A Study of the Molecular Compound between Inosine and Tryptophan

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Equilibria have been determined at 30 °C for the inosine–L-tryptophan–water and inosine–D-tryptophan–water systems. They show the formation of a molecular compound, which was demonstrated in U.S. Patent 3532684, over a wide range of variable ratios of the two components, inosine and tryptophan. The influence of some organic solvents on the formation of the molecular compound was examined, and it was shown that the water molecule is significantly involved in the formation of the crystalline molecular compound, and that solvent interactions play an important role. Similar crystalline molecular compounds were not formed by chemically closely related substances; the high specificity of the interaction between inosine and tryptophan was confirmed.

In 1968 it was demonstrated for the first time by two of the present authors and by others in a Japanese patent[1] that equimolecular amounts of inosine and tryptophan form a crystalline molecular compound which is sparingly soluble in water. It has also been pointed out in several subsequent patents[2,3] that the molecular compound of L-tryptophan with inosine is less soluble than that of D-tryptophan, and that DL-tryptophan is optically resolved by conversion to the molecular compound. It was also demonstrated in a Japanese patent of 1973[4] that the biological production of L-tryptophan is greatly increased by the addition of inosine to the reaction system, probably because the tryptophan produced in the solution is continuously removed, thus forming a sparingly soluble molecular compound with inosine. These facts suggest a specific strong interaction between inosine and tryptophan in an aqueous solution.

L-Tryptophan is known to show a molecular interaction in an aqueous solution with some purine ribonucleosides, such as adenosine, guanosine, cytidine, uridine, and thymidine.[5–7] However, none of them have ever given any stable crystalline molecular compound. In frozen aqueous solutions, some of these purine ribonucleosides form aggregates[8,9] with tryptophan; these aggregates are supposed to be 1 : 1 complexes, even though there is no complex formation at room temperature.[10] Recently, the equilibrium constants and the thermodynamic parameters for the complex between inosine and L-tryptophan have been reported by Ibáñez *et al.*[11]

This paper will describe the equilibria for the inosine–L-tryptophan–water and inosine–D-tryptophan–water systems, the importance of the water of crystallization in the formation of the crystalline molecular compound, and attempts to prepare an analogous crystalline molecular compound.

Results and Discussion

Tables 1 and 2 show the composition data of the equilibria at 30 °C for the inosine–L-tryptophan–water and inosine–D-tryptophan–water systems respectively. They show experimental errors within ±0.04% and ±0.02% (absolute value) for inosine and tryptophan respectively. The composition data of the solution in the equilibria for these two systems are illustrated in Fig. 1 (the isothermal solubility curves), which clearly demonstrates the formation of the molecular compound over a wide range of the two components, inosine and tryptophan. The molecular compound is readily formed, with a steep decrease in the solubility when a small amount of one of the components is added to the other component in the solution.

A small difference is observed between the solubility curves of inosine related to L-tryptophan and that related to D-tryptophan, as is shown in Fig. 1. This is significant for the resolution of the racemic compound of tryptophan. Information has already been given in the patents[3] that the molecular compound of inosine

TABLE 1. COMPOSITION DATA OF THE EQUILIBRIUM FOR THE INOSINE–L-TRYPTOPHAN–WATER SYSTEM AT 30 °C

Run No.	Solution (Wt %)		Wet residue[c] (Wt %)		Solid phase[b]
	Ino.[a]	L-Trp	Ino.[a]	L-Trp	
1	3.10	0.00	—	—	I
2	3.07	0.02	35.8	1.31	I+L-T·I·3H_2O
3	2.99	0.05	—	—	I+L-T·I·3H_2O
4	2.22	0.02	54.6	36.2	L-T·I·3H_2O
5	1.05	0.06	55.5	35.7	L-T·I·3H_2O
6	0.27	0.24	53.8	36.5	L-T·I·3H_2O
7	0.16	1.12	50.1	41.3	L-T·I·3H_2O
8	0.07	1.43	12.3	25.0	L-T·I·3H_2O+L-T
9	0.10	1.46	33.4	60.1	L-T·I·3H_2O+L-T
10	0.00	1.42	—	—	L-T

a) Inosine. b) I: Inosine in the α-form.[13] L-T·I·3H_2O: Molecular compound, L-Trp·Inosine·3H_2O. L-T: L-Trp. c) Inosine+L-Tryptophan+Water=100%.

