June, 1981] © *1981 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, *54, 1595—1599 (1981)* 1595

The Adsorption of Basic α-Amino Acids in an Aqueous Solution by Titanium(IV) Oxide

Susumu Okazaki,* Teruaki Aoki, and Koichi Tani
Department of Industrial Chemistry, Faculty of Engineering, Ibaraki University, Hitachi 316

(Received June 5, 1980)

TiO_2 showed a pronounced activity in the adsorption of basic α-amino acids, such as L-lysine and L-arginine, in an aqueous solution. The adsorptive activity of TiO_2 containing sulfate ions was lower than that of the sulfate ion-free TiO_2, and it was suppressed by phosphate ions. The pH of the aqueous solution hardly affected the adsorption, while the heat-treatment of TiO_2 above 500 °C noticeably lowered the adsorption. The Langmuir equation was applicable to the adsorption of L-lysine, showing that the amount of saturated adsorption was about 0.7 mmol/g TiO_2 heat-treated at 500 °C. The heat of adsorption of L-lysine, as determined from the adsorption isotherms at 10, 30, and 50 °C, was 2257 J/mol. A linear relationship was observed between the amount of L-lysine adsorbed and that of the surface OH groups.

The removal of excess α-amino acids in the blood is required for the treatment of certain kidney diseases.[1] The use of activated carbon has been an important removal method. Ito[2] has observed a marked adsorption of the basic amino acids, such as lysine, histidine, and arginine, by animal charcoals, and Wunderly[3] has obtained adsorption isotherms for phenylalanine, leucine, serine, and aspartic acid on animal and sugar charcoals. However, a systematic investigation of the adsorption of amino acids on Darco G-60 lignite charcoal by Cherdelin *et al.*[4] indicated that activated carbon was not effective for the adsorption of all kinds of α-amino acid, for the absence of the aromatic group in amino acids decreased the adsorption. Aliphatic amino acids, having a high solubility in water, can be expected to be better adsorbed by an adsorbent which is more polar than activated carbon.

In recent years, TiO_2 and its hydrate have been extensively used as catalysis,[5] catalyst-carriers,[6] and ion-exchangers.[7] They have also been observed to be excellent adsorbents for uranium in sea water.[8] However, no studies dealing with the adsorptions of α-amino acids by TiO_2 have been reported. The use of TiO_2 as an adsorbent for α-amino acids is, though, a matter of considerable interest because of its extremely low solubility in water, its nontoxicity, and its amphoteric nature.

In this paper, we wish to report our results concerning the activity of TiO_2 in the adsorption of basic α-amino acids in aqueous solutions. The adsorptive activities of alumina and activated carbon for α-amino acids were also examined for reference.

Experimental

Preparation of the Samples. Two TiO_2 samples were prepared: one by the hydrolysis of titanium tetraisoproxide (Wako Chemical Co.) at room temperature (designated as S-1), and the other, by the hydrolysis of titanium oxide sulfate (Nakarai Chemical Co.) at the temperature of boiling water (designated as S-2). After repeated washings and decantations, both samples were dried in air at 120 °C for 10 h. Although the complete removal of the sulfate ion was confirmed by the use of a $BaCl_2$ aqueous solution, S-2 contained a considerable amount of bound sulfate ion (for example, 0.5 mmol/g after heat-treatment at 400 °C). Therefore, in most experiments, S-1 was employed. Only in experiments relating to the effects of the heat-treatment temperature was S-2 used.

The alumina was prepared by the hydrolysis of aluminum isopropoxide (Wako Chemical Co.), followed by washing with water and drying at 120 °C. The activated carbons used were the commercial products of the Kureha Chemical Co. (MU and MU-E).

The various amino acids used in this study were commercial reagents of a special grade prepared by the Kokusan Kagaku Co.

Determination of the Amount of Surface OH Groups. The amount of surface OH groups was determined by a modification of Boehm's method.[9] The amount of OH on S-1 sample, which was heat-treated at 500 °C, was 0.7 mmol/g TiO_2.

