NOTES

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The Formation of Crystalline Complexes between Inosine and Several Hydroxybenzoic Acids

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Synopsis. Inosine was found to form 1 : 1 crystalline complexes with 2,4-, 2,5-, 2,6-, 3,4-, 3,5-dihydroxybenzoic acids, and with 3,4,5-trihydroxybenzoic acid, but was found to give no crystalline complex with benzoic acid, *o*-, *m*-, *p*-hydroxybenzoic acids, 2,3-dihydroxybenzoic acid, or 2,3,4-trihydroxybenzoic acid.

Several nucleic acid bases, such as adenine and cytosine, have been known to form crystalline intermolecular complexes with benzoic acid or monohydroxybenzoic acids.[1] One of the ribonucleotides, 5′-guanylic acid, has also been known to form crystalline complexes with several hydroxybenzoic acids.[2] On the basis of these facts, ribonucleosides have also been interested to form crystalline intermolecular complexes with benzoic acid or hydroxybenzoic acids.

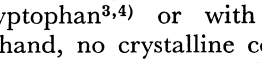
The present paper will deal with a study of the complex-formation of inosine or guanosine with several hydroxybenzoic acids. Inosine has been known to be a good counterpart for complex-formation with other organic substances, since only inosine, from among several ribonucleosides, has been demonstrated to form a 1 : 1 stable crystalline intermolecular complex with L-tryptophan[3,4] or with D-tryptophan.[3] On the other hand, no crystalline complex has ever been known between guanosine and other organic compounds, whereas interaction in the solution has been reported.[4]

An equimolar aqueous solution of the benzoic acid and the nucleoside was evaporated to dryness, and the resultant solid was examined by means of the X-ray powder diffraction method.

The results obtained are shown in Table 1. They are of interest, but not fully convincing. No acid—benzoic acid, *o*-, *m*-, or *p*-monohydroxybenzoic acid—showed any evidence of the complex-formation in the crystalline solid with inosine giving a mixture of the two components, whereas all of these benzoic acids are known to give crystalline complexes with adenine or cytosine.[1] The five dihydroxybenzoic acids (2,4-, 2,5-, 2,6-, 3,4-, and 3,5-), but not the 2,3-isomer and 3,4,5-trihydroxybenzoic acid, have been associated with inosine to give the corresponding crystalline complexes as is summarized in Tables 2 and 3, while 2,3,4-trihydroxybenzoic acid has not.

TABLE 1. THE FORMATION OF THE CRYSTALLINE INTERMOLECULAR COMPLEXES BETWEEN INOSINE AND SEVERAL HYDROXYBENZOIC ACIDS

Benzoic acid	−
o-Monohydroxybenzoic acid	−
m-Monohydroxybenzoic acid	−
p-Monohydroxybenzoic acid	−
2,3-Dihydroxybenzoic acid	−
2,4-Dihydroxybenzoic acid	+
2,5-Dihydroxybenzoic acid	+
2,6-Dihydroxybenzoic acid	+
3,4-Dihydroxybenzoic acid	+
3,5-Dihydroxybenzoic acid	+
2,3,4-Trihydroxybenzoic acid	−
3,4,5-Trihydroxybenzoic acid	+

The ionization constants of the benzoic acids are not available to aid in interpreting the specific capability of complex-formation between inosine and the selected benzoic acids. For example, the ionization constant of 2,3-dihydroxybenzoic acid (1.14×10^{-3})[5] exhibits no significant difference from those of the 2,4-isomer (5.16×10^{-4}),[5] the 2,5-isomer (1.3×10^{-3}),[5] and the 2,6-isomer (6×10^{-2}).[5] The information obtained in this study was that the phenolic hydroxyl groups of more than two in the benzoic acid may be important in forming crystalline complexes, and that such an arrangement in the structure as  may prevent the complex-formation with inosine. This information suggests that the hydrogen bonding may play an important role in the formation of the present crystalline molecular complexes.

The large crystals of the complexes were obtained from a dilute equimolar aqueous solution of the two components. The stoichiometry in the complexes was 1 : 1 without exception, while the number of the water of crystallization varied case by case between zero and three moles per mole of inosine (Table 2). Table 3 shows the X-ray powder diffraction data of the new complexes obtained in the present study.

Guanosine failed to form any crystalline molecular complex with any of those benzoic acids to give a mixtures of the two components. The characteristic properties of inosine to form a crystalline complex with several hydroxybenzoic acids were thus demonstrated in this study.

Experimental

Materials. The nucleosides and the benzoic acids were obtained from the Ajinomoto Co., Inc., and the Tokyo Kasei Co., respectively.