TABLE 2. COMPOSITION DATA OF THE EQUILIBRIUM FOR THE INOSINE–D-TRYPTOPHAN–WATER SYSTEM AT 30 °C

Run No.	Solution (Wt %)		Wet residue[c] (Wt %)		Solid phase[b]
	Ino.[a]	D-Trp	Ino.[a]	D-Trp	
1	3.10	0.00	—	—	I
11	3.24	0.07	57.6	38.1	I+D-T·I·H_2O
12	1.94	0.09	40.9	28.1	D-T·I·H_2O
13	0.49	0.27	32.0	24.4	D-T·I·H_2O
14	0.35	0.28	—	—	D-T·I·H_2O
15	0.23	0.55	53.8	41.9	D-T·I·H_2O
16	0.11	1.39	12.3	31.0	D-T·I·H_2O+D-T
10	0.00	1.42	—	—	(D-T)

a) Inosine. b) I: Inosine in the α-form.[13] D-T·I·H_2O: Molecular compound, D-Trp·Inosine·H_2O. D-T: D-Trp. c) Inosine+D-Tryptophan+Water=100%.

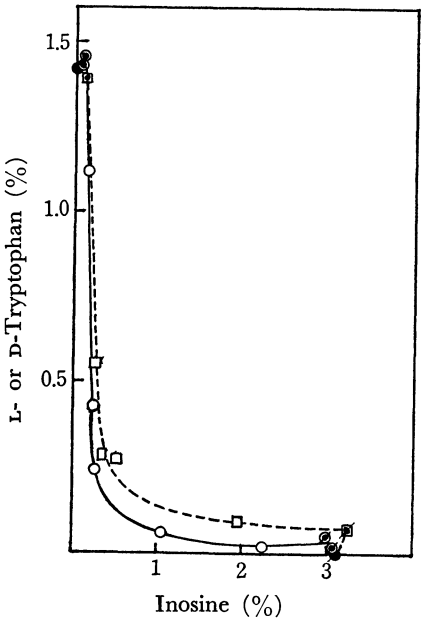


Fig. 1. An illustration of the composition of the solution phase in the equilibrium for the inosine-L-(or D-)tryptophan-water system at 30 °C.
The symbol marks of the solid phase are as follows:
○: L-Trp·Inosine·$3H_2O$ (molecular compound).
□: D-Trp·Inosine·H_2O (molecular compound).
●: L-Trp or D-Trp.
: Inosine (α-form).

with L-tryptophan is less soluble than that with D-tryptophan. The solubility difference of the curves in Fig. 1 is confirmed to be consistent with the above information over a wide region, even where the two components are not equimolar. Therefore, there is a possibility that the resolution of racemic tryptophan may be performed even when the amount of inosine is not equimolar.

It is known that racemic tryptophan is far less soluble than optically active ones. However, by the addition of a certain amount of inosine to the solution of racemic tryptophan, molecular compounds between inosine and optically active tryptophans were formed and precipitated because their solubilities are lower than the solubility of racemic tryptophan. The molecular compound of inosine with L-tryptophan was at first precipitated, leaving that of inosine with D-tryptophan in the solution.

It has been demonstrated[1,3] that the crystals of the molecular compound between inosine and L-tryptophan contain three molecules of water of crystallization, and that they lose two of them on heating in air, thus giving the monohydrate of the molecular compound. The experimental conditions used to obtain monohydrate from trihydrate were, for example, at 50 °C for 2 hr in air or at 110 °C for 40 min *in vacuo*.

