Discussion

Among the metabolites of cyclamate, cyclohexylamine probably formed in the first step, is thought to be more important in its toxicity than further oxidized metabolites, cyclohexanol and cyclohexanone, which were found too small in quantity to be determined by the present rutine procedure.

In the present study the fact of large variation of the convertion to cyclohexylamine in individuals which has been often reported by other researchers was again ascertained.

It should be noted that cyclohexylamine was excreted in urine later than cyclamate, having a peak in the second day or later which may depend on individuals and physiological conditions. It also suggests that the convertion in the body does not occur simultaneously with the adsorption of cyclamate, but that the degradating system is gradually or adaptively formed to attack cyclamate.

However, the site and mechanism of the biotransformation of cyclamate to cyclohexylamine have not been clarified. Kojima and Ichibagase[6] showed that cyclamate was converted to cyclohexylamine in liver from their experiment of rat liver homogenate, but the amine was only qualitatively detected and the amount was very small.

On the other hand the amount of cyclohexylamine excreted in individual urine was too variable to be explained by the degradating system in liver, remaining a possibility of the transformation by intestinal micro-flora. We rather presume a participation of the latter from our results concerning the incubation experiments of liver and cecal content of guinea pig which will be published elsewhere.

Acknowledgement The authors express their deep gratitude to Dr. Y. Ikeda and Dr. S. Horiuchi, Department of Toxicology of National Institute of Hygienic Sciences, for their helpful advice.

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Kinetics of Reaction of Dehydroacetic Acid. V.[1] Reaction with Primary Amines. (3)

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It has been pointed out that dehydroacetic acid (DHA), which is one of the officially recognized food preservatives in Japan, readily reacts with primary amines in solution to give initially the Schiff base-type compounds.[3] In a previous work,[4,5] β-phenethylamine was selected as the representative primary amine and the effect of acidity or basicity of the solution on the reaction rate was investigated in detail.

Numerous studies related to the formation of the Schiff base-type compounds support the following reaction path involving two steps.

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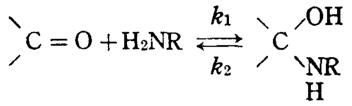
[1] Part IV: S. Goto, Y. Hirakawa, and S. Iguchi, *Shokuhin Eiseigaku Zasshi*, **10**, 194 (1969).

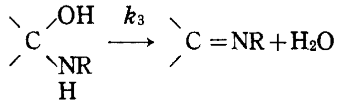
[2] Location: *Katakasu, Fukuoka.*

[3] S. Iguchi, K. Hisatsune, M. Himeno, and S. Muraoka, *Chem. Pharm. Bull.* (Tokyo), **7**, 323 (1959).

[4] S. Goto, S. Iguchi, A. Kono, and H. Utsunomiya, *J. Pharm. Sci.*, **56**, 579 (1967).

[5] S. Goto, A. Kono, and S. Iguchi, *J. Pharm. Sci.*, **57**, 791 (1968).


(a)


(b)

The first step (a) of the reaction involves an attack of the nitrogen base on the carbonyl compound to form an intermediate addition compound (carbinolamine), and the second step (b) consists of dehydration of the intermediate addition compound to form the Schiff base-type compound.

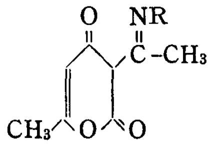
Result reported herein is concerned with substituent effect on the formation rate of the Schiff base–type compounds from the reaction of DHA with aliphatic and aromatic amines. Further, this result may serve to establish a probable reaction mechanism and to examine how a rate-determining step is influenced by the polar character of amines.

Experimental

Materials——Commercial EtOH was refined and redistilled, and water content of EtOH was measured by titrimetric (Karl Fischer) method. The obtained value was 3.097% (w/v). DHA (Taito Pfizer Co. Tokyo) was recrystallized from EtOH–H_2O to mp 109—110°. For amines, the reagent of extra pure grade was used without recrystallization or redistillation, but that of pure grade was recrystallized or redistilled before use.

Schiff Base-Type Compounds——To 20 ml of EtOH containing DHA (0.5 g) was added an equivalent mole of the amine and the mixture was kept at 80° on a steam bath for 10 hr. The obtained precipitate was recrystallized or, when the reaction solution was clear, the solvent was evaporated under reduced pressure and the residue was recrystallized, from EtOH or EtOH–H_2O. Analytical data are summarized in Table I.

