October, 1974] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 47(10), 2553—2555 (1974) 2553

The Equilibria for the Inosine–Calcium Hydroxide–Water and Inosine–Strontium Hydroxide–Water Systems

Yoshihisa Suzuki and Yasuichi Nomura
Central Research Laboratories, Ajinomoto Co., Inc., Suzuki-cho, Kawasaki 210

(Received March 1, 1974)

Solubility data have been obtained for the inosine-$Ca(OH)_2$-H_2O and inosine-$Sr(OH)_2$-H_2O systems at 10 °C. Three different solid phases of the calcium salt of inosine were found in the former system. The first is the normal hemi-calcium salt, $(\text{inosine})_2\cdot Ca\cdot 2H_2O$; the second, an abnormal salt, $(\text{inosine})_3\cdot Ca\cdot 3H_2O$, and the third, another abnormal salt, $(\text{inosine})_4\cdot Ca\cdot 9H_2O$. The former two salts have stable regions in the system, while the last one does not. The system including $Sr(OH)_2$ is simple, and no other salt-formation was found except for that of normal hemi-strontium salt, $(\text{inosine})_2\cdot Sr\cdot 2H_2O$. The X-ray powder diffraction data of the above four salts are given. The solubilities of the two normal hemi-alkaline-earth-metal salts of inosine in water were determined at 10—60 °C and formulated as follows:

$$\text{Ca-salt,}\quad \log S = 0.00406\cdot t - 0.4725$$

$$\text{Sr-salt,}\quad \log S = 0.00551\cdot t - 0.4116$$

where S represents the gram weight of anhydrous salt per 100 g of water.

In order to contribute to the process design of the separation or the purification of inosine, the two systems were examined and the solubility data for some alkaline-earth metal salts of inosine were obtained. No information has been found in the literature.

Results and Discussion

The results of the determination for the systems are presented in Tables 1 and 2, along with the experimental conditions. The mole ratio for the salt-formation between inosine and alkaline-earth metal is expected to be 2 : 1, in view of the acid-base valence. However, the system including calcium hydroxide is complicated because of the formation of two additional abnormal calcium salts of inosine besides the normal salt expected. One of them is estimated to be composed of three mol of inosine per mol of calcium atom with three mol of water of crystallization, and the other, of four mol of inosine per mol of calcium atom with nine mol of water. Their crystal habits are both feathery and fine-needle-like, thus hindering the precise determination of the water of crystallization. The above two abnormal salts of inosine can be explained as molecular compounds between the normal salts and free inosine. The former salt has a stable region in the system, while the latter does not and is metastable through-out the range. Their X-ray powder diffraction patterns are

Table 1. The equilibrium for the inosine-$Ca(OH)_2$-H_2O system at 10 °C

Exp. conditions[a)] (g material/50 ml H_2O)			Solution phase (Weight %)		Solid phase[b)]
I(α)[c)]	$Ca(OH)_2$	Salt	Inosine	$Ca(OH)_2$	
lit.[e)]	—	—	1.10	0	I2
2.0	—	A, 2.0	1.25	0.031	I2+C (m)
1.2	—	A, 1.0	1.18	0.030	C (m)
1.0	—	A, 1.0	0.860	0.034	C (m)
0.8	—	A, 1.0	0.678	0.052	C (m)
1.0[d)]	—	B, 0.5+C, 0.5	0.803	0.029	I2+B
0.18	—	B, 1.0	0.667	0.028	B
—	—	B, 2.0	0.418	0.040	B
—	0.01	B, 1.0	0.455	0.063	B (m)
0.2	—	A, 1.0	0.748	0.048	A (m)
0.4	—	A, 1.0	0.691	0.046	A+C
0.11	—	A, 1.0	0.580	0.044	A (m)
0.11	—	A, 1.0+B, 1.0	0.422	0.044	A+B
—	—	A, 2.0	0.339	0.052	A
—	0.05	A, 2.0	0.389	0.137	A
—	0.08	A, 1.0	0.503	0.220	A
—	1.0	A, 1.0	0.658	0.330	A+$Ca(OH)_2$
0.15	1.0	—	0.296	0.240	$Ca(OH)_2$
lit.[f)]	—	—	0	0.175	$Ca(OH)_2$

a) The equilibria were approached by dissolving the materials in water. b) I2: Inosine·$2H_2O$. A: $(\text{Inosine})_2\cdot Ca\cdot 2H_2O$, B: $(\text{Inosine})_3\cdot Ca\cdot 3H_2O$, C: $(\text{Inosine})_4\cdot Ca\cdot 9H_2O$, m: metastable c) I(α): Inosine in the α-form.[1)] d) I2 was used. e) Reference 1. f) The mean value: H. Bassett, *J. Chem. Soc.*, **1934**, 1270.