It is found that the crystals of monohydrate thus obtained absorb moisture in air rapidly at first and gradually at the end, returning to original trihydrate in a few days. This process can be followed quantitatively by means of the X-ray powder diffraction spectra. Two strong peaks corresponding to the spacings of 7.63 Å and 6.33 Å are specific for the trihydrate, and two other strong peaks corresponding to the spacings of 7.25 Å and 6.92 Å are specific for the monohydrate. The change in the intensity of these four specific peaks reflects an increase and a decrease of these two forms in the crystals. However, when trihydrate crystals were very rapidly heated in air, it sometimes occurred that the X-ray diffraction spectra of trihydrate were unchanged even though the weight of the crystals was decreased by an amount as much as would correspond to two moles of water. This fact suggests that monohydrate crystals are sometimes able to retain the structure of original trihydrate crystals. Therefore, the two moles of water are likely to be nearly zeolitic water.

On the other hand, the crystals of the molecular compound between inosine and D-tryptophan were always obtained in monohydrate, and never in trihydrate. It is supposed that one mole of water in both molecular compounds is significantly involved in the formation of the molecular compound.

Trihydrate crystals of the molecular compound between inosine and L-tryptophan can be dried into anhydrous crystals by heating at 140 °C for 30 min *in vacuo*. The anhydrous crystals thus obtained also increased in weight because of the absorption of moisture when they were left in air. However, it was found that they never returned to being original hydrous crystals in air. Rather, they became amorphous.

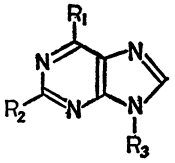
The fact that one mole of water may play an important role in the formation of the crystalline molecular compound suggests the presence of solvent interaction in the intermolecular bonding between inosine and tryptophan. We examined the influence of several organic solvents on the trihydrate crystals of the molecular compound between inosine and L-tryptophan. Their crystals were practically insoluble; they remained unchanged in acetone, dioxane, tetrahydrofuran, and 1-butanol, while they were somewhat changed in appearance in methanol, ethanol, and 1-propanol. It was also confirmed by the X-ray method that the crystals of the trihydrate were partly changed into those of monohydrate in 85—95% (v/v) aqueous ethanol or aqueous 1-propanol, and that the crystals of L-tryptophan were found in the residue in addition to those of monohydrate in pure ethanol or pure 1-propanol. As was established in the patents,[3] the crystals added in methanol were changed into those of inosine. This last observation can be explained by the dissolution of the molecular compound, followed by the crystallization of inosine (leaving tryptophan in methanol).

It has been reported that the presence of ethanol destablizes the flavin–indole complexes.[12] The aggregates, which are supposed to be molecular complexes between nucleic acid derivatives and aromatic amino acids, in frozen aqueous solutions have been reported to be inhibited by the addition of organic solvents such as ethanol.[10] These destabilizing effects of the organic solvents on the molecular compounds demonstrate that solvent interactions play an important role in the formation of the molecular compound, especially in the present case when a water molecule is necessary to form the stable crystals.

Arcaya *et al.*[7] reported the interaction of L-aromatic amino acids including tryptophan with some ribonucleosides, and proposed a possible ring-ring-type interaction between an amino acid and nucleoside. This interaction is also possible in the present crystalline molecular compound between the imidazole ring in tryptophan and the purine ring in inosine. The fine hair-like appearance of the crystals of the molecular compound in this study suggests ring-ring vertical stacks with an alternative sequence of inosine and tryptophan. The growth of the linear vertical stacks would make for a long, slender crystal.

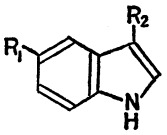
Besides such a ring-ring-type interaction based on the hydrophobic moiety in the molecule, the role of the hydrophilic moiety, such as the ribosyl or hydroxyl groups in the inosine molecule and the side-chain group in the tryptophan molecule, can never be disregarded in the formation of the crystalline molecular compound. Aiming to get some information about the role of such side chains, some compounds analogous to inosine and tryptophan were examined, and we attempted to make crystalline molecular compounds between them.