Method of Adsorption. One or 2 g of the adsorbent were added to 50 or 200 ml of an amino solution of 400 or 200 mg dm^{-3}, and then the mixture was continuously shaken for 3 h at 30 °C using a shaker. Prior to the adsorption, the TiO_2 and Al_2O_3 were ground so as to pass through a 150-mesh sieve and then fabricated to granules of a 14—20 mesh size under a pressure of 40 MPa. The solutions were buffured at pH 7.3 by using KH_2PO_4 and Na_2HPO_4. To ascertain the effect of pH, KH_2PO_4/Na_2HPO_4 ratio was adjusted to give the solution pH values of 5.2 and 8.3. On the other hand, to find the effect of the phosphate-ion concentration on the adsorption, its concentration was adjusted to 0.025, 0.050, and 0.083 mol dm^{-3}. The adsorption isotherm was determined in deionized water, since phosphate ions affected the adsorption of amino acid.

Analysis. The analysis of the amino acids was carried out by using a Total Carbon Detector (Toshiba-Beckmann Co., Type 915) or the ninhydrin method.[10]

Results and Discussion

Comparisons of Adsorptive Activities of Adsorbents for Amino Acids. The results obtained for the adsorption of various types of α-amino acid are listed in Table 1. The adsorptive activities of other metal oxides, such as ZrO_2, MgO, and SiO_2, were also examined, but their activities appeared to be considerably less than that of TiO_2.

As is shown in Table 1, TiO_2 exhibited the highest activity for the adsorption of all the basic amino acids except L-tryptophan. Its activity for the neutral or acidic α-amino acids was far less than those of activated carbons. The high activities of TiO_2 in the adsorption of L-lysine·HCl and L-ornithine·HCl may be due to the high degree of hydrolysis of these salts in solution.

Effects of Phosphate Ion and pH on Adsorption. A

Table 1. Effect of acid-base property of amino acid on adsorption

Adsorbate	Degree of adsorption/%[a)]			
	Al_2O_3[b)]	S-1[b)]	Activated carbon MU-LE	Activated carbon MU-L
Neutral				
Glysine	18.5	22.9	24.4	23.3
L-Alanine	22.2	21.5	25.0	16.5
L-Valine	12.9	16.9	50.6	46.0
α-Amino-isobutyric acid	0	0	18.1	15.8
DL-Leucine	0	0	84.1	79.5
L-Isoleucine	20.6	0	81.6	84.8
Acidic				
DL-Glutamic acid	0	0	20.8	22.6
DL-Aspartic acid	0	0	18.1	12.2
Basic				
L-Lysine	12.0	83.4	20.5	15.1
L-Arginine	1.8	72.6	73.5	71.1
L-Histidine	8.6	86.2	92.2	94.3
L-Tryptophan	7.8	18.1	100	99.5
Salt				
L-Lysine·HCl	1.1	73.4	24.9	20.7
L-Citrulline·HCl	0	11.9	83.9	80.4
L-Ornithine·HCl	15.3	87.1	26.1	14.2

a) Adsorption temperature: 30 °C; time: 3 h; initial concentration of amino acid: 400 mg/l; phosphate buffer: pH 7.3 (0.033 mol/l). b) Heat-treated at 150 °C for 3 h immediately before use.

considerable change in the pH of the solutions was observed during the course of adsorption. The adsorption experiments in phosphate buffer solutions were carried out at pH values close to that of the blood. However, since the phosphate ion is adsorbed on TiO_2,[11)] the effect of the phosphate ion on the adsorption of amino acid was examined, together with the effect of the pH of the solutions. L-Lysine was selected as representative of aliphatic and basic α-amino acids because of the ease of analyzing.

As is shown in Fig. 1, both the rate of adsorption and the amount of L-lysine adsorbed at the equilibrium were reduced by the presence of the phosphate ion. In the absence of the phosphate ion, the pH of the solution dropped from the initial value of 9.7 to 7.4 after 24 h. However, the effect of the pH on the adsorption of the amino acid was slight in the pH range of 5.2—8.3 when the concentration of phosphate ion was kept constant. The amount of L-lysine adsorbed at the equilibrium decreased linearly with the increase in the phosphate concentration as is shown in Fig. 2. This fact suggests that the α-amino acid and the phosphate would be adsorbed on identical sites. Thus, it appeared that the adsorption of the α-amino acids proceeded in competition with the phosphate ion. However, a preferential adsorption of the amino acid on TiO_2 was observed at higher phosphate concentrations (0.0033 mol dm^{-3}) and at dilute amino acid concentrations (0.0027 mol dm^{-3}).

