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# Analysis of Solubility Properties of L-Tryptophan in Aqueous Solution based on the Effects of Third Component on Optical Rotatory Dispersion and on Adsorption by Carbon Black[1,2]

HIDEAKI UMEYAMA, TSUNEJI NAGAI, and HISASHI NOGAMI

*Faculty of Pharmaceutical Sciences, University of Tokyo*[3]

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Solubility properties of L-tryptophan in aqueous solution was discussed on the basis of the effects of 3rd component on the optical rotatory dispersion (ORD) and on the adsorption by carbon black.

ORD data showed the optical rotation of L-tryptophan around 450 mμ was suitable in analyzing the effect of 3rd component on it. Adsorption data indicated the respective hydrophobicities of the present 3rd components. Alcohols and glycols were classified into three types according to the similarities in hydrophobicity and in structure.

Except for urea, formamide and dimethylsulfoxide, there was found a correlation between the ORD and the adsorption data. Alcohols were considered to give effect selectively on the indole group of L-tryptophan, while glycols to give effect also on the moiety other than the indole group. Urea and formamide might give effect on the hydrophilic moiety of L-tryptophan to result in the decrease in optical rotation, but this effect was considered to have no relation to the increase in solubility.

Analyzing the relationship between the ORD and the adsorption data, the present 3rd components were classified into three types, corresponding to the previous classification according to solubility studies. Finally, the results verified the concept regarding the contact of 3rd component with L-tryptophan to result in the increase in solubility in aqueous solution.

In previous papers,[4,5] discussing the effect of 3rd component (additive) on the water structure around tryptophan, the increase in the solubility of tryptophan in water upon addition of various organic compounds was explained on the basis of the following mechanism: such a 3rd component comes in contact with the hydrophobic moiety of tryptophan to result in a simultaneous structural change of the iceberg around that moiety, accompanying an increase of its affinity to water.

1) This paper forms part XIX of "Physico-chemical approach to Biopharmaceutical Phenomena." Preceding paper, Part XVIII: H. Umeyama, T. Nagai, H. Nogami, and T. Oguma, *Chem. Pharm. Bull.* (Tokyo), **19**, 412, (1971).
2) A part of this work is presented at the 90th Annual Meeting, of the Pharmaceutical Society of Japan Sapporo, July, 1970.
3) Location: *Hongo, Bunkyo-ku, Tokyo.*
4) H. Nogami, T. Nagai, and H. Umeyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 328 (1970).
5) H. Nogami, T. Nagai, and H. Umeyama, *Chem. Pharm. Bull.* (Tokyo), **18**, 335 (1970).

Regarding L-tryptophan, it can be considered that the electron cloud of this optically active compound is influenced by the contact of 3rd component mentioned above,[6] resulting in a change in optical rotatory dispersion (ORD).

The decrease in adsorption of L-tryptophan by carbon black from aqueous solution with addition of EtOH also was explained on the basis of the contact between EtOH and carbon black.[4,7] Therefore, it is expected that the effect of 3rd component on the ORD has relation to that on the adsorption by carbon black.

In previous papers,[8] it was demonstrated that the more hydrophobic the drug, the more it was adsorbed by carbon black from aqueous solution. Thus the adsorbability on carbon black may indicate the extent of the hydrophobicity. Although there were many experimental difficulties in obtaining the adsorption isotherms of the present 3rd components themselves by carbon black, the effect of such 3rd components on the hydrophobic interaction between L-tryptophan and carbon black surface could be evaluated by the decrease in adsorbability of L-tryptophan on carbon black,[4] as might be related to the extent of the interaction between 3rd component and carbon black or the hydrophobicity of the same 3rd component.

The present study was attempted to investigate the effect of 3rd component on ORD of L-tryptophan in aqueous solution in relation to the hydrophobicity of 3rd component evaluated by the adsorbability of L-tryptophan on carbon black from aqueous solution containing the same 3rd component, and finally to make sure of the concept regarding the contact of such a 3rd component described above.

## Experimental

**Materials**——L-Tryptophan and the materials of the purest reagent grade used as 3rd components were obtained commercially. Carbon black used was the same as described in a previous paper.[9]

**ORD Measurement**——This was done by a Jasco Model ORD/UV-5 spectropolanimeter, using a 5 cm cell at approximately 27°. The concentration of L-tryptophan was $1.64\times10^{-3}$g/ml containing 4 M of each 3rd component, except for concentration dependence studies. The absorbance was always less than 2.

