TABLE II—SUBSTITUTENT GROUP CONTRIBUTION TO HYPOGLYCEMIC ACTIVITY

Position(s)	Group[a]	Contribution[b]
R_1 and R_2	H	-8.99
R_1 and R_2	CH_3	3.56
R_1 and R_2	C_2H_5	2.20
R_1 and R_2	OCH_3	1.17
R_1 and R_2	$(CH_2)_2$	0.38
R_1 and R_2	$(CH_2)_{2.5}$	-3.54
R_3	$(CH_2)_5$	-2.47
R_3	$(CH_2)_6$	-3.17

[a] See *Footnote a*, Table I. [b] See *Footnote b*, Table I.

$0.38\% = 0.76\%$), and the pentamethylene group, $(CH_2)_5$ $(2 \times -3.54\% = -7.08\%)$. At the R_3 position, both the pentamethylene and hexamethylene groups have a negative contribution $(-2.47\%$ and -3.17%, respectively).

It is apparent from Table I that reasonable agreement exists between the calculated and observed hypoglycemic activities. The *F*-ratio between the variance of the estimated activity and the variance of the observed activity is significant at the 99% level. The coefficient of the multiple correlation is 0.977. In four of the 12 compounds (No. 2, 4, 10, and 12), however, the differences between the calculated and observed values are greater than the standard deviations reported by McManus and Gerber (5).

The primary interest in this type of study is the selection of untested molecules which have a high probability of possessing useful therapeutic activity. The seven untested molecules which are predicted to be more active than chlorpropamide [activity as determined by McManus and Gerber (5), 35 ± 3.3%], are 1,1-pentamethylene-4-(4-ethyl-4-methyl-1-piperidinesulfamyl)semicarbazide; 1,1 - pentamethylene - 4 - (4 - methoxy - 4 - methyl-1 - piperidinesulfamyl)semicarbazide; 1,1 - hexamethylene - 4 - (4 - methoxy - 4 - methyl - 1 - piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4,4-diethyl - 1 - piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4-ethyl- 4 - methoxy-1-piperidinesulfamyl)semicarbazide; 1,1-hexamethylene-4-(4-ethyl-4-methoxy-1-piperidinesulfamyl)semicarbazide; 1,1-pentamethylene-4-(4,4-dimethoxy-1-piperidinesulfamyl)semicarbazide (No. 11, 13, 14, 15, 17, 18, and 19, respectively). These compounds appear to be worthy of synthesis and testing as possible hypoglycemic agents.

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Kinetics of Reaction of Dehydroacetic Acid I

Reaction with Primary Amines I

### By SHIGERU GOTO, SADAO IGUCHI, AKIRA KONO, and HIDEKO UTSUNOMIYA

Dehydroacetic acid (DHA), one of the officially recognized food preservatives in Japan, has been found in some instances to readily react with amino compounds under mild and physiological conditions to produce Schiff's base type compounds. The kinetic study of the reaction between DHA and β-phenethylamine (PE-NH), with the formation of 3-(1-phenethylamino)ethylidene-6-methyl-3H-pyran-2,4-dione, was investigated in various water–ethanol mixtures, and a linear relationship was observed between the reciprocal of the dielectric constant of the solvent and log *k*. The *k*–pH profiles for the reaction in 80 per cent ethanol solution at 40 and 60° were completed in the acetate buffer regions. No general acid-base catalysis was observed but salt effects were not negligible. The rate-determining step in 80 per cent ethanol solution was probably the attack of free PE-NH on the protonated carbonyl carbon atom in free DHA.

DEHYDROACETIC ACID (DHA) (I) has been accepted as one of the officially recognized food preservatives in Japan and is at present being used widely not only in some foodstuffs and refreshing drinks but also in pharmaceutical preparations as an antiseptic. It has been pointed out that one of the most remarkable chemical behaviors of DHA is its affinity toward compounds possessing an amino radical. When

they coexist in solution, Schiff's base type compounds (II) are initially readily produced by the reaction between DHA and amino compounds, and then finally transformed, in some cases, to lutidone derivatives (V). This has been reported in the preceding papers (1, 2) and the mechanism described in Scheme I was proposed.

In some instances the conversion proceeds under mild and physiological conditions.

Other similar qualitative and synthetic investigations (3–5) have been published, but no

R = CH₃, C₂H₅, CH₂C₆H₅, and (CH₂)₂C₆H₅
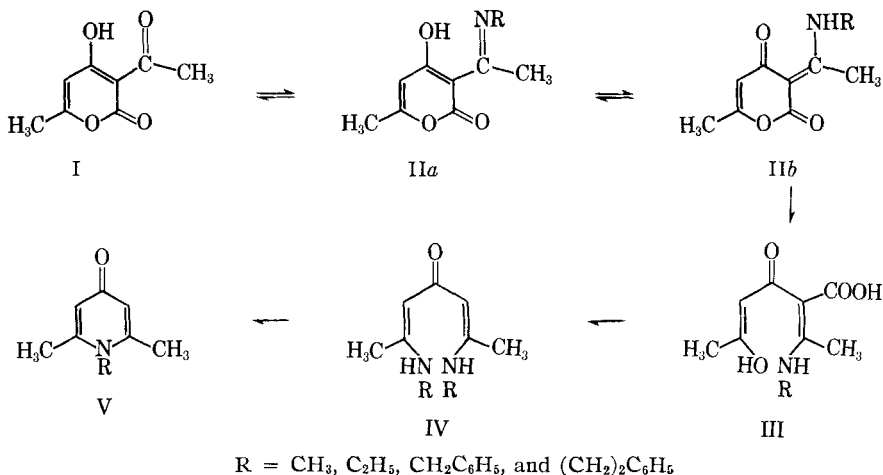
Proposed Route for Reaction of Dehydroacetic Acid with β-Phenethylamine

Scheme I

kinetic data on this transformation have been reported. It seems natural, based on the unique chemical behavior of DHA, especially from a kinetic viewpoint, to extend studies to cover the re-estimation of its practical usages in Japan.