Table I. Analytical Data for Representative Schiff Base-Type Compounds



R	mp(°C)	Formula	Analysis (%)					
			Calculated			Found		
			C	H	N	C	H	N
$-CH_2CH_2OH$	119—120	$C_{10}H_{13}O_4N$	56.9	6.16	6.63	56.7	6.20	6.55
$-CH_2C_6H_5$	79—81	$C_{15}H_{15}O_3N$	70.0	5.83	5.41	69.9	5.89	5.50
$-CH_2CH_2C_6H_5$	89—91	$C_{16}H_{17}O_3N$	70.8	6.27	5.16	70.6	6.17	5.13
$-C_6H_5$	121—122	$C_{14}H_{13}O_3N$	69.1	5.35	5.78	69.3	5.37	5.62
$-C_6H_4CH_3(p)$	156—158	$C_{15}H_{15}O_3N$	70.0	5.83	5.41	70.0	5.89	5.36
$-C_6H_4CH_3(m)$	123—124	$C_{15}H_{15}O_3N$	70.0	5.83	5.41	70.2	5.91	5.52
$-C_6H_4Cl(p)$	139—140	$C_{14}H_{12}O_3NCl$	60.5	4.32	5.03	60.2	4.39	4.80
$-C_6H_4Cl(m)$	126—128	$C_{14}H_{12}O_3NCl$	60.5	4.32	5.03	60.6	4.30	5.03
$-C_6H_4OCH_3(p)$	178—180	$C_{15}H_{15}O_4N$	65.9	5.50	5.13	66.2	5.71	5.04
$-C_6H_4OCH_3(m)$	113—114	$C_{15}H_{15}O_4N$	65.9	5.50	5.13	66.1	5.46	5.07
$-C_6H_4OH(m)$	201—203	$C_{14}H_{13}O_4N$	64.9	5.02	5.40	64.9	5.02	5.36

Kinetic Procedures——The reaction rate was determined by measuring the decrease of DHA in EtOH solution using a Hitachi photoelectric spectrophotometer, Model EPU-2A, or a Shimadzu photoelectric spectrocolorimeter, Model Spectronic 20. The analitical procedure was stated in previous report.[4] All the reactions were carried out at 40°. A required amount (2×10^{-2}M) of the amine was weighed into a 100 ml measuring flask and dissolved in EtOH. The flask was placed in a thermostat for at least 30 min and EtOH solution of DHA was added to the flask from a pipette. The concentration of DHA was also maintained at 2×10^{-2}M.

Thin-Layer Chromatographic Analysis——The thin-layer plates (20×20 cm) were coated with 0.4 mm layer of silica gel B-5 (Wako pure reagent) and a 100 μl reaction solution was spotted on the plate at regular time intervals. The plates were developed with benzene–AcOH–H_2O (1:1:2) and the solvent front was allowed to advance 13 cm. Detection of DHA and the Schiff base-type compound was made under ultraviolet ray at 235.6 mμ. Each spot was scraped and extracted with warm EtOH in a small galss vessel for about 5 min. The extracts were filtered through a filter paper, the filtrate was diluted with EtOH, and an absorbance of the solution was measured by spectrophotometry. Results of analyses by colorimetry and by thin-layer chromatography agreed well. Since the sum of the molar concentrations of DHA and the Schiff base at each time interval would be equal to the initial molar concentration of DHA, the reaction was terminated when the Schiff base-type compound was formed. The result for the reaction with hexylamine is given in Table II. The same result was obtained in the reaction between DHA and aniline.

Table II. Concentration of Residual DHA and formed the Schiff Base in Reaction with Hexylamine as a Function of Time

Time (min)	Concentration ($\mathrm{M}\times10^{-2}$)		
	Residual DHA	Schiff base formed	Total
0	1.00	0.00	1.00
10	0.77	0.20	0.97
70	0.37	0.64	1.01
160	0.19	0.87	1.06
250	0.15	0.90	1.05
1270	0.00	1.05	1.05

Result and Discussion

From the results obtained, the experimental rate equation is represented as follows:

$$v=k_{\mathrm{app}}[\mathrm{DHA}][\mathrm{RNH_2}] \tag{1}$$

When the initial concentrations of DHA and amines are the same, Eq (1) is simplified to

$$v=k_{\mathrm{app}}[\mathrm{DHA}]^2 \tag{2}$$

or

$$\frac{1}{a-x}=k_{\mathrm{app}}t+\frac{1}{a} \tag{3}$$

where, v is the reaction rate, k_{app}, the apparent second-order rate constant, a, the initial concentration of DHA or amine, and x, the concentration of DHA or amines consumed at time t. Accordingly, Eq. (3) indicates that a linear ralationship exists between $1/(a-x)$ and t. Typical second-order plots for the reaction of *sec*-butylamine with DHA is shown in Fig. 1.

