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Polymorphism of Inosine. III. The Equilibrium for the Inosine–Dimethyl Sulfoxide–Water System

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The phase diagram for the inosine-dimethyl sulfoxide-water system was determined at 30 °C. Inosine in the α-form is more soluble than that in the β-form in aqueous DMSO. The difference in the solubilities between the two forms is small when the concentration of DMSO is below 50%. However, it becomes several times larger in 70% DMSO than in water. This is the reason why inosine in the β-form is often obtainable with ease from 70% DMSO in the absence of seed β-crystals, while it is with difficulty obtained only from water. When the concentration of DMSO becomes higher than 82%, a new solid phase is obtained. It consists of inosine with two mol of DMSO of crystallization. Inosine in the β-form is also obtainable with ease through the decomposition of these new crystals.

Inosine is usually crystallized in the α-form from water; only when some seed crystals in the β-form are present it is crystallized in the β-form. However, the crystals in the β-form are directly obtained without seed crystals when inosine is crystallized from 70% (w/w) aqueous dimethyl sulfoxide (DMSO) at 30 °C.1) In order to explain the above fact, the equilibrium for the inosine–DMSO–water system was studied. There is a datum on the solubility of inosine in pure DMSO at 30 °C by Rosenthal.2)

Results and Discussion

Figure 1 shows the phase diagram for the inosine–DMSO–water system at 30 °C, and in Fig. 2 the details are plotted with the experimental data in terms of the solubility of inosine per 100 g of aqueous DMSO.

The solubility of inosine in the α-form in aqueous DMSO is higher than that in the β-form over the whole range. Consequently, β-crystals are more stable than α-crystals in aqueous DMSO, as is the case in water. However, a notable distinction was found between the aqueous DMSO system and the water system. It is the difference in the solubility between the two forms. It increases with an increase in the DMSO, with a steep ascent beyond 50% DMSO, while it is small and constant below 50%. In 75% DMSO it reaches about six times that in water. This large difference in the solubility is sufficient to cause the quick transition from the α-form to the β-form without any seed crystals. This is the reason why inosine is crystallized in the β-form from the 70% DMSO solution, whereas it is crystallized in the α-form from water.

In high concentration of DMSO near 80%, a new solid phase was obtained. It was found to be inosine, with two mol of DMSO of crystallization. It appears consecutively on the line of the β-crystal phase, as is shown in the figures. The composition of the critical point is DMSO : water : inosine=75.0 : 16.1 : 8.9(Point A in Fig. 2). When the concentration of DMSO is higher than that value, the new solid phase is most stable.

These inosine crystals with the DMSO of crystallization are incongruent and are decomposed by pure water. This property suggests another new method for the direct preparation of β-crystals. When these new crystals are treated with a small portion of water or aqueous DMSO (below 80%), they are transformed into inosine anhydrous crystals in the β-form. It has been pointed out that there is a region where the new crystals are less soluble than α-crystals and more solu-

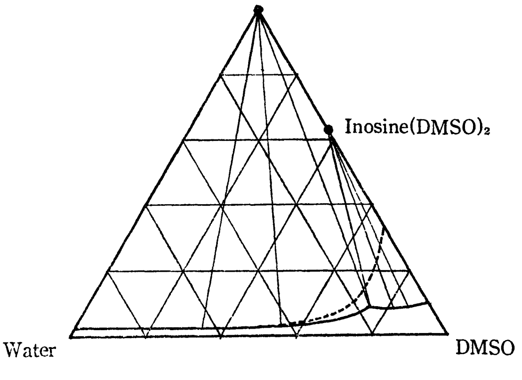

Fig. 1. Phase diagram for the inosine-dimethylsulfoxide-water system at 30 °C.

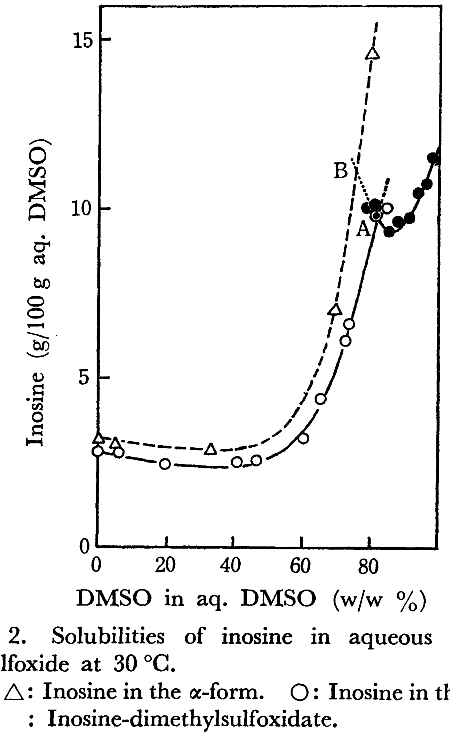

Fig. 2. Solubilities of inosine in aqueous dimethylsulfoxide at 30 °C.
△: Inosine in the α-form. ○: Inosine in the β-form.
: Inosine-dimethylsulfoxidate.