Table 2. The equilibrium for the inosine-$Sr(OH)_2$-H_2O system at 10 °C

Exp. conditions[a] (g material/50 ml H_2O)			Solution phase (Weight %)		Solid phase[b]
I(α)[c]	Sr8[b]	S[b]	Inosine	$Sr(OH)_2$	
lit.[e]	—	—	1.10	0	I2
2[d]	0.05	—	1.29	0.044	I2
2	—	2	1.61	0.092	I2+S
2[d]	—	2	1.56	0.091	I2+S
0.3	—	2	1.02	0.086	S
—	—	2	0.369	0.074	S
—	0.3	2	0.475	0.376	S
—	0.6	2	0.699	0.700	S
—	2	2	0.856	0.875	S+Sr8
0.2	2	—	0.405	0.733	Sr8
lit.[f]	—	—	0	0.57	Sr8

a) The equilibria were approached by dissolving the materials in water. b) I2: Inosine·$2H_2O$. S: $(Inosine)_2\cdot Sr\cdot 2H_2O$, Sr8: $Sr(OH)_2\cdot 8H_2O$. c) I(α): Inosine in the α-form.[1] d) I2 was used. e) Ref. 1. f) C. Scheibler, *J. Pharm. Chim.*, **8**, 540 (1883).

Table 3. X-Ray powder diffraction data of some abnormal calcium salts of inosine

$(Inosine)_3\cdot Ca\cdot 3H_2O$		$(Inosine)_4\cdot Ca\cdot 9H_2O$	
d(Å)	I/I_0[a]	d(Å)	I/I_0[a]
15.00	90	10.05	100
8.76	5	7.56	40
8.08	40	6.00	10
7.44	100	5.73	20
7.11	5	5.28	20
6.13	40	5.13	5
4.93	70	5.04	25
4.40	40	4.97	20
4.23	30	3.88	20
4.04	70	3.75	20
3.90	30	3.58	20
3.28	20	3.50	20
3.24	20	3.36	30

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

shown in Table 3; they are characteristic enough to enable us to distinguish these crystals from other crystals.

The system including strontium hydroxide is simple, and no other salt-formation was found except the normal hemi-strontium salt of inosine.

The normal hemi-calcium salt and hemi-strontium salt of inosine possess stable regions in the systems. They are both obtained in the mono-hydrate form with respect to one mol of inosine and a half of the alkaline-earth metal. Their X-ray powder diffraction patterns are similar (Table 4), and it may be supposed that they have almost the same crystal structure except for the unit-cell dimensions, which are controlled by the atomic size of the alkaline-earth metal. The crystal appearances are also similar, both being fine hard pillars.

Table 4. X-Ray powder diffraction data of normal alkaline-earth metal salts of inosine

$(Inosine)_2\cdot Ca\cdot 2H_2O$		$(Inosine)_2\cdot Sr\cdot 2H_2O$	
d(Å)	I/I_0[a]	d(Å)	I/I_0[a]
9.72	100	10.05	100
7.83	20	8.04	20
6.11	20	6.24	20
5.47	5	5.54	15
4.87	5	4.85	15
4.77	5	4.65	20
4.58	20	4.44	40
4.35	30	3.99	20
3.92	10	3.69	15
3.44	10	3.34	5
3.15	10	3.13	20
3.07	10	3.05	10

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

Table 5. Solubilities of normal alkaline-earth metal salts of inosine in water

Temp. (°C)	S (g anhydrous salt/100 g water)	
	$(Inosine)_2\cdot Ca\cdot 2H_2O$	$(Inosine)_2\cdot Sr\cdot 2H_2O$
10	0.364	0.430
30	0.458	0.590
40	—	0.664
50	0.530	—
60	—	0.801

Table 5 shows the solubilities of these two normal hemi-alkaline-earth-metal salts of inosine in water. Each logarithm of the solubility is linear to the temperature; they are expressed as follows:

$$\text{Ca-salt} : \log S = 0.00406\cdot t - 0.4725$$

$$\text{Sr-salt} : \log S = 0.00551\cdot t - 0.4116$$

where S is the gram weight of anhydrous salt per 100 g of water. The solubility-behavior of these two salts is similar. They are less soluble than free inosine.[1]

The two phase diagrams plotted with the data in Tables 1 and 2 are roughly similar figures in spite of the difference in the salt-formation. This is confirmed when they are superimposed with the same co-ordinates. The inosine-contents in solution give nearly equal minima when the mole ratio of inosine and alkaline-earth metal is 2 : 1. They both gradually increase with an increase in the alkaline-earth metal hydroxide content from that point. They both also show a steep descent with an increase in the alkaline-earth metal hydroxide content from a point where the inosine and alkaline-earth metal salts of inosine share the solid phase. The solubilities of calcium- and strontium-hydroxide in water increase at the same slope with an increase in the addition of inosine.

On the other hand, there is one remarkable difference between these two systems. The solubility of inosine in water always begins to increase with an increase in the strontium hydroxide content. However, two cases are possible in the system concerning calcium hydroxide:

in the one case the addition of calcium hydroxide to a saturated aqueous solution of inosine causes an increase in the solubility of inosine, while in the other case it causes a decrease. These two cases are incompatible while the same solid phase (inosine dihydrate) is present in the system. A different solubility is acceptable when different solid phases (calcium salt of inosine, B and C in Table 1) appear in the system. Actually a decrease in the solubility is observed when calcium hydroxide is added to a saturated aqueous solution of inosine, because a decrease in the solubility produces a stable calcium salt of inosine, whereas an increase of that produces a metastable calcium salt.

