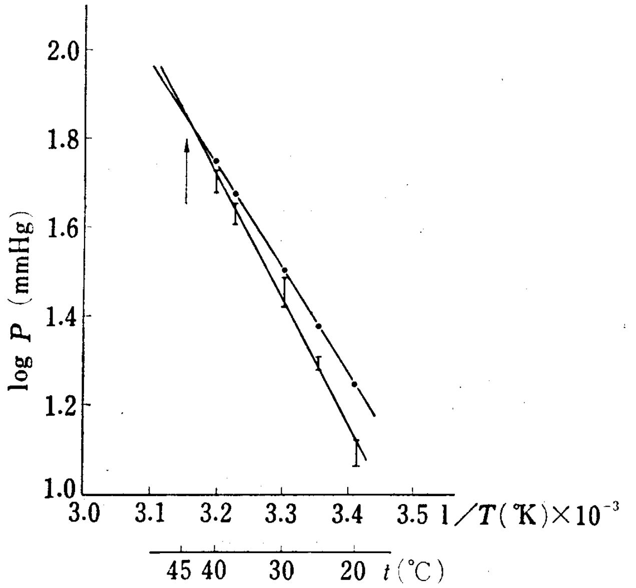

Fig. 6. Plots of Clausius-Clapeyron Equation

●: saturated pressure of water in atmosphere
I: equilibrium pressure of mepyrizole dihydrate in atmosphere

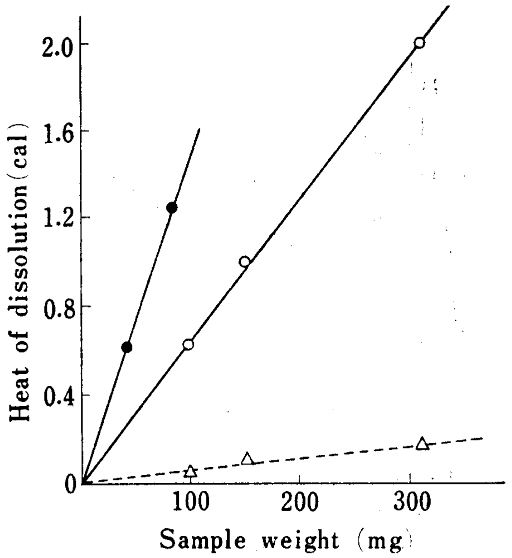

Fig. 7. Heat of dissolution of Mepyrizole Anhydride and Dihydrate into Water at 37°

●: mepyrizole dihydrate
○: mepyrizole anhydride
△: hydration energy of the anhydride

shown in Fig. 8. The dissolution rate of mepyrizole anhydride is very fast as presented in Fig. 9, so that the mepyrizole anhydride dissolves completely in water before the abnormal endothermic reaction starts after the maximum point.

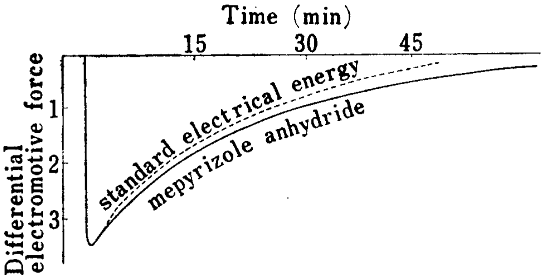

Fig. 8. Comparison of Thermodiagram between Mepyrizole Anhydride and Standard Electrical Energy

Sample weight of mepyrizole anhydride; 151.5 mg, full scale; 500 μV, chart speed; 20 cm/hr

From these results, it can be explained that the endothermic reaction observed after the maximum point of the thermodiagram is due to the hydration reaction of mepyrizole anhydride. The hydration energy of the anhydrous form was calculated by integration of the abnormal endothermic reaction energy as 0.13 kcal/mole.

Process of Dissolution in Water

From the results of calorimetric analysis, the dissolution phenomenon of mepyrizole anhydride can be explained as follows;

$$[\mathrm{MP}]_{\mathrm{solid}} \rightarrow [\mathrm{MP}]_{\mathrm{aqueous}} \rightarrow [\mathrm{MP}\cdot 2\mathrm{H_2O}]_{\mathrm{aqueous}}$$

Mepyrizole dihydrate dissolves in water as mepyrizole dihydrate. Mepyrizole anhydride $[\mathrm{MP}]_{\mathrm{solid}}$ first dissolves quickly in water as anhydride $[\mathrm{MP}]_{\mathrm{aqueous}}$ and then gradually changes into dihydrate $[\mathrm{MP.2H_2O}]_{\mathrm{aqueous}}$.