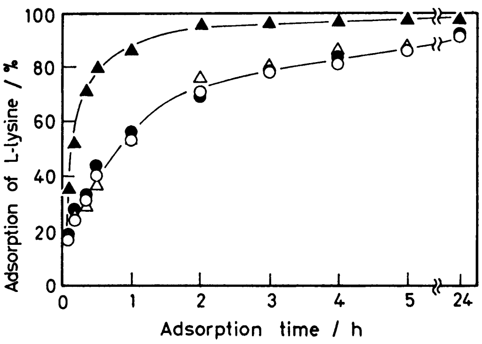


Fig. 1. Effect of pH on adsorption.
L-Lysine (20 mg) and S-1 (2 g) were added to 50 ml of the solntions buffered at pH 5.2 (○), 7.3 (●), and 8.3 (△). ▲ Denotes the result of the adsorption carried out in deionized water.

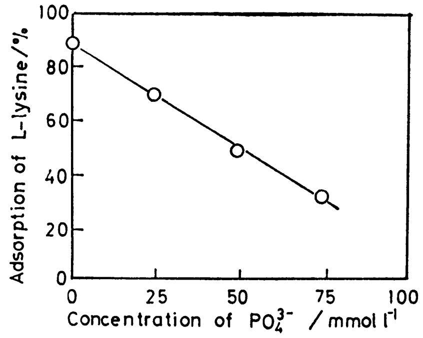


Fig. 2. Effect of concentration of phosphate ion on adsorption. S-1 (1 g) was added to 50 ml of the solution buffered at pH 7.3, and the adsorption was carried out 5 h. Other conditions were the same as those in Fig. 1.

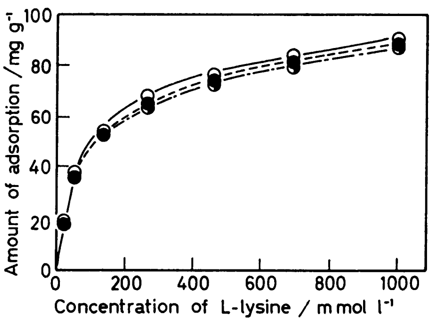


Fig. 3. Adsorption isotherm of L-lysine in deionized water. Adsorption were carried out for 70 h at 10 °C (○), 30 °C (●), and 50 °C (◑). Other conditions were the same as those Fig. 2.

Adsorption Isotherms in Deionized Water. Figure 3 shows the adsorption of L-lysine at 10, 30, and 50 °C. The effect of the temperature on the adsorption was slight. As is shown in Fig. 4, a linear relation was obtained between the logarithm of the equilibrium constant of adsorption, K_c, and $1/T$. Here, the equilibrium constant was calculated by dividing the amount of the amino acid adsorbed by that in the solution at the equilibrium (*i.e.*, about 1000 mg dm^{-3}). At this concentration, the surface of TiO_2 appeared to be saturated almost completely with the amino acid at

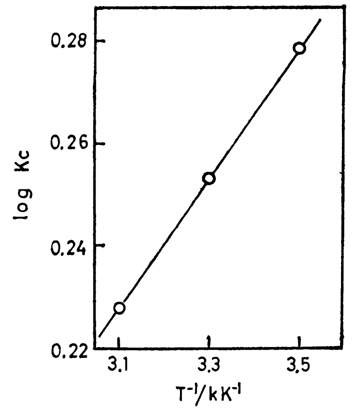


Fig. 4. Linear relationship between $1/T$ and log K_c. K_c denotes the equilibrium constant of adsorption of L-lysine.

these temperatures. The heat of adsorption, H_a, was calculated to be 2257 J/mol by using the van't Hoff equation; this value indicates a weak interaction of the amino acid with the TiO_2 surface.

The linear relation between the concentration of L-lysine and the value of the concentration divided by the amount of adsorption at the equilibrium (Fig. 5) indicates that the Langmuir adsorption equation is applicable. The values of the monomolecular adsorption, V_m, were calculated to be 0.70 (at 10 °C), 0.67 (at 30 °C), and 0.65 (at 50 °C) mmol/g from the reciprocal of the slope, namely, $\Delta C/\Delta(C/V)$, shown in Fig. 5.

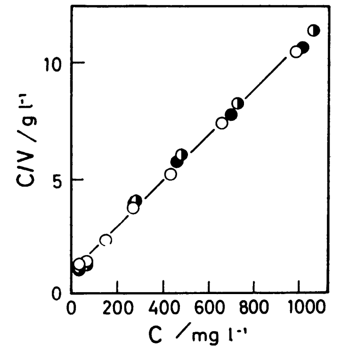


Fig. 5. Application of Langmuir equation to the adsorption shown in Fig. 3.

