A Study of the Inosine–Hydrochloric Acid–Water and Inosine–Sulfuric Acid–Water Systems

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Solubility data have been obtained for the inosine–HCl–H_2O and inosine–H_2SO_4–H_2O systems at 5 °C. The acid hydrolysis of inosine was satisfactorily prevented under the present conditions. Three new acid salts of inosine have been obtained: (1) $C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$ in the A-form, (2) $C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$ in the B-form, and (3) $C_{10}H_{12}N_4O_5 \cdot H_2SO_4 \cdot 2H_2O$. Their X-ray powder diffraction data are given. All of them are incongruent and decomposed by pure water; however, they are stable in air during storage at room temperature.

In order to contribute to the process design of the separation or purification of inosine, its solubility in several mineral acid solutions was studied. Inosine is known to be susceptible to acid hydrolysis, giving hypoxanthine and D-ribose. However, the hydrolysis was practically negligible at 5 °C within certain periods. The equilibria for the inosine–HCl–H_2O and inosine–H_2SO_4–H_2O systems were satisfactorily determined. No data have been found in the literature concerning the subject of the present study.

Results and Discussion

Table 1 shows the composition data for the inosine–hydrochloric acid-water system at 5 °C. The equilibrium was achieved by the dissolution of the crystals to acid solutions. The mixture was usually shaken overnight at 5 °C. These conditions were generally sufficient to achieve the equilibrium. However, there was a fear of the hydrolysis of inosine in high concentrations of hydrochloric acid, so the shaking period for the equilibrium was shortened to 5 hr when the concentration of hydrochloric acid was higher than 16% (Runs No. 9—11). In spite of the above treatment, we could not completely prevent the hydrolysis in high concentrations of hydrochloric acid. The data obtained in these runs had some deviations (within 10%). On the other hand, there was no significant hydrolysis when the concentration of hydrochloric acid was less than about 10%.

TABLE 1. COMPOSITION DATA FOR THE INOSINE–HYDROCHLORIC ACID–WATER SYSTEM AT 5 °C

Run No.	Solution (Weight %)			Wet residue (Weight %)		Solid phase[a]
	Inosine	HCl	Hx[b]	Inosine	HCl	
lit.[c]	0.76	0	—	—	—	I2
1	4.50	1.77	—	63.18	0.54	I2
2	6.82	2.67	—	62.50	0.82	I2
3	13.76	4.06	—	72.80	6.44	I2+A
4	13.35	4.11	—	70.61	4.77	I2+A
5	13.86	4.03	—	44.73	4.74	I2+A
6	6.40	7.72	—	58.39	10.51	A
7	4.44	13.15	+	55.68	11.72	A
8	4.24	15.28	+	56.26	11.07	A
9	4.03	16.42	0.2	59.80	12.79	A+B
10	4.03	17.48	0.2	53.60	11.40	A+B
11	4.65	19.71	0.3	56.66	13.01	B

a) I2: Inosine di-hydrate, $C_{10}H_{12}N_4O_5 \cdot 2H_2O$. A: Inosine hydrochloride in the A-form, $C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$. B: Inosine hydrochloride in the B-form, $C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$. b) Hx.: Hypoxanthine. c) Y. Suzuki, This Bulletin, **47**, 2549 (1974).

The system shows the formation of two new solid phases which possess the same molecular formula, but different crystal structures. They are both inosine monohydrochloride hemihydrate. The one stable in lower concentrations of hydrochloric acid was named the A-form, and the other, stable in higher concentrations, the B-form. Their X-ray powder diffraction data are shown in Table 2.