Examination of the Complex-Formation. An equimolar amount of inosine (or guanosine) and one of the benzoic acids were dissolved together in water, and the solution was evaporated to dryness in a desiccator. The resultant

Table 2. The analytical results of the crystalline intermolecular complexes between inosine and several hydroxybenzoic acids

Complex	Mp (°C)	Component (%)			Elementary analysis (%)		
		I[1]	HBA[2]	W[3]	C	H	N
Found Inosine·2,4-DihydroxyBA[4]	187—189	65.4	36.6	0.2	47.93	4.33	13.34
Calcd for $C_{10}H_{12}N_4O_5 \cdot C_7H_6O_4$		63.5	36.5	0.0	48.34	4.30	13.27
Found Inosine·2,5-DihydroxyBA	86—90	57.9	35.0	8.2	44.61	4.92	12.19
Calcd for $C_{10}H_{12}N_4O_5 \cdot C_7H_6O_4 \cdot 2H_2O$		58.5	33.6	7.9	44.54	4.84	12.22
Found Inosine·2,6-DihydroxyBA	81—83	57.3	34.5	10.0	44.02	5.32	11.85
Calcd for $C_{10}H_{12}N_4O_5 \cdot C_7H_6O_4 \cdot 2.5H_2O$		57.5	32.9	9.6	43.68	4.96	11.98
Found Inosine·3,4-DihydroxyBA	189—193	58.8	33.2	8.3	44.46	4.83	11.80
Calcd for $C_{10}H_{12}N_4O_5 \cdot C_7H_6O_4 \cdot 2H_2O$		58.5	33.6	7.9	44.54	4.84	12.22
Found Inosine·3,5-DihydroxyBA	110—113	56.6	31.6	11.4	42.92	5.22	11.49
Calcd for $C_{10}H_{12}N_4O_5 \cdot C_7H_6O_4 \cdot 3H_2O$		56.3	32.4	11.3	42.86	5.08	11.76
Found Inosine·3,4,5-TrihydroxyBA	163—165	58.1	34.7	7.4	42.98	5.02	11.94
Calcd for $C_{10}H_{12}N_4O_5 \cdot C_7H_6O_5 \cdot 2H_2O$		56.5	35.9	7.6	43.04	4.67	11.81

1) I: Inosine. 2) HBA: Hydroxybenzoic acid. 3) W: Water. 4) BA: Benzoic acid.

Table 3. The x-ray powder diffraction data of several inosine-hydroxybenzoic acid complexes

1[a]		2[b]		3[c]		4[d]		5[e]		6[f]	
d (Å)	I/I_0	d (Å)	I/I_0	d (Å)	I/I_0	d (Å)	I/I_0	d (Å)	I/I_0	d (Å)	I/I_0
5.83	70	13.00	15	10.53	80	8.59	15	10.78	50	10.05	40
5.87	30	10.53	60	8.67	70	8.04	20	9.72	40	9.61	30
5.34	20	8.67	80	6.56	30	7.44	20	6.46	100	6.81	20
4.77	100	6.97	40	6.46	30	7.95	50	4.65	20	6.37	40
4.17	20	6.51	30	5.79	50	5.72	50	3.97	20	5.40	30
3.87	30	5.68	100	5.64	90	4.98	30	3.43	20	4.77	50
3.62	30	5.28	30	4.87	30	4.72	40	3.34	20	4.48	30
3.53	50	4.87	30	4.82	30	4.23	40	3.23	20	4.40	50
		4.80	30	3.27	100	4.13	40	3.19	15	3.63	100
		4.35	40	3.23	70	3.44	100	3.15	15	3.49	70
		3.27	70								
		3.21	70								

The scale of intensity (I/I_0) is so chosen as to make the most intense line have the value 100.
a) Inosine—2,4-Dihydroxybenzoic acid Complex (anhydrate). b) Inosine—2,5-Dihydroxybenzoic acid Complex (2 hydrate). c) Inosine—2,6-Dihydroxybenzoic acid Complex (2.5 hydrate). d) Inosine—3,4-Dihydroxybenzoic acid Complex (2 hydrate). e) Inosine—3,5-Dihydroxybenzoic acid Complex (3 hydrate). f) Inosine—3,4,5-Trihydroxybenzoic acid Complex (2 hydrate).

solids were examined by means of the X-ray powder diffraction method, and their data were compared with those of mechanical mixtures of the two components.

The Crystallization of the Complexes. Inosine (13.4 g) was dissolved in water (160 ml) with heating, and then one of the hydroxybenzoic acids (0.05 M) was stirred, drop by drop, into this solution at 40 °C over a 2-hr period. After the mixture had then been cooled to 0—5 °C for 3 hr, the crystals precipitated were filtered and analyzed.

Analysis. Paper chromatography was carried out on Toyo-Roshi No. 51A, with the *n*-butanol—acetic acid—water (4 : 1 : 1) solvent system, and the components of the crystals were determined by comparing the UV absorption at the maximum wavelength with the standard references. The water of crystallization was determined by Karl-Fischer titration.

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