Effects of Heat-treatment Temperature of TiO_2 and the Amount of Surface OH Groups on the Adsorptive Activity. Figure 6 shows the effects of the heat-treatment temperature of S-1 and S-2 on the adsorption of L-lysine. The adsorptive activity of S-1 showed a maximum at 150 °C, which is close to the dehydration temperature (160 °C) of titanium hydroxide or $TiO_2 \cdot H_2O$.[12] Almost all the adsorptive activity was lost upon heat-treatment at 700 °C, which is the temperature of the conversion from anatase to rutile.[12]

The adsorptive activity of S-2, which contains sulfate ions, also decreased with the increase in the heat-treatment temperature. However, the adsorptive activity of S-2 was less than that of S-1, and it was lost upon heat-treatment at 500 °C. Since it has been established that TiO_2 containing sulfate ions has acid sites,[13] the adsorptive activity of TiO_2 toward α-amino acid is not related merely to the surface acidity. If the sulfate ions in TiO_2 diffuse to the surface at higher temperature and are lost as SO_3 at about 600 °C,[14] the surface of S-2 treated at temperatures near 600 °C may be covered by sulfate ions, which are then decomposed to SO_3 at slightly higher temperatures (about 600 °C). These sulfate ions on the TiO_2 surface possibly depress the adsorptive ability due to Ti^{4+} ions, O^{2-} ions, and/or OH groups on the surface, resulting in the observed decrease in the adsorptive activity. Since it is known that the Brönsted acid sites due to OH groups on the TiO_2 surface are able to interact with NH_3 and pyridine,[15] the OH groups may react also with amino acids through the interaction with lone-pair electrons of the N atom. The amount of surface OH groups was determined, therefore, to find the correlation between OH and the adsorptive activity.

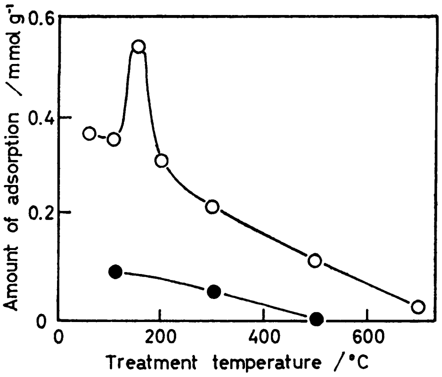


Fig. 6. Effect of heat-treatment temperature of TiO_2 on adsorption.
One gram of S-1 (○) or S-2 (●) was added to 50 ml of the buffered solution containing 2000 mg of L-lysine per litter, and the adsorption were carried out for 50 h at 30 °C.

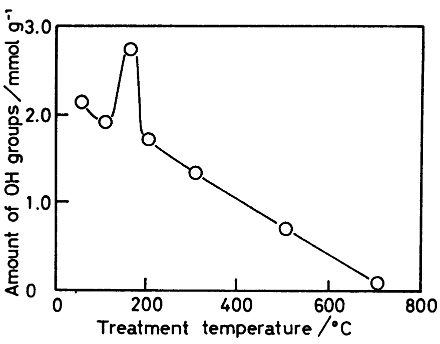


Fig. 7. Effect of heat-treatment temperature of TiO_2 on the amount of surface OH groups.

Figure 7 shows that the amount of surface OH groups was at its maximum when the sample was heat-treated at 150 °C, as was the activity of adsorption (Fig. 6). The linear relation between the amount of adsorption and the amount of surface OH groups shown in Fig. 8 indicates that the adsorptive activity of TiO_2 is largely dependent on the amount of surface OH

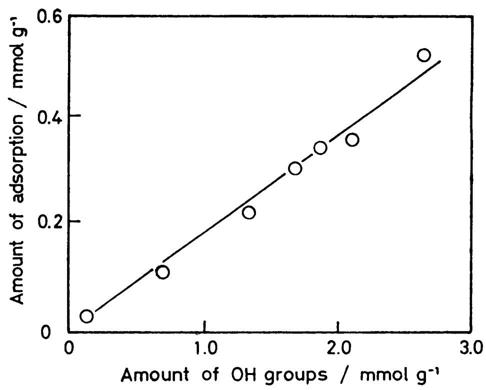


Fig. 8. Amount of L-lysine adsorbed *vs.* amount of surface OH groups for S-1.

groups. The amount of monomolecular adsorption of amino acid on TiO_2 was calculated as 0.65—0.70 mmol/g TiO_2 in the preceding section. This value almost coincides with the amount of surface OH groups on TiO_2 treated at 500 °C; the amount is shown to be 0.72 mmol/g TiO_2 in Fig. 7. This fact further supports the view that the adsorptive activity depends on the surface OH groups.