**Procedure for Determination of the Amount of L-Tryptophan adsorbed by Carbon Black**——This was done in the same way as described in previous papers.[4,9]

**Quantitative Determination of L-Tryptophan**——This was done according to UV absorption method in the same way as described in previous papers,[4,9] using a Hitachi 124 spectrophotometer.

## Result and Discussion

### Effect of 3rd Component on ORD of L-Tryptophan in Aqueous Solution

The ORD curve of L-tryptophan in aqueous solution is shown in Fig. 1. Where the wavelength was longer than 500 mμ, the optical rotation was too low to give reliable data valuable in analyzing the effect of 3rd component on it.

Next, plotting the data according to the single term Drude equation, *i.e.*, $[\alpha]=K/(\lambda^2-\lambda_0^2)$ where $[\alpha]$ is the specific rotation at the wavelength $\lambda$, $\lambda_0$ the wavelength of the closest absorption maximum[10] and $K$ the constant, the plots deviated from the straight line to the lower

6) The changes in ultraviolet (UV) spectrum of L-tryptophan and in refrective index of solvent were not so remarkable with addition of 3rd component under the experimental conditions that might be unimportant as the factors contributing to the change in optical rotation.

7) Since the carbon black surface is much more hydrophobic than the hydrophobic moiety of L-tryptophan, the contact between EtOH and carbon black was considered to form the predominant factor affecting the adsorption of L-tryptophan.

8) *a*) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **17**, 168 (1969); *b*) *Idem*, *ibid.*, **17** 176 (1969); *c*) H. Nogami, T. Nagai, and S. Wada, *ibid.*, **18**, 342 (1970); *d*) *Idem*, *ibid.*, **18**, 348 (1970); *e*) H. Nogami, T. Nagai, and N. Nambu, *ibid.*, **18**, 1643 (1970).

9) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2248 (1968).

10) The wavelength 266 mμ was applied to $\lambda_0$ for the present case on the consideration of the circular dichroism curve: M. Legrand and R. Viennet, *Bull. Soc. Chim. France*, **1965**, 679.

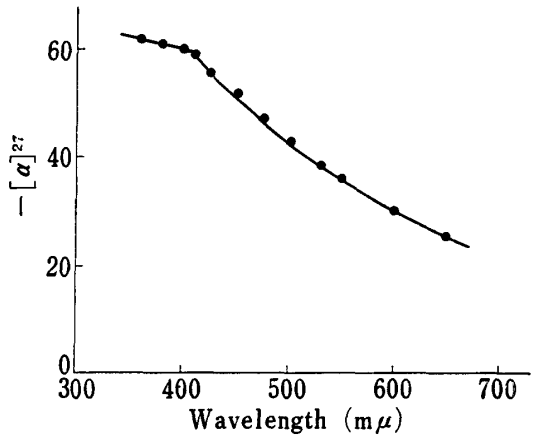


Fig. 1. ORD of L-Tryptophan in Aqueous Solution, observed in Concentration $1.64\times10^{-3}$ g/ml

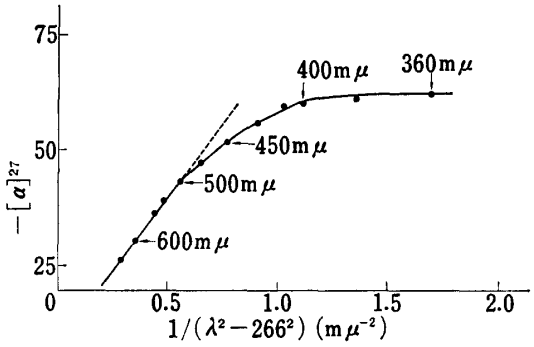


Fig. 2. Plot of Specific Rotation, $[\alpha]_{,}^{27}$ of L-Tryptophan according to the Single Term Drude Equation, where $\lambda$ is the Wavelength, observed in Concentration $1.64\times10^{-3}$ g/ml in Aqueous Solution

part in the region below 530 mμ, as shown in Fig. 2. Therefore, the single electron moving in the helicoidal surface[11] containing the indole group is considered to have relation to the optical rotation where the plot is on the straight line in Fig. 2, while the additional contribution of such electrons as included in carboxyl and amino groups to the optical rotation also seems to get remarkable with the decrease in wavelength where the plot deviates, suggesting the data in the region below a certain wavelength also are valueless in analyzing the effect of 3rd component on the optical rotation of L-tryptophan.