Because the attack of primary amines, one of the nucleophilic reagents, on the carbonyl component is considered generally as one of the acid-base reactions, it seems reasonable to consider that the higher rate for carbonyl-amine reaction is due mainly to the higher degree of nucleophilicity for amines with a strong basicity. The replacement of amine component of the Schiff base of salicylaldehyde by another amine has been investigated by Muto.[6] The reaction *via* two steps involving a *gem*-diamine as an intermediate proceeded in EtOH solution and the attack of amine having a stronger basicity on the Schiff base derived from weaker amine produced replacement of the amine component. .Such a fact may suggest that the

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[6] Y. Muto, *Nippon Kagaku Zasshi*, 76, 252 (1955).

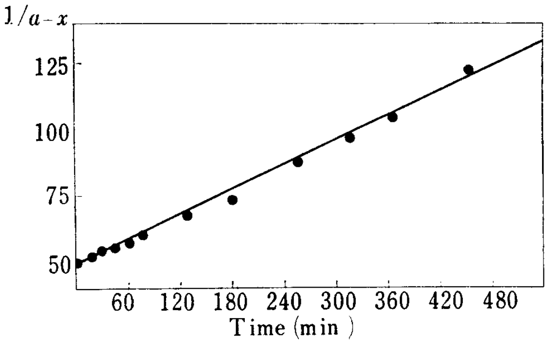
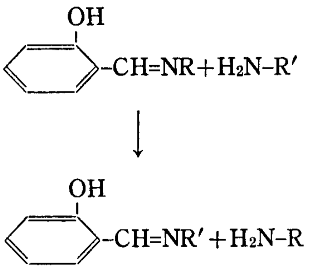


Fig. 1. Typical Second-order Plots for the Reaction of *sec*-Butylamine with Dehydroacetic Acid at 40°

attacking rate of amines on carbonyl component is dependent on the basicity of amines.



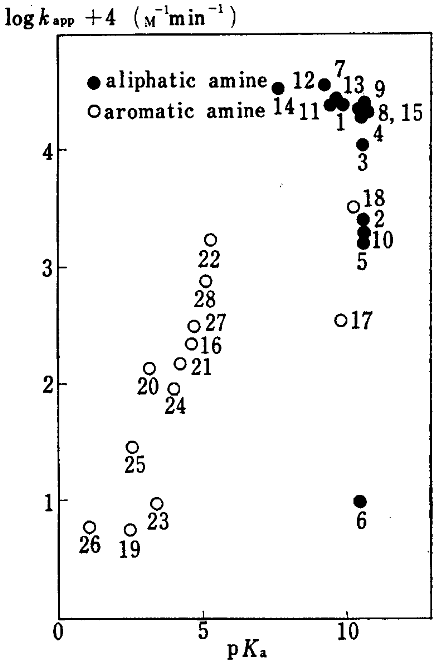


Fig. 2. Relationship between Second-order Rate Constant and pK_a

1 propylamine, 2 isopropylamine, 3 butylamine, 4 isobutylamine, 5 *sec*-butylamine, 6 *tert*-butylamine, 7 allylamine, 8 hexadecylamine, 9 laurylamine, 10 cyclohexylamine, 11 monoethanolamine, 12 benzylamine, 13 β-phenethylamine, 14 β-aminopropylnitrile, 15 hexylamine, 16 aniline, 17 *m*-aminophenol, 18 *p*-aminophenol, 19 *p*-aminobenzoic acid, 20 *m*-aminobenzoic acid, 21 *m*-anisidine, 22 *p*-anisidine, 23 *m*-chloraniline, 24 *p*-chloraniline, 25 *m*-nitroaniline, 26 *p*-nitroaniline, 27 *m*-toluidine, 28 *p*-toluidine

First, the logarithm of second-order rate constants for the Schiff base formation were plotted as a function of pK_a values⁷⁾ of the amines. No simple and linear correlation was found between them as shown in Fig. 2. Therefore, it became obvious that the rate could not be controlled by the basicity of amines alone.