According to Kobayashi *et al.*,[11] TiO_2 heat-treated at 160 °C showed a maximum activity for the adsorption of phosphate ions. This temperature (160 °C) almost coincides with that at which the amount of OH was at its maximum, suggesting that the phosphate ions also may be adsorbed on the surface OH groups. The suppression of the adsorption of L-lysine by phosphate ions is possibly due to the competition between the phosphate ions and the amino acid for the interaction with surface OH groups.

Table 2. Comparison of adsorptions of various amino acids

Adsorbate	Degree of adsorption/%[a]			
	Al_2O_3[b]	S-1[b]	Activated carbon	
			MU-LE	MU-L
α-Aminoisobutyric acid	1.4	10.9	21.8	22.9
DL-α-Aminobutyric acid	0	15.7	32.1	33.2
γ-Aminobutyric acid	8.7	38.2	26.8	29.1
L-Lysil-L-lysine·2HCl	5.3	7.9	45.3	47.9

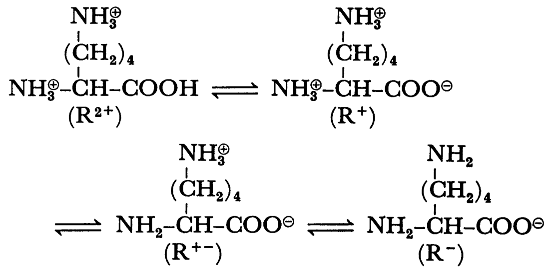
a) The adsorptions were carried out in deionized water. Adsorption temperature: 30 °C; time: 5 h; initial concentration of amino acid: 400 mg/l. b) Heat-treated at 150°C for 3h immediately before use.

Effect of Structure of Amino Acid on the Adsorption. In order to obtain some structural interpretation of the adsorption, the adsorption of three kinds of amino butyric acid were carried out under the same conditions. Table 2 shows the results, including also those of the dimer of L-lysine. γ-Amino acid is preferably adsorbed over α-amino acid, indicating that the end position is more favored for the interaction than is the α-position. The difference in the activity for the interaction between the α and γ position may be ascribed, at least in part, to steric factors. Also, the fact that the iso-type isomer and the dimer were adsorbed by less butyric acid than normal would seem to support this explanation.

Table 3. Acid-base parameters of some basic amino acids in an aqueous solution[16]

Amino acid	pK_{a1}	pK_{a2}	pK_{a3}	pH_I
Lysine	2.18	8.95 (α-Amino)	10.53 (ε-Amino)	9.74
Arginine	2.17	9.04 (α-Amino)	12.48 (Guanigino)	10.76
Histidine	1.82	6.00 (Imidazol)	9.17	7.58
Tryptophane	2.38	9.39		5.88

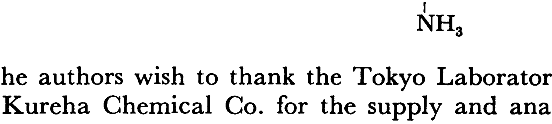
Behavior of Lysine on the TiO_2 Surface. In aqueous solutions, most amino acid molecules take one of these forms, NH_3^+RCOOH, $NH_3^+RCOO^-$, or NH_2RCOO^-, depending on the pH of the solution. The dissociation constants of the carboxyl group (pK_{a1}) and amino groups (pK_{a2}, pK_{a3}) in various basic amino acids listed in Table 3, together with the isoelectric points (pH_I). The pH_I values show that lysine and arginine should exist as cations in the pH range (5.2—8.3) of this study. For example, lysine should exist in the form of R^+, as is shown below.



$$\underset{(R^{2+})}{\mathrm{NH_3^{\oplus}{-}\overset{\substack{NH_3^{\oplus}\\|\\(CH_2)_4\\|}}{C}H{-}COOH}} \rightleftharpoons \underset{(R^{+})}{\mathrm{NH_3^{\oplus}{-}\overset{\substack{NH_3^{\oplus}\\|\\(CH_2)_4\\|}}{C}H{-}COO^{\ominus}}}$$
$$\rightleftharpoons \underset{(R^{+-})}{\mathrm{NH_2{-}\overset{\substack{NH_3^{\oplus}\\|\\(CH_2)_4\\|}}{C}H{-}COO^{\ominus}}} \rightleftharpoons \underset{(R^{-})}{\mathrm{NH_2{-}\overset{\substack{NH_2\\|\\(CH_2)_4\\|}}{C}H{-}COO^{\ominus}}}$$