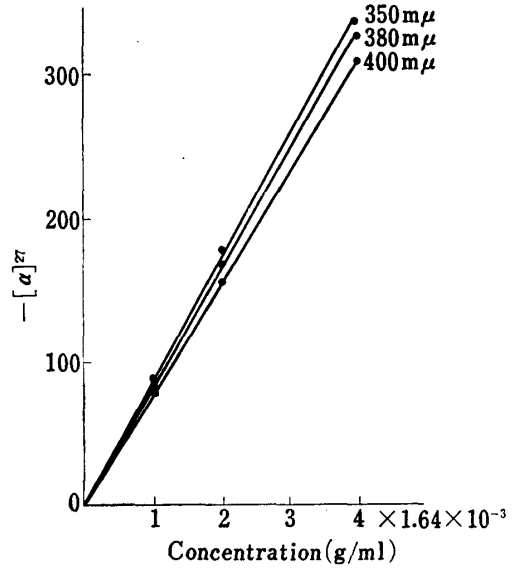


Fig. 3. Relationship between Specific Rotation and Concentration of L-Tryptophan at Different Wavelengths in Aqueous Solution Containing 4M *n*-PrOH

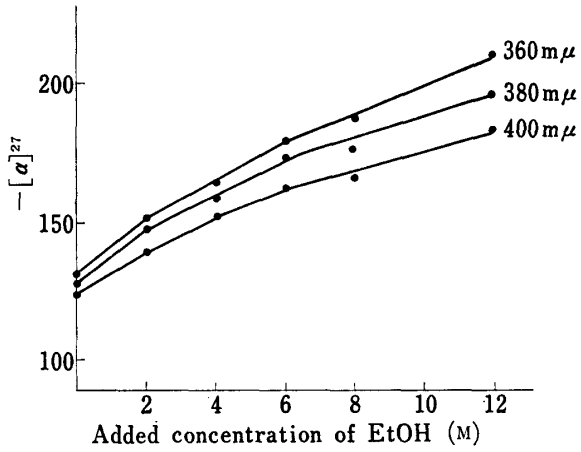


Fig. 4. Changes in Specific Rotation of $3.28\times10^{-3}$ g/ml of L-Tryptophan at Different Wavelengths with Added Concentration of EtOH in Aqueous Solution

Considering these circumstances, the optical rotation around 450 mμ seems to be suitable to the present study. Fig. 3 shows that the optical rotation was proportional to the concentration of L-tryptophan in aqueous solution containing 4M *n*-PrOH at 350, 380 and 400 mμ, in the similar way to the case containing no 3rd component. Fig. 4 shows that the optical

11) K. Imahori, "Senkōsei—Sono Riron to Ōyo," Tokyo-Kagaku-Dojin, Tokyo, 1960, p. 41.

rotation of 164 mg/50 ml of L-tryptophan increased almost linearly with the concentration of EtOH at 360, 380 and 400 mμ, and thus it was considered that EtOH gave effect on the electron in the helicoidal surface in proportional to its concentration. These concentration dependences as shown in Fig. 3 and 4 might be common to the cases of other 3rd components used in the present study.

Table I shows the ORD data of L-tryptophan in various solutions containing 4M of 3rd components listed in the same order as in Table II, which will be discussed later.

Table I. ORD[a] of L-Tryptophan in Aqueous Solution Containing 4M of 3rd Component