Since the rate constants for the Schiff base formation could be rather poorly correlated to pK_a values, Hammett's and Taft-Ingold's equations (ρ—σ plots) were applied for substituted aromatic and aliphatic amines, respectively. A linear relationship was observed as shown in Fig. 3 for aromatic amines and the Hammett's equation was as follows:

$$\log \frac{k_{app}}{k_0} = -2.32\sigma \qquad (4)$$

The rate constant for aniline is denoted by k_0. The negative value of ρ indicates that the introduction of an electron-releasing group significantly increases the reaction rate. Moreover, it may be suggested that the addition (a) of aromatic amines to the carbonyl group of DHA becomes the rate-limiting step for the Schiff base formation.

Unfortunately, a simple Taft-Ingold rule was not applicable to the reaction of eight aliphatic amines as shown in Fig. 4.

If the steady-state approximation is used for the given consecutive reaction scheme, the concentration of carbinolamine (y) would be expressed as follows:

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7) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," Methuen and Co., London, 1962.

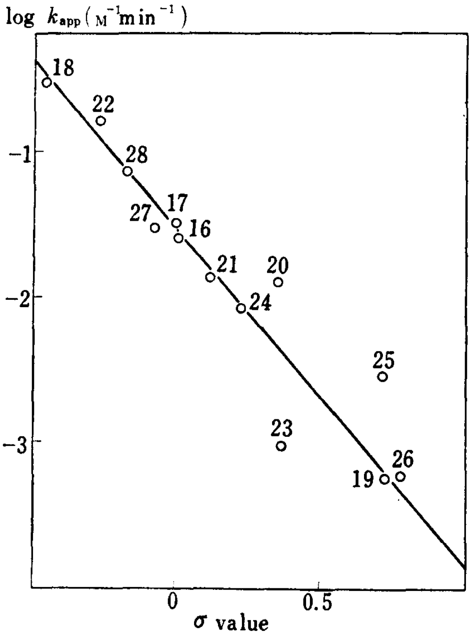

Fig. 3. Hammett Plot for the Reaction between Aromatic Amine and Dehydroacetic Acid at 40°

Refer to Fig. 2 for the numbers noted in this graph.

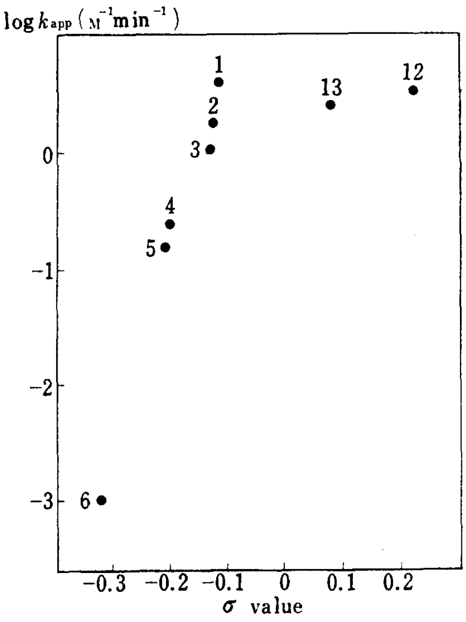

Fig. 4. Taft-Ingold Plot for the Reaction between Aliphatic Amine and Dehydroacetic Acid at 40°

Refer to Fig. 2 for the numbers noted in this graph.