The following equation is applicable if it is assumed that the hydration reaction of mepyrizole is a first order reaction,

$$\frac{dC_h}{dt} = \mathrm{K}\cdot C_a = \mathrm{K}\cdot(C_{ao}-C_h) \qquad (1)$$

where C_h and C_a are the concentrations of mepyrizole hydrate and anhydride in the aqueous solution at time t. C_{ao} is the initial concentration of mepyrizole anhydrate, K is the rate constant of the hydration reaction. When equation (1) is integrated, it becomes

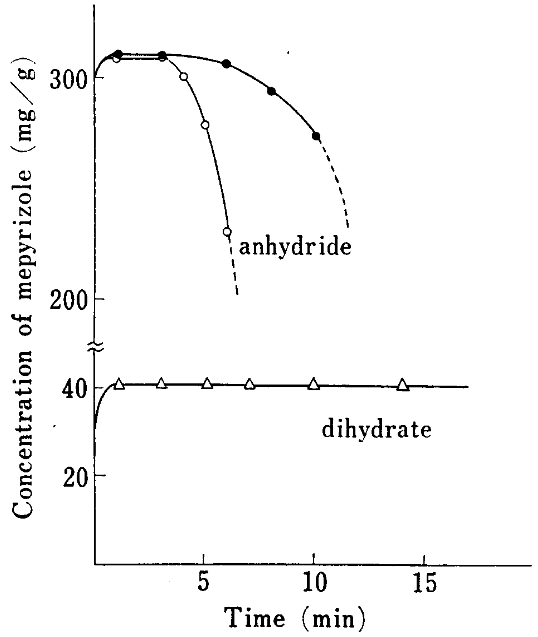

Fig. 9. Dissolution Behaviours of Mepyrizole Anhydride and Dihydrate

sample amount: anhydride 60 g, dihydrate 10 g added in 100 ml of water
—○— and —●—: anhydride
—△—: dihydrate

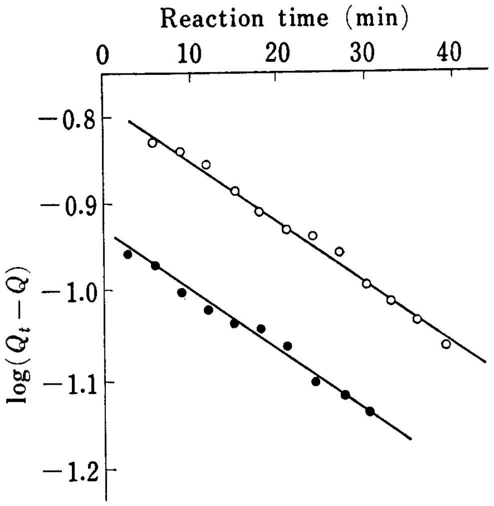

Fig. 10. Relationship in Eq. 3 between $\log\,(Q_t-Q)$ and Reaction Time for Mepyrizole Anhydride

○: 311.6 mg of the sample weight
●: 151.5 mg of the sample weight

$$\ln\,(C_{ao}-C_h) = -\,\mathrm{K}t + \mathrm{const} \qquad (2)$$

From equation (2), ln $(C_{ao}-C_h)$ is proportional to time t. C_h can not be measured directly but it can be assumed that C_h is proportional to the amount of heat (Q) liberated in t minutes by the endothermic reaction of hydration in the solution.

When hydration is completed, the concentration of the hydrate C_h should be equal to the initial concentration of the anhydride C_{ao} and the liberated heat Q is equal to the total hydration energy Q_t. Consequently $(C_{ao}-C_h)$ in equation (2) can be replaced by (Q_t-Q) and the following equation is obtained.

$$\ln\,(Q_t-Q) = -\,\mathrm{K}t + \mathrm{const} \qquad (3)$$

Fig. 10 shows the ln (Q_t-Q)-t plot. An approximately linear relation is indicated and confirms the assumption that hydration in an aqueous solution proceeds as a first order reaction. The rate constant K is calculated as 16.7×10^{-3} min.$^{-1}$

General Discussion

Heat of hydration of mepyrizole in the solid phase is 2.6 kcal/mole when calculated as the difference between the heat of dissolution of the anhydrous form and that of the dihydrated form. Heat of hydration was also calculated as 2.3 kcal/mole from the results of the hygroscopic equilibrium, using the Clausius–Clapeyron equation. The unstability of mepyrizole dihydrate is explained from the low value of the heat of hydration.

Armarego et al carried out studies on the ultraviolet absorption spectra of quinazolines,[10] purines[11] and other heterocyclic compounds. The change in the ultraviolet absorption spectra over a wide range of pH values can be explained by the addition of water by the

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10) W.L.F. Armarego and R.E. Willette, *J. Chem. Soc.*, **1965**, 1258.
11) A. Albert, *J. Chem. Soc.* (*B*), **1966**, 438.

covalent bond by way of the C=N double bond of the fused heterocyclic ring. In this case, however, hydration by covalent is obstructed if methyl group *etc.* is substituted in the ring near the C=N double bond.

Mepyrizole is composed of two single rings of pyrimidine and pyrazole. Moreover, the position of the C=N double bond is substituted with methyl and methoxyl groups. A large change in the ultraviolet absorption spectra of aqueous solutions of mepyrizole was not observed over a wide range of pH values. It is believed that the hydration of mepyrizole is not due to the covalent bond but to the weak hydrogen bond between the four nitrogen atoms in the pyrimidine and pyrazole rings.

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