TABLE 1. X-RAY POWDER DIFFRACTION DATA OF INOSINE-DIMETHYLSULFOXIDATE

Inosine·$(DMSO)_2$	
d(Å)	I/I_0[a]
11.63	10
9.71	30
5.47	30
5.22	10
4.80	100
4.35	30
4.15	30
4.10	10
3.83	30
3.20	20

a) The scale is so chosen as to make the most intense line have the value 100.

ble than β-crystals. This region is shown between Point A and Point B in Fig. 2. When the new crystals are dissolved in this region, the β-crystals will be preferentially nucleated.

These new crystals are unstable in air and are gradually changed into β-crystals within a few days at room temperature, probably because of the influence of the humidity in the air. This transition of crystals in air is accelerated on heating.

Table 1 shows the X-ray powder diffraction data of these new crystals. It is of interest that inosine can be crystallized with two mol of DMSO of crystallization, while it is crystallized with the same moles of water of crystallization, and that DMSO is able to combine with inosine at room temperature, while water does so only at low temperatures.[1]

The solubility of inosine in pure DMSO reported by Rosenthal agreed with that for inosine in the α-form. However, it is obvious that the equilibrium is unstable and that inosine-dimethylsulfoxidate will be crystallized with a faint stimulation.

Experimental

Materials. The inosine used, of a commercial A-grade, was obtained from the Ajinomoto Co., Inc. The DMSO was of a reagent grade; its water content was checked by Karl Fischer titration.

Preparation of the Crystals. Inosine anhydrous crystals in the α-form and in the β-form were prepared according to the method reported in the preceding paper.[1] Inosine-dimethylsulfoxidate crystals were obtained as follows: inosine (12 g) was dissolved in DMSO (98%, 50 ml) with heating, after which mixture was stirred for two days at room temperature. The precipitated prism crystals were filtered, washed with ether, and analyzed immediately. Yield, 12 g; mp 88—89 °C. Found: C, 39.31; H, 5.69; N, 13.41%. Calcd for $C_{10}H_{12}N_4O_5\cdot(C_2H_6OS)_2$: C, 39.61; H, 5.70; N, 13.26%. Inosine content calculated from the optical density (250 nm in 0.1 M HCl) compared with the standard inosine, 64.0% (theoretical, 63.3%).

Solubility Measurements. A sufficient amount of the crystals selected among inosine-dimethylsulfoxidate and inosine in the α-form and in the β-form were mixed with aqueous DMSO to make a slurry in tightly-capped glass bottles. The equilibria were achieved by tumbling the bottles for 16 hr at 30 °C. Then, the mixtures were filtered by means of a glass filter. The UV absorbances at 250 nm in 0.1 M HCl were measured for the filtrates and the residues. The inosine contents were calculated from the molar extinction coefficient defined as 11,800 under the above conditions. The UV absorbances due to DMSO are negligible compared with those of inosine. The water contents in the solution phase were determined by Karl Fischer titration. The DMSO was determined by means of the difference. The residues on a filter were examined by the X-ray powder diffraction method in order to identify the crystal form.[3]

Decomposition of Inosine-dimethylsulfoxidate. (a) Inosine-dimethysulfoxidate (25 g) was added to aqueous DMSO (70%, 100 ml) to make a slurry. After the mixture had been shaken for 5 hr, it was left to stand overnight. The crystals suspended were filtered and identified by the X-ray method. (β-Form). (b) The crystals of inosine-dimethylsulfoxidate were precipitated according to the method described above. Immediately after the crystals had been removed from the mother liquor with a filter, they were washed with ether and left to stand in air for 3 hr at room temperature. Then, they are dried for 1 hr at 90 °C. They were identified by means of the X-ray method (β-Form).

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References

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2) N. A. Rosenthal, Fr. 1468708 (1967).
3) Y. Suzuki and N. Nagashima, This Bulletin, **43**, 1600 (1970).