Several of the ribonucleosides illustrated in Fig. 2 were examined for interaction with L-tryptophan (**13**). Adenosine (**2**), guanosine (**3**), xantosine (**4**), hypoxanthine (**5**), and 9-β-D-ribopyranosyl hypoxanthine (**6**) all did not give any crystalline molecular compound



	R_1	R_2	R_3
1.	OH	H	β-D-Ribofuranose
2.	NH_2	H	β-D-Ribofuranose
3.	OH	NH_2	β-D-Ribofuranose
4.	OH	OH	β-D-Ribofuranose
5.	OH	H	H
6.	OH	H	β-D-Ribopyranose

Fig. 2. Purine ribonucleosides examined.



	R_1	R_2
7.	H	H
8.	COOH	H
9.	H	COOH
10.	H	CH_2COOH
11.	H	CH_2CH_2COOH
12.	H	$CH_2CH_2CH_2COOH$
13.	H	$CH_2CHCOOH$ NH_2
14.	H	$CH_2CHCOOH$ $NHCOCH_3$
15.	H	$CH_2CH_2NH_2$
16.	H	CH_2CH_2OH

Fig. 3. Indole derivatives examined.

except crystalline mixtures of two original components. It is of interest that the isomerization of the ribose moiety in inosine from the furanosyl form to the pyranosyl form inhibited the formation of the molecular compound. Some nucleosides, such as cytidine, uridine, and 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide, also gave negative results. Several indole derivatives illustrated in Fig. 3 were examined with inosine (**1**). Indole (**7**), indole-5-carboxylic acid (**8**), indole-β-carboxylic acid (**9**), β-indolyl acetic acid (**10**), β-indolyl propionic acid (**11**), β-indolyl butyric acid (**12**), acetyl-L-tryptophan (**14**), tryptamine (**15**), and tryptophol (**16**) all failed to give any crystalline molecular compound. It was thus confirmed that the interaction between inosine and tryptophan is highly specific.

Experimental

Materials and Equipment. The inosine (α-form),[13] guanosine, adenosine, 5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide, hypoxanthine, L-tryptophan, and acetyl-L-tryptophan were obtained from the Ajinomoto Co., Inc. The 9-β-D-ribopyranosyl hypoxanthine was prepared according to the method of a previous paper.[14] The D-tryptophan was presented by Dr. Takekazu Akashi of the Ajinomoto Co., Inc. The other materials were commercially purchased. The X-ray (Cu*Kα*) powder diffraction data for the determination of crystal forms were obtained by means of a Rigaku-denki diffractometer.

Preparation of the Crystals of the Molecular Compounds. (1) The trihydrate crystals of the molecular compound between inosine and L-tryptophan were prepared according to the patents.[1] (2) The monohydrate crystals of the molecular compound between inosine and D-tryptophan were prepared by evaporating an equimolar aqueous solution of the two components to dryness *in vacuo*. The analytical data were coincident with those in the patents.[3]

Determination of Equilibria. *Procedure*: The equilibria were approached by dissolving the crystals in water at 30± 0.5 °C. The details in Table 1 show the results obtained by dissolving inosine (Run No. 1), by dissolving L-tryptophan (Run No. 10), by dissolving both inosine and L-tryptophan (Runs No. 3, 4, 5 and 7), by dissolving the molecular compound trihydrate of inosine with L-tryptophan (Run No. 6), by dissolving both inosine and the molecular compound trihydrate (Run No. 2), and by dissolving both L-tryptophan and the molecular compound trihydrate (Runs No. 8 and 9). The data in Table 2 show the results obtained by dissolving both inosine and D-tryptophan (Runs No. 13, 15 and 16), and by dissolving the molecular compound monohydrate of inosine with D-tryptophan in water (Run No. 14) or that in aqueous inosine solution (Run No. 12). It took 16 hr for the equilibrium to be reached by tumbling the bottles containing the mixture on a water bath at a constant temperature. The mixture in the bottles was quickly filtered in a chamber kept at 30 °C with a glass filter. The wet residues and the filtrates were sampled by weight, diluted to appropriate volumes, and submitted to analysis. *Analysis*: The composition data were determined by the measurement of the UV absorption of the samples in 0.1 M HCl at 250 nm and at 280 nm, which are the specific wavelengths giving the maximum absorption for inosine and tryptophan respectively. The UV absorbances of the two materials, inosine and tryptophan, had previously been measured under our experimental conditions; the molar extinction coefficients were obtained for inosine at 250 nm ($\varepsilon_{I\ 250}$, 11700) and at