| No. | 3rd component | $-[\alpha]^{27}$ | | | |
|---|---|---|---|---|---|
| | | 450 mμ | 400 mμ | 380 mμ | 350 mμ |
| 1 | water | 51.8 | 59.6 | 61.0 | 59.2 |
| 2 | MeOH | 59.8 | 72.0 | 74.4 | 75.2 |
| 3 | formamide | 46.2 | 55.6 | 58.6 | 56.2 |
| 4 | ethylene glycol | 55.0 | 62.2 | 65.8 | 65.2 |
| 5 | glycerol | 58.0 | 65.0 | 69.6 | 73.2 |
| 6 | EtOH | 59.6 | 72.8 | 76.2 | 78.0 |
| 7 | dimethyl sulfoxide | 51.0 | 58.0 | 61.0 | 58.6 |
| 8 | propylene glycol | 56.4 | 66.6 | 70.0 | 71.2 |
| 9 | diethylene glycol | 53.2 | 64.0 | 66.6 | 67.8 |
| 10 | urea | 30.6 | 32.6 | 30.8 | 24.0 |
| 11 | triethylene glycol | 61.4 | 64.0 | 65.8 | — |
| 12 | methyl cellosolve | 59.8 | 71.4 | 76.4 | 81.8 |
| 13 | iso-PrOH | 58.6 | 70.2 | 75.6 | 79.8 |
| 13 | *t*-BuOH | 74.0 | 81.0 | 86.2 | 91.8 |
| 15 | dioxane | 65.8 | 75.0 | 78.0 | — |
| 16 | hexylene glycol | 63.4 | 75.6 | 82.2 | 84.2 |
| 17 | *n*-PrOH | 65.8 | 79.4 | 84.2 | 87.8 |

*a*) observed in the solution of $1.64\times10^{-3}$ g/ml of L-tryptophan

Table II. Adsorbed Amount of L-Tryptophan by Carbon Black from Aqueous Solutions of Different Equilibrium Concentrations ($C_e$) Containing 4M of 3rd Component

| No. | 3rd component | $C_e=3\times10^{-3}$M | $C_e=4\times10^{-3}$M | $C_e=5\times10^{-3}$M |
|---|---|---|---|---|
| 1 | water | 1.265 | 1.330 | 1.372 |
| 2 | MeOH | 0.862 | 0.923 | 0.965 |
| 3 | formamide | 0.737 | 0.811 | 0.867 |
| 4 | ethylene glycol | 0.743 | 0.802 | 0.847 |
| 5 | glycerol | 0.703 | 0.759 | 0.802 |
| 6 | EtOH | 0.561 | 0.631 | 0.686 |
| 7 | dimethyl sulfoxide | 0.547 | 0.613 | 0.667 |
| 8 | propylene glycol | 0.528 | 0.595 | 0.648 |
| 9 | diethylene glycol | 0.481 | 0.567 | 0.632 |
| 10 | urea | 0.471 | 0.551 | 0.622 |
| 11 | triethylene glycol | 0.365 | 0.425 | 0.472 |
| 12 | methyl cellosolve | 0.334 | 0.391 | 0.436 |
| 13 | iso-PrOH | 0.234 | 0.283 | 0.327 |
| 14 | *t*-BuOH | 0.172 | 0.235 | 0.288 |
| 15 | dioxane | 0.178 | 0.208 | 0.221 |
| 16 | hexylene glycol | 0.123 | 0.147 | 0.169 |
| 17 | *n*-PrOH | 0.124 | 0.147 | 0.163 |

**Effect of 3rd Component on Adsorption of L-Tryptophan by Carbon Black from Aqueous Solution**

Fig. 5 shows that the adsorbed amount of L-tryptophan by carbon black varied according to 3rd components. The adsorbed amounts of L-tryptophan obtained from the adsorption isotherms in Fig. 5 are listed in Table II according to the decreasing order of the data in the equilibrium concentration $4\times10^{-3}$M.[12] This decreasing order is considered to correspond to the increasing order of the hydrophobicity of 3rd components.

Although the hydrophobic property and related phenomena have been discussed, any evaluation of the hydrophobicity has never been given. From the results shown in Table II, it was recognized that urea is of almost the same hydrophobicity as diethylene glycol, propylene glycol, dimethyl sulfoxide and EtOH.

There was found an interesting fact that alcohols and glycols were classified into three according to the similarities in hydrophobicity and structure as: 1) MeOH, ethylene glycol and glycerol of low hydrophobicity—the same number of carbon as oxygen; 2) EtOH, propylene glycol and diethylene glycol of medium hydrophobicity—the number of carbon is one more than that of oxygen; 3) triethylene glycol, methyl cellosolve and iso-PrOH of high hydrophobicity—the number of carbon is two more than that of oxygen. In this connection, MeOH, ethylene glycol and glycerol in the above first type gave a similar effect on the hydrolysis rate of aspirin,[13] though these showed different viscosities in aqueous solution, and thus it was suggested the hydrolysis of aspirin might be influenced by the hydrophobicity of 3rd component.