As is generally known, basic amino acids, which exist as cations in a neutral solutions, are selectively adsorbed by weakly acidic ion-exchange resins.[17] Accordingly, TiO_2 may show a high adsorptive activity for lysine and arginine, for the surface OH groups on TiO_2 show weak Brönsted acidity.[5] On the other hand, Al_2O_3, which has only Lewis acidity,[18] did not show a high adsorptive activity for the basic amino acids.

The fact that γ-aminobutyric acid was preferably adsorbed over a α-type isomer (Table 2) suggests that the activity of $\omega\text{-}NH_3^+$ is higher than that of $\alpha\text{-}NH_3^+$. Thus, the adsorption of lysine on TiO_2 is considered to proceed as follows:



$$\mathrm{{-}\overset{|}{\underset{|}{Ti}}{-}OH + H_3N^{\oplus}{-}(\underset{\substack{|\\NH_3}}{C}H_2)_4{-}COO^{\ominus}} \longrightarrow \mathrm{Ti{-}^{\ominus}O{-}^{\oplus}N{-}(CH_2)_4{-}\underset{\substack{|\\NH_3}}{C}H{-}COOH}$$

The authors wish to thank the Tokyo Laboratory of the Kureha Chemical Co. for the supply and analysis of some amino acids; they are also grateful to Mr. Atsumu Nishimura for helpful discussions of the experimental results.

References

1) K. Sanjo *et al.*, *Jinko Jinzo*, **5**, 231 (1976).
2) T. Ito, *J. Agric. Chem. Soc.*, **12**, 204 (1936).
3) K. Wunderly, *Helv. Chim. Acta*, **17**, 523 (1934).
4) V. H. Cheldelin and R. J. Williams, *J. Am. Chem. Soc.*, **64**, 1513 (1942).
5) S. Okazaki, "Kinzoku-sankabutsu To Fukugo-sankabutsu," ed by K. Tanabe, T. Seiyama, and K. Fueki, Kodansha, Tokyo (1978), p. 102.
6) S. Okazaki, *Kagakukogyo*, **22**, 56 (1978).
7) V. V. Pekrek, *Talanta*, **19**, 219 (1972); C. H. Wirguin and A. A. Yaron, *J. Appl. Chem.*, **15**, 445 (1965).
8) N. J. Keen, *Chem. Ind.*, **1977**, 579; N. Ogata, *Nippon Kaisui Gakkaishi*, **30**, 3 (1976).
9) H. P. Boehm, *Angew. Chem.*, **78**, 617 (1966).
10) N. Takahashi, "Seikagaku Jikken Koza," ed by Nippon Seikagakukai, Tokyokagakudojin, Tokyo (1978), Vol 1 (II), p. 69.
11) E. Kobayashi, M. Sugai, and M. Higuchi, *Nippon Kagaku Kaishi*, **1979**, 39.
12) J. Barksdale, "Titanium, Its Occurence, Chemistry, and Technology," The Ronald Press. Co., New York (1966), p. 71.
13) A. Kurosaki and S. Okazaki, *Nippon Kagaku Kaishi*, **1976**, 1816; K. Morishige, H. Hattori, and K. Tanabe, *Bull. Chem. Soc. Jpn.*, **48**, 3088 (1975).
14) T. Chinone and S. Okazaki, *Nippon Kagaku Kaishi*, **1978**, 1327.
15) G. D. Parfitt, J. Rambotham, and C. H. Rochester, *Trans. Faraday Soc.*, **67**, 841 (1971); **67**, 1500 (1971).
16) H. R. Mahler and E. H. Cordes, translated by F. Egami *et al.*, "Basic Biological Chemistry," Tokyokakudojin, Tokyo (1973), p. 43.
17) K. Satake, "Tanpakushitsu," Asakurashoten, Tokyo (1975), p. 25.
18) H. Pines and W. O. Haag, *J. Am. Chem. Soc.*, **82**, 2471 (1960); E. P. Parry, *J. Catal.*, **2**, 371 (1963).