TABLE 2. X-RAY POWDER DIFFRACTION DATA OF SOME MINERAL ACID SALTS OF INOSINE

$C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$ A-Form		$C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$ B-Form		$C_{10}H_{12}N_4O_5 \cdot H_2SO_4 \cdot 2H_2O$	
d(Å)	I/I_0[a]	d(Å)	I/I_0[a]	d(Å)	I/I_0[a]
8.30	40	8.01	100	8.54	100
6.19	15	6.11	5	6.11	10
5.40	20	5.64	5	5.13	25
4.84	5	5.25	10	4.87	5
4.60	5	4.80	5	4.48	5
4.13	10	4.33	5	4.29	25
3.67	100	4.13	5	4.06	5
3.28	10	4.00	10	3.72	60
3.22	15	3.85	5	3.62	10
3.18	10	3.53	90	3.43	10
3.05	10	3.28	10	3.33	15

a) The scale of the intensity (I/I_0) is so chosen as to make the most intense line have the value 100.

The crystals in the A-form appeared in the region where the concentration of hydrochloric acid is between about 4% (Runs No. 3—5 in Table 1) and about 17% (Runs No. 9—10 in Table 1), while the B-form appears above about 17%. Neither of these two salts could be crystallized from an equimolar solution of inosine and hydrochloric acid. They could be obtained from a solution containing a large excess of hydrochloric acid. Therefore, the salts were concluded to be incongruent and decomposed by pure water. An excess amount of the salts added to pure water could not remain in the salt-form in the residue, but changed into the inosine-dihydrate-form.

On the other hand, they were stable in air during storage for at least one month at room temperature when the mother liquor was completely removed from the crystal surface by washing with an organic solvent such as ethanol. When the removal of the mother liquor was insufficient, the crystals of the salts gradually became dark, probably because the acid in the mother liquor reacted with the ribose moiety of the inosine in the crystal. The solubility of inosine increased with an increase in the concentration of hydrochloric acid up to about 4%, and then gradually decreased with a further increase in the acid concentrations; it became constant beyond a 14% concentration of the acid. It is of interest that the solid phase changes from inosine dihydrate to inosine hydrochloride hemihydrate at a point where a sharp change in the solubility is observed, while inosine hydrochloride in the solid phase changes from the A-form to the B-form at a point where an almost constant solubility is observed.

Table 3. Composition data for the inosine–sulfuric acid–water system at 5 °C

Run No.	Solution (Weight %)			Wet Residue (Weight %)		Solid Phase[a]
	Inosine	H_2SO_4	Hx.[b]	Inosine	H_2SO_4	
lit.[c]	0.76	0	—	—	—	I2
1	2.31	1.37	—	54.24	—	I2
2	9.11	5.31	—	74.51	1.35	I2
3	11.03	8.02	—	75.68	1.91	I2
4	15.88	10.12	—	—	—	I2+ S
5	17.44	9.50	—	55.33	20.56	S (m)
6	13.28	10.87	—	54.77	21.73	S
7	7.29	14.00	—	56.89	22.08	S
8	2.90	23.39	0.03	54.83	24.43	S
9	2.00	30.00	+	53.83	24.71	S

a) I2: Inosine di-hydrate, $C_{10}H_{12}N_4O_5 \cdot 2H_2O$. S: Inosine sulfate, $C_{10}H_{12}N_4O_5 \cdot H_2SO_4 \cdot 2H_2O$. m: metastable.
b) Hx.: Hypoxanthine. c) See Table 1.

Table 3 shows the composition data for the inosine–sulfuric acid–water system at 5 °C. The hydrolysis of inosine in this system was much less than that in the hydrochloric acid system, so, practically, it could be disregarded. This system gave a new solid phase, the monosulfuric acid salt of inosine dihydrate. This acid salt of inosine could be obtained when the concentration of sulfuric acid was higher than 10% (Runs No. 4 in Table 3). These crystals had a plate-like appearance. Although they are incongruent and are decomposed by pure water, they are stable in air during storage for at least one month at room temperature, as in the case of the hydrochloric acid salt of inosine.

The solubility of inosine increased with an increase in the concentration of sulfuric acid until the concentration of sulfuric acid reached about 10%; it gradually decreased beyond that point.

It is interesting that both hydrochloric acid and sulfuric acid form salts with inosine, keeping an equivalent mole ratio in spite of the different valences of the acids.