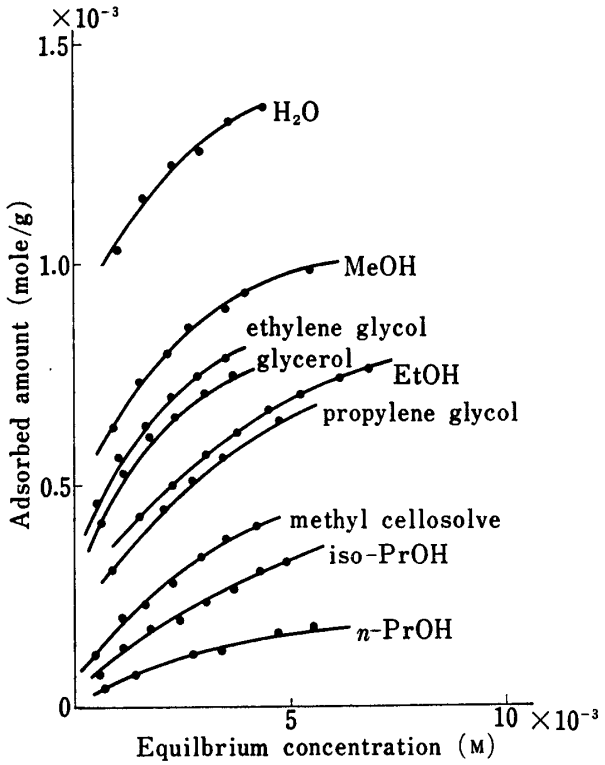


Fig. 5. Effect of 3rd Component (4M) on the Adsorption of L-Tryptophan by Carbon Black from Aqueous Solution at 30°

Formamide showed the similar hydrophobicity to MeOH. Therefore, the increase in hydrophobicity due to the substitution of $-NH_2$ for -H of formamide to give urea seemed similar to that due to the substitution of $-CH_3$ for -H of MeOH to give EtOH, because urea and EtOH showed the similar hydrophobicity.

**Relationships between the Data of ORD and Adsorption**

Fig. 6—9 show different plots of adsorption data against ORD ones, where both the data are combined at random to discuss the general correlation. Actually, it seems impossible to obtain a complete correlation between both the data, because ORD may originally be influenced more or less by various factors.

As shown in Fig. 6, a good correlation was found at 450 mμ except for the data for urea, formamide and dimethylsulfoxide, demonstrating that 3rd component gave effect on the indole group according to its hydrophobicity. The similar correlation was observed at 380, 400

12) There is no remarkable difference in the decreasing order among the data in the three different equilibrium concentrations.

13) H. Umeyama, T. Nagai, and H. Nogami, *Yakuzaigaku*, **30**, 255 (1970).

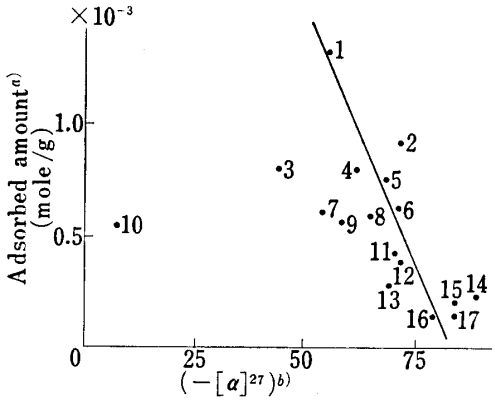


Fig. 6. Plot of the Adsorbability in Equilibrium Concentration $4\times10^{-3}$M against the Specific Rotation at 450 mμ of L-Tryptophan upon Addition of 3rd Component represented by the Same No. as in Tables I and II

*a*) at 30°
*b*) in the solution of $1.64\times10^{-3}$ g/ml of L-tryptophan containing 4M of 3rd component (except for the cases of 2M *t*-BuOH and 2M hexylene glycol)

