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The Polymorphism of Inosine. II. The Measurement of Solubilities in Water

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The solubilities of anhydrous inosine crystals in the α-form and in the β-form in water were determined in the temperature range between 20 °C and 60 °C. The results may be formulated as follows:

α-form $\log S = 0.0190\,t - 0.065$

β-form $\log S = 0.0198\,t - 0.161$

These data indicate that the β-crystals are more stable than the α-crystals over the whole temperature range examined. However, the difference in solubility is too small to induce the transition without seed crystals in the β-form, so that the α-crystals themselves are practically stable in water. The rate of the transition from the α-form to the β-form varies in proportion to the amount of the seed β-crystals. At low temperatures the dihydrate form of inosine is more stable than both of these two anhydrous forms. The solubility may be formulated as follows:

dihydrate-form $\log S = 0.0314\,t - 0.276$

The polymorphism of anhydrous inosine crystals was briefly presented in the preceding paper.[1] The crystals in an orthorhombic system are called α-form, and those in a monoclinic system, β-form. The solubility data of inosine in water were reported for the α-form.[2] This paper will describe the solubilities in the two forms and the transition from the α-form to the β-form.

Experimental

Material. The inosine used was of a commercial A-grade of the Ajinomoto Co., Inc.

Preparation of the Crystals. *Inosine Dihydrate*: The commercial inosine was recrystallized in water at 5 °C. *Inosine Anhydrous in the α-Form*: The commercial inosine was recrystallized in water at 40 °C. *Inosine Anhydrous in the β-Form*: The commercial inosine was recrystallized in water at 40 °C in the presence of the seed crystals in the β-form. The seed crystals were obtained as follows: the commercial inosine (1.2 g) was dissolved in a 70% (w/w) dimethyl sulfoxide solution (10 ml), after which the wall of the glass vessel was scraped with a glass rod at room temperature until the β-crystals has been nucleated.

Solubility Measurement. A sufficient amount of the crystals was mixed with water to make a slurry in tightly-capped glass bottles. The equilibrium was achieved by tumbling the bottles at a constant temperature (±0.2 °C) for 16 hr. The supernatant clear solutions were sampled with a cotton-filtered pipet, weighed, diluted to appropriate volumes, and submitted to the measurement of the UV absorbance in 0.1 M HCl at 250 nm. The concentrations of the inosine were calculated from the molar extinction coefficient of 11800 under the above conditions. The residues were filtered and examined by means of the X-ray powder diffraction method in order to confirm the crystal form of the solid phase.

α-β Transition in Water. *Analysis*: For a brief quantitative analysis of the mixture of the two forms, the relative peak heights of the X-ray diffraction patterns were compared. The intensity of the 8.37 Å line (I_α) and that of 7.59 Å line (I_β) were measured. The β-form content was defined as $\{I_\beta/(I_\alpha+I_\beta)\}\times 100\%$. The calibration curve was almost linear. *In the Presence of the Seed Crystals*: Inosine crystals in the α-form (10 g) were added to 100 ml of water to make a slurry at room temperature. About three grams of inosine were dissolved, and seven grams remained undissolved. After the inosine crystals in the β-form (0.7 g or 1.4 g) had been added, the slurry was tumbled on a shaker at 30 °C. The suspended residues were sampled at successive intervals for X-ray examination. *In the Absence of the Seed Crystals*: Inosine crystals in the α-form (100 g) were added to 100 ml of water. After the resultant slurry had been vigorously stirred at 95 °C for 24 hr, the residue was examined by means of the X-ray diffraction method.

α-β Transition on the Drying of the Dihydrate Crystals. The dihydrate crystals of inosine were dried in air at 60 °C overnight with a little portion of the seed crystals in the β-form or without them. The resultant crystals were examined by means of X-ray analysis.