Experimental

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

Analysis. The inosine was determined with UV-absorbance at 250 nm in 0.1 M HCl, using the molar extinction coefficient of 11,800 under the above conditions. The calcium was determined by EDTA-titration with a BT indicator and strontium by displacement titration with EDTA-Mg, using BT. The chlorine was checked by the Volhard method. The X-ray powder diffraction patterns (CuK_α) were used for the identification of the solid phase in equilibrium. The degree of hydration of the crystals was determined by Karl Fischer titration.

Preparation of the Crystals. *Normal Calcium Salt of Inosine (A)*: Two solutions were prepared; one was inosine (26.8 g) in 1/12 M NaOH (1.2 l), and the other, $CaCl_2 \cdot 2H_2O$ (7.35 g) in water (10 ml). The latter solution was gradually stirred into the former solution at 40 °C. After the subsequent mixture had been cooled to room temperature, the precipitated needle crystals were filtered and dried in air. Mp 265 °C (colored), 290—295 °C (decomp. with bubbling). Found: Ca, 6.78; H_2O, 5.33; Cl, 0.00%; $\varepsilon_{250\,\mathrm{nm}}$ in 0.1 M HCl, 10,370. Calcd for $(C_{10}H_{11}N_4O_5)_2 \cdot Ca \cdot 2H_2O$: Ca, 6.56; H_2O, 5.90; Cl, 0%; ε, 10,370. *Abnormal Calcium Salt of Inosine (B)*: Two solutions were prepared; one was inosine (20.1 g) in 1/6 M NaOH (300 ml), and the other, $CaCl_2 \cdot 2H_2O$ (3.68 g) in water (5 ml). The latter solution was gradually stirred into the former at 60 °C. After the mixture had been stirred for 30 min at that temperature, the precipitated crystals were filtered and dried in air. Mp 249—51 °C (decomp. with darkening). Found: Ca, 4.50; H_2O, 6.61%; $\varepsilon_{250\,\mathrm{nm}}$ in 0.1 M HCl, 10,590. Calcd for $(C_{10}H_{11}N_4O_5)_2 \cdot C_{10}H_{12}N_4O_5 \cdot Ca \cdot 3H_2O$: Ca, 4.77; H_2O, 6.03%; ε, 10,400.

Abnormal Calcium Salt of Inosine (C): Finely powdered $Ca(OH)_2$ (0.17 g) was dissolved in cold water (100 ml), and then inosine (3.4 g) was stirred into this solution at 5—10 °C. When a few fine crystals appeared, they were quickly filtered and dried in air. Mp 146 °C(began to melt), 156 °C(half-melted), and 164—167 °C(decomp. keeping white). Found: Ca, 3.22; H_2O, 12.59%: $\varepsilon_{250\,\mathrm{nm}}$ in 0.1 M HCl, 9,940. Calcd for $(C_{10}H_{11}N_4O_5)_2 \cdot (C_{10}H_{12}N_4O_5)_2 \cdot Ca \cdot 9H_2O$: Ca, 13.5; H_2O; 12.74%; ε, 9,760. *Normal Strontium Salt of Inosine* In water (500 ml), $Sr(OH)_2 \cdot 8H_2O$(21.3 g) was dissolved at 60 °C, and then powdered inosine crystals (43 g) were stirred into this solution. After the mixture had been kept for 30 min at 60 °C, the precipitated crystals were filtered and dried in air. Mp 326—8 °C(decomp. with darkening and bubbling). Found: Sr, 13.3; H_2O, 5.3%; $\varepsilon_{250\,\mathrm{nm}}$ in 0.1 M HCl, 9.680. Calcd for $(C_{10}H_{11}N_4O_5)_2 \cdot Sr \cdot 2H_2O$: Sr, 13.3; H_2O, 5.5%; ε, 9.620.

Determination of Equilibria. Definite amounts (see Tables 1 and 2) of materials selected from among inosine, its calcium salts and $Ca(OH)_2$, or inosine, its strontium salts and $Sr(OH)_2 \cdot 8H_2O$ were added to water (50 ml) in tightly-capped glass bottles. The equilibrium was achieved by tumbling the bottles for 16 hr on a water bath at 10 °C. Then, the mixtures were quickly filtered with a glass filter, and the solution and the solid were submitted to analysis.

Solubility Measurements of Normal Alkaline-earth Metal Salts of Inosine. A sufficient amount of normal calcuim- or strontium-salt of inosine was added to water in a tightly-capped glass bottle, and the bottle was tumbled for 16 hr at a constant temperature(10—60 °C). Then the mixture was quickly filtered, and the UV absorbance of the filtrate was measured. The residue was checked by X-ray analysis.

The authors wish to thank Mr. Takashi Sato for his technical assistance.

References

1) Y. Suzuki, This Bulletin, **47**, 2549 (1974).