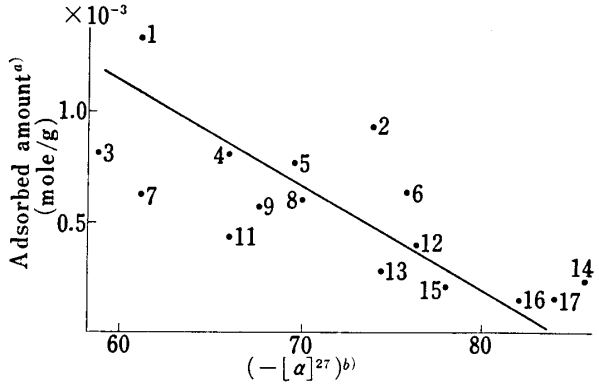


Fig. 7. Plot of the Adsorbability in Equilibrium Concentration $4\times10^{-3}$M against the Specific Rotation at 380 mμ of L-Tryptophan upon Addition of 3rd Component represented by the Same No. as in Tables I and II

*a*) at 30°
*b*) in the solution of $1.64\times10^{-3}$ g/ml of L-tryptophan containing 4M of 3rd component (except for the cases of 2M *t*-BuOH and 2M hexylene glycol)

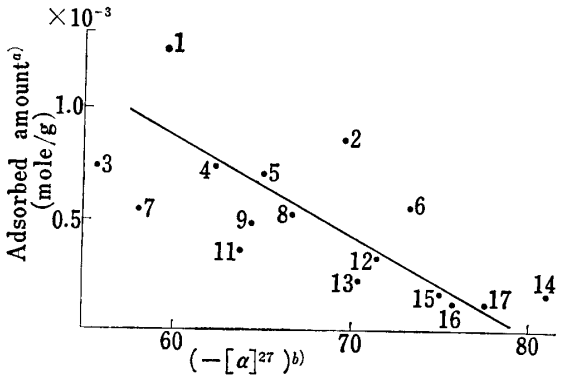


Fig. 8. Plot of the Adsorbability in Equilibrium Concentration $3\times10^{-3}$M against the Specific Rotation at 400 mμ of L-Tryptophan upon Addition of 3rd Component represented by the Same No. as in Tables I and II

*a*) at 30°
*b*) in the solution of $1.64\times10^{-3}$ g/ml of L-tryptophan containing 4M of 3rd component (except for the cases of 2M *t*-BuOH and 2M hexylene glycol)

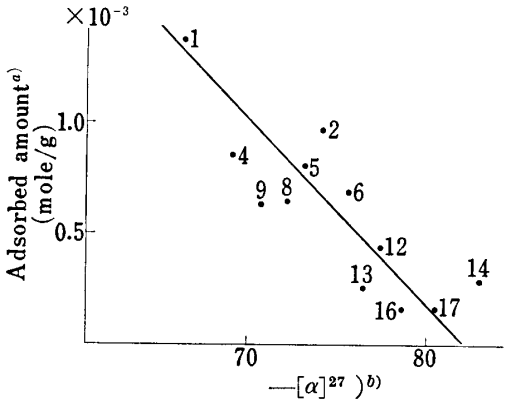


Fig. 9. Plot of the Adsorbability in Equilibrium Concentration $5\times10^{-3}$M against the Specific Rotation at 350 mμ of L-Tryptophan upon Addition of 3rd Component represented by the Same No. as in Tables I and II

*a*) at 30°
*b*) in the solution of $1.64\times10^{-3}$ g/ml of L-tryptophan containing 4M of 3rd component (except for the cases of 2M *t*-BuOH and 2M hexylene glycol)

and 350 mμ, as shown in Fig. 7—9, informing that 3rd component might give effect on the indole group also at these wavelengths.

Regarding the data for alcohols and glycols including dioxane in Fig. 7, the correlation coefficient for the compounds of $C_2$ to $C_4$ was −0.89.[14] Even if MeOH and triethylene glycol were included, the coefficient was −0.73.[14] Fitting the data to a straight line by the method of least squares, MeOH, EtOH and *n*-PrOH are in the upper part from the straight line. Therefore, these alcohols were considered to give effect selectively on the indole group. However, as glycols generally are in the lower part from the straight line, these might give effect

14) Significant by *t*-test at 5% level.

also on the moiety other than the indole group, *i.e.*, the hydrophilic moiety. The above result was not contradictory to the previous report that the effect of ethylene glycol on the hydrophilic moiety of L-tryptophan was larger than that of EtOH from the solubility studies.[5]