Results and Discussion

Table 1 shows the solubilities of inosine in water. The logarithms of the solubilities are linear to the temperature and are expressed as follows:

$$\alpha\text{-form:}\quad \log S = 0.0190\,t - 0.065 \qquad (1)$$

$$\beta\text{-form:}\quad \log S = 0.0198\,t - 0.161 \qquad (2)$$

where S represents the gram weight of inosine per 100 g of water and where t is the temperature (°C). These results show that inosine in the β-form is less soluble than that in the α-form, and that, therefore, the β-form is more stable than the α-form. Accordingly, it can be expected that the α-crystals will be transformed into the β-crystals in water. However, the difference in solubility between them is too small to induce the transition without the seed crystals in the β-form, so that the α-crystals themselves are practically stable in water. During the measurement of the solubility, the transition of the crystals in the residue was not observed. As two lines, represented by Eq. (1) and Eq. (2), cross at nearly 120 °C, the stability may be reversed beyond that temperature.

At low temperatures the dihydrate form of inosine is more stable than either of the two anhydrous forms. On the assumption that the same linear relation between log S and t as in Eq. (1) or Eq. (2) holds good, the solubility of the dihydrate form is expressed from the data in Table 1 as follows:

$$\text{dihydrate-form:}\quad \log S = 0.0314\,t - 0.276 \qquad (3)$$

Table 1. Solubilities of inosine in water[a)]

Temp. (°C)	S (g/100 g water)		
	Dihydrate[b)]	Anhydride	
		β-Form	α-Form
0	0.52	—	—
10	1.10	—	—
20	—	1.75	2.14
30	—	2.71	3.29
40	—	4.20	4.72
50	—	6.73	7.79
60	—	10.5	11.8

a) Average of two measurements with deviations within ±5% except for the data at 30 °C and 40 °C which are single. b) Solubilities are expressed in terms of anhydride.

where S represents the gram weight of inosine in terms of anhydride per 100 g of water. Two cross-points are obtained, the one formed by the two lines derived from Eq. (1) and Eq. (3), and the other, from those derived from Eq. (2) and Eq. (3). They represent the transition temperatures from the dihydrate-form to the α-form, and from the dihydrate-form to the β-form, respectively. The former is estimated to be about 17 °C, and the latter, about 10 °C. The dihydrate-form is stable below these temperatures.

The transition from the α-form to the β-form was observed when some seed β-crystals were added to a slurry of the α-crystals in water. The rate is in proportion to the amount of the seed β-crystals. When the amount of the seed β-crystals added was 20% of the amount of the α-crystals in the slurry, the transition was observed to begin a little after 1 hr, to be about half completed after 4 hr, and to be over completely within 20 hr. A slower rate was observed when the seed crystals were decreased to 10%. In this case, the transition was slightly observed after 6 hr, and it took 40 hr for the complete conversion. On the other hand, in the absence of the seed β-crystals a slurry of the α-crystals did not show any transformation, not even at 95 °C after 24 hr.

The addition of the seed β-crystals also has a significant influence on the drying of the dihydrate crystals in air. When the dihydrate crystals are dried in air with heating, they lose the water of crystallization with ease, giving anhydrous crystals in the α-form. However, they give the crystals in the β-form when some seed crystals in the β-form are mixed in before heating.

Inosine crystallizes usually in the α-form from an aqueous solution; this fact is consistent with the law of successive reactions proposed by Ostwald. However, the crystals in the β-form were found to be often directly obtainable from about a 70%(w/w) dimethyl sulfoxide solution without the seed β-crystals. Since the solubility gap between the two forms causes the transition, any difference in the solubility between them in a 70% dimethyl sulfoxide solution must be larger than that in water. This will be discussed in the next paper.

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References

1) Y. Suzuki and N. Nagashima, This Bulletin, **43**, 1600 (1970).

2) Y. Suzuki, T. Toki, and T. Nakamura, Presented orally at 18th Annual Meeting of the Chemical Society of Japan, Osaka, 1965.