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The Formation of Crystalline Molecular Complexes between Inosine and Several Phenols

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Synopsis. Inosine has been found to form 1:1 crystalline molecular complexes with phenol, *p*-nitrophenol, and 1,2,3-trihydroxybenzene respectively, and to be inactive with *o*-, *m*-nitrophenol, 1,2,4-, 1,3,5-benzenetriol, benzenediols, aminophenols, and cresols.

Since the formation of a crystalline molecular complex between inosine and tryptophan was shown for the first time between 1968 and 1970 by Suzuki *et al.*,[1,2] inosine is known to have a strong ability of complex-formation among several ribonucleosides. The formation of a specific crystalline molecular complex is useful for industrial isolation processes. It is also known that flavins with indoles[3] including tryptophan form molecular complexes similar to the flavin-phenol complexes.[4] Indoles are considered to be poorer electron donors to flavins than are phenols. These facts suggested the probability of some crystalline complex-formation of inosine with phenols. This paper will deal with the formation of molecular complexes between inosine and several phenols, which are of interest in relation to the above background.

Results and Discussion

Table 1 shows the results of the crystalline complex formation between inosine and several phenols. Phenol, *p*-nitrophenol, and 1,2,3-benzenetriol (pyrogallol) were confirmed to form 1:1 complexes with inosine. Table 2 shows their X-ray powder diffraction data.

The inosine-*p*-nitrophenol complex is easily obtained from an equimolecular aqueous solution of inosine and *p*-nitrophenol. However, both inosine–phenol and inosine–pyrogallol complexes are incongruent and could not be obtained in a pure form from an equimolecular aqueous solution of the two components, from which the crystals of inosine were usually precipitated with or without those of the complexes. The crystallization of the complex without any contamination of the inosine crystals was performed from an aqueous solution which contained 1 mol of inosine and 2 mol (or more) of phenol, or 1 mol of inosine and 4 mol (or more) of pyrogallol, respectively.

Table 1. The crystalline complex formation between inosine and several phenol compounds

Phenol	+
o-Nitrophenol	—
m-Nitrophenol	—
p-Nitrophenol	+
o-Aminophenol	—
m-Aminophenol	—
p-Aminophenol	—
o-Cresol	—
m-Cresol	—
p-Cresol	—
o-Benzenediol	—
m-Benzenediol	—
p-Benzenediol	—
1,2,3-Benzenetriol (Pyrogallol)	+
1,2,4-Benzenetriol	—
1,3,5-Benzenetriol	—

Table 2. The X-ray powder diffraction data of several inosine–phenol complexes

Inosine–phenol complex		Inosine–*p*-nitrophenol complex		Inosine–pyrogallol complex	
d(Å)	I/I_0[a]	d(Å)	I/I_0[a]	d(Å)	I/I_0[a]
7.73	20	7.83	10	7.83	30
6.66	70	6.92	10	6.97	10
5.70	20	5.87	10	6.37	20
5.59	20	5.50	30	5.87	20
5.45	40	4.90	20	5.19	20
4.87	50	4.29	10	4.93	10
3.44	50	3.75	10	4.31	20
3.33	100	3.62	20	4.02	20
3.29	30	3.44	100	3.92	100
3.02	50	3.28	30	3.40	40
				3.33	30

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

Inosine is known to crystallize with such solvents of crystallization as water,[5] pyridine,[6] *N*,*N*-dimethylformamide,[6] and dimethyl sulfoxide.[7] Among the several phenols investigated in the present study, only phenol is liquid at room temperature and can be used as a solvent of inosine. The complex between inosine and phenol was also obtained from a pure phenol solution of inosine as well as from the aqueous phenol solution described above. Considering these facts, the phenol in the complex may be interpreted as a solvent of crystallization.

The presence of the specific interactions of inosine with *p*-nitrophenol and phenol in the present study, and the absence of that with *o*-nitrophenol, are of interest in view of the facts that *p*-nitrophenyl phosphate and phenyl phosphate were good donors, while *o*-nitrophenyl phosphate was not, in the phosphorylation of inosine by the bacterial nucleoside phosphotransferase.[8,9]

The information now available to us is insufficient to explain why these three phenols form crystalline complexes with inosine from among many other phenol

compounds. The ionization constants of phenol (1.3×10^{-10})[10] and pyrogallol (9.68×10^{-10})[11] are of the same order as those of unreacted phenols, such as 1,3,5-benzenetriol (4.5×10^{-10}),[10] *o*-aminophenol (2.18×10^{-10})[12] and benzenediol (1.1—3.6×10^{-10}).[10] *p*-Nitrophenol has an ionization constant (6.5×10^{-8})[13] similar to those of the *o*-isomer (7.5×10^{-8})[13] and *m*-isomer (1.0×10^{-8}).[13] These facts suggest that acid-base interaction makes little contribution to these molecular complex-formations. The effect of hydrogen bonding or sterical arrangement may be proposed.