Urea, formamide and dimethylsulfoxide showed very low optical rotations compared with their adsorbabilities and the plots in Fig. 6—9, especially for urea, are far away from the straight line. Since it is known that the change of $-COO^-$ to -COOH or of $-NH_3^+$ to $-NH_2$ accompanies a decrease in optical rotation,[15] the above result might be due to the effect of 3rd component on these hydrophilic groups. In previous papers,[4,5] the solubility of L-tryptophan increased linearly with the added concentration of urea or formamide and this increase was considered to come from the effect of these compounds on the hydrophobic moiety of L-tryptophan, considering that the solubilities of glycine and alanine decreased gradually with the addition of urea.[16] In other words, the water around the hydrophilic moiety of L-tryptophan was considered to be substituted by urea without accompanying the change in hydrophilic nature of that moiety. Therefore, it was concluded that the effect of urea or formamide on the hydrophilic moiety of L-tryptophan has relation to the decrease in optical rotation, but has no relation to the increase in solubility.

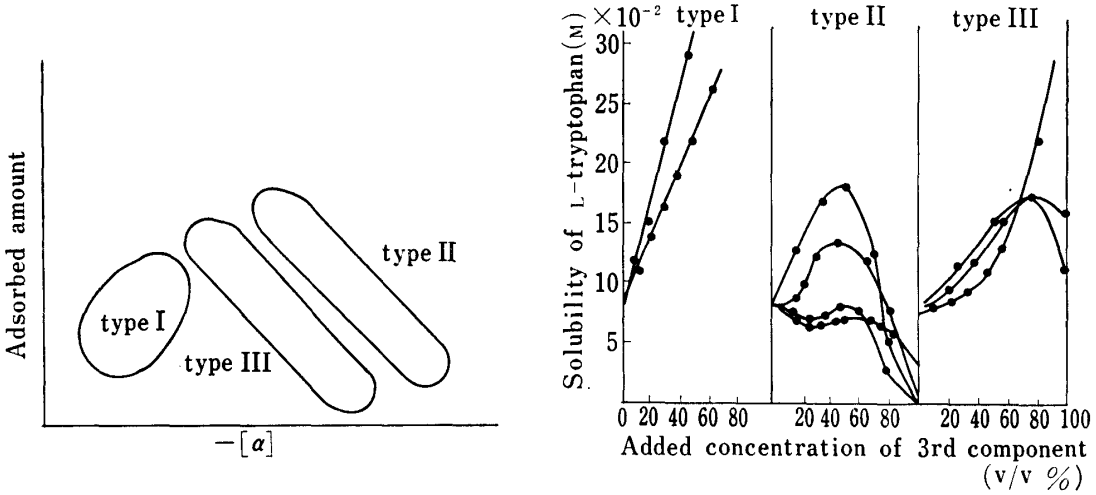


Fig. 10. Classification of 3rd Components according to the Relationship between the Effects on the Adsorbability and on the Optical Rotation of L-Tryptophan, Comparing with the Previous Classification according to Solubility Studies[5]

type I: urea, formamide
type II: MeOH, EtOH, *n*-PrOH, dioxane
type III: ethylene glycol, diethylene glycol, triethylene glycol

From the results shown in Fig. 6—9, the present 3rd components are classified into three types, as shown roughly in Fig. 10. This classification corresponded to the previous classification according to solubility studies.[5] Considering that the change in optical rotation may be due to the change in the motion of electron in the helicoidal surface, it is reasonably understood that 3rd component comes in contact with L-tryptophan molecule. Therefore, the relationship between solubility and optical rotation upon addition of 3rd component as described above verified the concept regarding the contact of 3rd component with L-tryptophan to result in the increase in solubility in aqueous solution,[4,5] moreover extending to the effect of 3rd component (denaturant) on the denaturation of globular protein.[5]

**Acknowledgement** The authors gratefully acknowledge the award of Research Grants from Naito Foundation (to T.N.). Thanks are also given to Miss Yoshie Uchida of the Analysis Center of this Faculty for measurement of ORD spectra.

15) Concluded from the data for L-tryptophan described in "The Merck Index," 8th ed., Merck & Co., Inc. 1968.
16) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, **238**, 4074 (1963).