$$\underset{x}{\text{DHA}}+\underset{x}{\text{amine}} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \underset{y}{\text{Carbinolamine}} \xrightarrow{k_3} \underset{z}{\text{Schiff base}}$$

$$y = \frac{k_1}{k_2+k_3}x^2 \tag{5}$$

Since the DHA concentration obtained by the colorimetric method[4] is considered as the sum of the concentration of DHA (x) and that of carbinolamine (y),[8] the decreasing rate is expressed as follows:

$$-\frac{d(x+y)}{dt} = k_1x^2-k_2y \tag{6}$$

Substitution of Eq.(5) into Eq.(6) will give

$$-\frac{d(x+y)}{dt} = \frac{k_1k_3}{k_2+k_3}x^2 \tag{7}$$

$$= k_{\text{app}}(x+y)^2 \tag{8}$$

Thus, k_{app} is represented by

$$k_{\text{app}} = \frac{k_1k_3}{k_2+k_3}\frac{x^2}{(x+y)^2} \tag{9}$$

$$= \frac{k_1k_3(k_2+k_3)}{(k_2+k_3+k_1x)^2} \tag{10}$$

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8) When ammonia gas is passed through an ether solution of DHA, a stable carbinolamine compound precipitates out (S. Iguchi, S. Goto, and Y. Kodama, *Yakugaku Zasshi*, **79**,1100 (1950)), The colorimetric assay developed with $TiCl_3$ was also found to be applicable for this carbinolamine.[4] And the molecular absorption coefficient of carbinolamine at 550 mμ agreed with that of DHA.

In the steady state approximation, $k_2+k_3 \gg k_1 x$ is considered. Then Eq. (10) can be reduced to

$$k_{app} = \frac{k_1 k_3}{k_2+k_3} \tag{11}$$

Assuming that $k_3 \gg k_2$, Eq. (11) can be simplified to

$$k_{app} = k_1 \tag{12}$$

Eq. (12) may be applied to the reaction of DHA with aromatic amines. If $k_2 \gg k_3$ is assumed, Eq. (11) can be reduced to

$$k_{app} = \frac{k_1}{k_2} k_3 = K \cdot k_3 \tag{13}$$

In the case of the reaction of aliphatic amines, Eq. (13) may be used. The equilibrium constant, K, may probably show a linear and negative logarithmic correlation with Taft-Ingold's substituent constants. In contrast, the rate constant of dehydration, k_3, may show a positive ρ value, indicating that this step is aided by electron-withdrawal from the reaction center. Since the apparent rate constant, k_{app}, depends on both the equilibrium constant, K, and the rate constant for dehydration of carbinolamine, k_3, as shown in Eq. (13), these two substituent effects cancel each other and apparent rate constant, k_{app}, shows almost no variation with change in electron-withdrawing substituents (benzylamine and β-phenethylamine in Fig. 4). A break occurs in the curve at near the point of $\sigma=0$, as shown in Fig. 4, and the $\sigma-\rho$ relationship for the reaction with six other aliphatic amines shows that there is a significant decrease in the rate with electron-donating substituent (Fig. 4). It may be suggested that the dehydration of carbinolamine becomes the rate-limiting step in the Schiff base formation from these aliphatic amines.

Noyce[9] has stated that the most likely cause of failure to obtain a linear $\sigma-\rho$ orrelation with substituted amines in the formation of semicarbazones is that the rate constant of dehydration step in the reaction sequence is almost comparable to that of the addition step. The equation for the observed relative rate is expressed as:

$$\log \frac{k_{app}}{k_0} = (\rho_1+\rho_3-\rho_2)\sigma - \log \left[\frac{1 + \dfrac{k_3^0}{k_2^0} 10^{(\rho_3-\rho_2)\sigma}}{1 + \dfrac{k_3^0}{k_2^0}} \right] \tag{14}$$

where K_2° and K_3° are the rate constants for standard amines. This function is not linear in σ, and may be shown to pass through a maximum by successive differentiation of σ. A similar equation may be applicable to the formation of the Schiff base between DHA and aliphatic amines.

It may be concluded that (1) the rate constants for the Schiff base formation are poorly correlated to pK_a values of amines, (2) Hammett's plot is applicable to the reaction of DHA with aromatic amines and give a negative ρ value (-2.32), and that (3) data from the reaction with aliphatic amines do not follow the simple Taft-Ingold's equation. A possible mechanistic interpretation with respect to the Schiff base formation is hereby presented, and the balance between the factor of addition step and that of subsequent dehydration step is responsible for the occurrence of a break in the Taft-Ingold's plot.

Above explanation is only consistent with the observation in the Hammett's and Taft-Ingold's plots, and further investigation and consideration will be necessary.

Acknowledgement The authors thank Professor S.Iguchi for his helpful advice.

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9) D.S. Noyce, A.T. Bottini, and S.G. Smith, *J. Org. Chem.*, **23**, 752 (1958).