Experimental

Materials. The inosine used was of a commercial A-grade of the Ajinomoto Co., Inc.; the other materials were of a reagent grade.

Analysis. The inosine was determined by means of the UV absorbance at 250 nm in 0.1 M HCl using the molar extinction coefficient of 11,800. The acids were determined by titration with standard NaOH, using a methyl red-bromocresol green indicator. The chlorine was determined by the Volhard method. The X-ray (Cu$K\alpha$) powder diffraction patterns were used for the identification of the solid phase in equilibrium. The degree of hydration of the crystals was determined by Karl Fischer titration. One-dimensional ascending paper chromatography (PC) was carried out on Toyo-Filterpaper No. 51A, the following solvent system being used: *n*-butanol–acetic acid–water (4 : 1 : 1). The hydrolysis of inosine into hypoxanthine was checked by PC (R_f, inosine, 0.25; hypoxanthine, 0.42), using UV-light.

Preparation of the Crystals. *Inosine Monohydrochloride Hemihydrate in the A-Form:* Inosine (6.0 g) was dissolved in aqueous 13% (w/w) HCl (50 ml), and the resultant solution was cooled in an ice-box for a week. The large prism crystals thus precipitated were filtered out and then dried in air. Yield, 2.1 g; mp 112 °C (colored)-130 °C (decomp. with darkening). One UV-absorbing spot was observed on PC (R_f, 0.25). Found: H_2O, 3.44; Cl, 11.1; Acid for HCl, 11.6%; $\varepsilon_{250\,nm}$ in 0.1 M HCl, 9,940. Calcd for $C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$: H_2O, 2.87; Cl, 11.32; Acid for HCl, 11.65%; ε, 10090.

Inosine Monohydrochloride Hemihydrate in the B-Form: Inosine (8.6 g) was dissolved in aqueous 21% (w/w) HCl (50 ml), and the resultant solution was cooled in an ice-box overnight. The precipitated fine prism crystals were filtered out, washed with ethanol and ether, and then dried in air. Yield, 5.9 g; mp 122 °C (colored)-130 °C(decomp. with darkening). One UV-absorbing spot was observed on PC (R_f, 0.25). Found: H_2O, 2.44; Cl, 11.2; Acid for HCl, 11.7%; $\varepsilon_{250\,nm}$ in 0.1 M HCl, 9,940. Calcd for $C_{10}H_{12}N_4O_5 \cdot HCl \cdot (1/2)H_2O$: H_2O, 2.87; Cl, 11.32; Acid for HCl, 11.65%; ε, 10090.

Inosine Monosulfate Dihydrate: Inosine (6.2 g) was dissolved in aqueous 31% (w/w) H_2SO_4 (45 ml), and the resultant solution was cooled in an ice-box overnight. The precipitated plate crystals were filtered out and dried in air. Yield, 7 g; mp 108—112 °C. One UV-absorbing spot on PC was observed (R_f, 0.25). Found: H_2O, 7.47; Acid for H_2SO_4, 25.1%; $\varepsilon_{250\,nm}$ in 0.1 M HCl, 7,890. Calcd for $C_{10}H_{12}N_4O_5 \cdot H_2SO_4 \cdot 2H_2O$: H_2O, 8.96; Acid for H_2SO_4, 24.38%; ε, 7,870.

Solubility Measurement. A sufficient amount of the crystals, usually inosine was added to aqueous HCl (0—20%) or aqueous H_2SO_4 (1—20%) to make a slurry in tightly-capped glass bottles at 5 °C. Inosine hydrochloride in the A-form was used for Runs No. 3, 8 and 10 in Table 1, and the B-form, for Run No. 9. Inosine sulfate was used for Runs No. 5—8 in Table 2. The mixtures were shaken at 5 °C for 16 hr except for Runs No. 9—11 (for 5 hr) in Table 1. Then, the mixtures were quickly filtered with a glass filter. The clear solutions and the wet residues were sampled, diluted to appropriate volumes, and submitted to analysis. The residues were examined by means of the X-ray method.

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