Experimental

Examination of the Complex-Formation. Inosine and one of the phenols were dissolved (in a molar ratio of 1:1) into water, and the mixture was evaporated to dryness in a desiccator. The resultant solids were examined with the X-ray (CuK_α) powder diffraction method. When their data did not coincide with those of the mechanical mixture of the two components, the complex may be formed; further confirmation was done.

The Crystallization of the Complexes. *Inosine–Phenol Complex*: Inosine (3.3 g) was dissolved in a mixture of water (6 ml) and 90% aqueous phenol (15 ml) with heating. When the resultant solution was cooled to room temperature, prism crystals were precipitated. They were filtered and analyzed.

Inosine–p-Nitrophenol Complex: Inosine (6.7 g) was dissolved in water (80 ml) with heating, and then *p*-nitrophenol (3.25 g) was stirred into this solution at 40 °C over a 2-hr period. The crystals thus precipitated were filtered and analyzed.

Inosine–Pyrogallol Complex: Inosine (13.4 g) was dissolved in water (160 ml) with heating, and then pyrogallol (25.2 g) was added to this solution. The resultant mixture was stirred for 2 hr at 5 °C. The crystals thus precipitated were filtered and analyzed.

Paper Chromatography. The components of the crystals were determined by paper chromatography on Toyo-Roshi No. 51A, with the *n*-butanol–acetic acid–water (4:1:1) solvent system. The UV absorbance of the spots was determined at the maximum wavelength compared with that of the standard references.

Analysis. *Inosine–Phenol Complex*: Mp 143—145 °C. Found: C, 53.02; H, 5.07; N, 15.28%. Calcd for $C_{10}H_{12}N_4O_5\cdot C_6H_6O$: C, 53.03; H, 5.01; N, 15.46%.

Inosine–p-Nitrophenol Complex: Mp 189—191 °C. Found: C, 47.18; H, 4.21; N, 17.15; Inosine, 66.3; *p*-Nitrophenol, 33.4%. Calcd for $C_{10}H_{12}N_4O_5\cdot C_6H_5NO_3$: C, 46.91; H, 4.31; N, 17.19; Inosine, 65.9; *p*-Nitrophenol, 34.1%.

Inosine–Pyrogallol Complex: Mp 167—171 °C. Found: C, 51.22; H, 4.58; N, 14.33; Inosine, 70.4; Pyrogallol, 30.4%. Calcd for $C_{10}H_{12}N_4O_5\cdot C_6H_6O_3$: C, 51.80; H, 4.60; N, 14.21; Inosine, 68.1; Pyrogallol, 31.9%.

References

1) Y. Suzuki, T. Hirahara, and T. Nakamura, Japanese Pat. 43-29010 (1968).
2) Y. Suzuki, T. Hirahara, T. Akashi, and T. Nakamura, U.S. Pat. 3532684 (1970).
3) J. F. Pereira and G. Tollin, *Biochim. Biophys. Acta.* **143**, 79 (1967).
4) D. E. Fleischman and G. Tollin, *Proc. Natl. Acad. Sci. U.S.*, **53**, 237 (1965).
5) C. E. Bugg, U. T. Thewalt and R. E. Marsh,, *Biochem. Biophys. Res. Commun.*, **33**, 436 (1968).
6) Y. Suzuki, K. Hara, and T. Hirahara, Japanese Pat. 46-18758 (1971).
7) Y. Suzuki and K. Hara, This Bulletin, **47**, 2551 (1974).
8) K. Mitsugi, *Agr. Biol. Chem.*, **28**, 659 (1964).
9) K. Mitsugi, A. Kamimura, M. Kimura, and S. Okumura, *ibid.*, **29**, 1109 (1965).
10) "Kagakudaijiten," Vol. 7, Kyoritsu Shuppan, Tokyo (1960), p. 727.
11) C. T. Abichandani and S. K. K. Jatkar, *J. Indian Inst. Sci.*, **21A**, 417 (1938).
12) "Kagakudaijiten," Vol. 1, Kyoritsu Shuppan, Tokyo (1960), p. 290.
13) "Kagakudaijiten," Vol. 6, Kyoritsu Shuppan, Tokyo (1960), p. 788.