280 nm ($\varepsilon_{I\ 280}$, 2450), and for tryptophan at 250 nm ($\varepsilon_{T\ 250}$, 1390) and at 280 nm ($\varepsilon_{T\ 280}$, 5620). The analytical data of the mixture of inosine and tryptophan were obtained by the use of the following two equations:

$$A_{250} = \varepsilon_{I\ 250}\frac{C_I \times 10^{-3}}{MW_I} + \varepsilon_{T\ 250}\frac{C_T \times 10^{-3}}{MW_T}$$

$$A_{280} = \varepsilon_{I\ 280}\frac{C_I \times 10^{-3}}{MW_I} + \varepsilon_{T\ 280}\frac{C_T \times 10^{-3}}{MW_T}$$

where A_{250} is the UV absorbance at 250 nm, A_{280} is that at 280 nm, C_I(mg/l) is the concentration of inosine, C_T(mg/l) is that of tryptophan, MW_I is the molecular weight of inosine (268), and MW_T is that of tryptophan (204). C_I and C_T were thus determined by the measurement of A_{250} and A_{280}. The confirmation of the crystalline form in the residues was performed by means of the X-ray powder diffraction method.[3]

Behavior of the Molecular Compound in Several Organic Solvents. Finely powdered trihydrate crystals of the molecular compound between inosine and L-tryptophan (0.5 g) were added to one of several organic solvents (10 ml). After the mixtures had been vigorously shaken, the undissolved crystals were observed with a microscope. Some of them were examined also by means of the X-ray method in order to determine the crystal form.

Attempts at the Preparation of the Analogous Crystalline Molecular Compound. One of the nucleosides and L-tryptophan, or one of the indole derivatives and inosine, were dissolved (in a molar ratio of 1 : 1) in water. Then the mixture was evaporated to dryness *in vacuo* in a desiccator. The resultant solid was examined by means of X-ray diffraction. When the solid is the mixture of the two original components, its spectra may be consistent with those of a mechanical mixture of them. If any molecular compound is present in the solid, though, its spectra may show novel and specific peaks.

References

1) Y. Suzuki, T. Hirahara, and T. Nakamura, Japan. Patent 43 29010 (1968).
2) Y. Suzuki, T. Akashi, and T. Nakamura, Japan. Patent 44 20983 (1969).
3) Y. Suzuki, T. Hirahara, T. Akashi, and T. Nakamura, Brit. Pat. 1180635 (1970); U.S. Pat. 3532684 (1970); West Ger. Offen. 1645977 (1970); Fr. Pat. 1601066 (1970).
4) H. Enei, H. Nakazawa, H. Matsui, S. Okumura, and H. Yamada, Japan. Patent Kokai 48 80785 (1973).
5) M. Pieber, C. Romero, C. Tirapegui, and J. C. Tohá, *Z. Naturforsh.* **24b**, 508 (1969).
6) M. Pieber, C. Romero, and J. C Tohâ, *Arch. Biol. Med. Exp.* **6**, 58 (1969).
7) G. Arcaya, M. E. Pantoja, M. Pieber, C. Romero, and J. C. Tohá, *Z. Naturforsh.* **26b**, 1026 (1971).
8) C. Helene, *Biochem. Biophys. Res. Commun.*, **22**, 237 (1966).
9) C. Helene, R. Santus, and M. Ptak, *C. R. Acad. Sci. Ser. C*, **262**, 1349 (1966).
10) T. Montenay-Garestier and C. Helene, *Nature*, **217**, 844 (1968).
11) I. Ibáñez, M. Pieber, and J. C. Toha, *Z. Naturforsh. C*, **28**, 385 (1973).
12) J. F. Perira and G. Tollin, *Biochim. Biophys. Acta*, **143**, 79 (1967).
13) Y. Suzuki and N. Nagashima, This Bulletin, **43**, 1600 (1970).
14) Y. Suzuki, *ibid.*, **47**, 898 (1974).