In this experiment the authors selected β-phenethylamine (PE-NH) as a representative primary amine because of its easiness of treatment. Reactions of DHA were kinetically studied in water–ethanol mixtures.

This paper considers the kinetics in the first step of the transformation reaction, the formation of 3-(1-phenethylamino)-ethylidene-6-methyl-3H-pyran-2,4-dione (NR-DHA).

EXPERIMENTAL

Materials—Ethanol of superior special grade for precision analysis was used. PE-NH (Wako pure reagent) was redistilled twice (b.p. 178–179°). DHA (Taito Pfizer Co.) was recrystallized from ethanol–water, m.p. 109–110°. Commercial acetic acid was redistilled, and sodium acetate and sodium chloride of pure reagent grade were used for the kinetic studies.

Determination of DHA—A 1-ml. sample was diluted with 9 ml. of cold distilled water. Three drops of a 10% hydrochloric acid solution and 0.1 ml. of a 17% titanium chloride solution were added to the sample solution. The observed pH of the final sample solution was in the range of 1.5 to 2.0. The absorbance of the sample was measured against a blank solution at 550 mμ using 1-cm. cells in a Hitachi photoelectric spectrophotometer of the EPU 2A type. A plot of absorbance *versus* concentration gave a straight line graph over the range 1×10^{-4} to 2×10^{-3} M. The percentage standard deviation for repeated determinations was $\pm 1.0\%$. The coexistences of NR-DHA (II), lutidones (V), 2,6-bis(phenethylamino)-2,5-heptadien-4-one (IV), and PE-NH did not interfere with DHA determinations.

Kinetic Studies—The rates were measured by following the decrease of DHA. Twenty-five milliliters of a 2×10^{-2} M DHA solution in an appropriate acetate buffer was mixed with 25 ml. of a 2×10^{-2} M PE-NH solution in a 50-ml. glass volumetric flask, and thermostated at the temperature of the prospective kinetic study. The pH of the reaction mixture was measured over the entire period of a kinetic run, and the average of these pH values was used for further kinetic calculations. The ionic strengths of the reactions were generally constant at 0.1. The effect of change in the ionic strength on the reaction was studied by changing the ionic strength of the solutions with sodium chloride, and the effect of the general acid-base catalysis was evaluated in eightfold ranges of acetate buffer concentration at the constant ionic strength ($\mu = 0.1$). Aliquots (1 ml.) were pipeted out at regular time intervals, and the above for the DHA assay was used. Verification of the fact that the spectrophotometric absorption band at 550 mμ characterized the intact DHA was obtained by thin-layer chromatographic determination of an aliquot of the residual DHA and the reaction product, NR-DHA, in 99.7% ethanol at 40°. Calibration curves of known concentrations (1×10^{-5} to 2×10^{-4} M) for the two components in ethanol were prepared by spectrophotometric absorbances at 305 mμ for DHA and 314 mμ for NR-DHA. The thin-layer plates (20 × 20 cm.) were coated with a 0.4-mm. layer of silica gel B-5 (Wako pure reagent), and a 100-μl. sample solution was spotted on the plate. The plates were developed with benzene–acetic acid–water (1:1:2) twice. The solvent front was allowed to advance 13 cm. Detection for DHA and NR-DHA was performed under ultraviolet light at 253.6 mμ. Each spot was cut off and extracted with warm ethanol in a small glass apparatus for about 5 min. The extracts were filtered through filter paper (Toyo Roshi Co.). The residues on the paper were washed twice by warm ethanol. The washings were added to the filtrates, and the total solutions were adequately diluted by ethanol and measured by the spectrophotometric method. The results of the analysis from colorimetric and thin-

layer chromatographic methods coincided well. Since the sum of the mole concentrations of DHA and NR-DHA at each time interval was equal to the initial mole concentration of DHA, the reaction was terminated at the formation of the Schiff's base type compounds.

RESULTS AND DISCUSSION

Reaction in 99.73 Per Cent v/v[1] Ethanol Solution— A mixture of DHA and an equivalent mole of PE-NH $(1 \times 10^{-2}M)$ was placed in the constant-temperature bath, and the proceeding reaction was studied by estimating the concentration of DHA by means of the color assay developed with titanium chloride. The reaction rate was expressed as:

$$v = k_1 (\text{DHA})(\text{PE-NH}) \qquad (\text{Eq. 1})$$

and satisfied the rate equation,

$$dx/dt = k_1 (a - x)^2 \qquad (\text{Eq. 2})$$

and the integrated form is

$$1/(a - x) = k_1 t + 1/a \qquad (\text{Eq. 3})$$

where a is the initial concentration for DHA and PE-NH, x represents the consumed concentration at t for DHA and PE-NH, and k_1 is the apparent second-order rate constant. The second-order plots for the reaction are given in Fig. 1.

Effect of Addition of Water—The addition of water retarded the apparent rate constant, k_1, and accelerated the hydrolysis of NR-DHA (k_2).

$$\text{PE-NH} + \text{DHA} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{NR-DHA} + \text{H}_2\text{O}$$
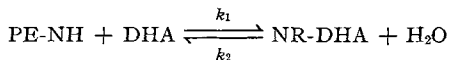
The observed apparent equilibrium constant, K, in the presence of an excess amount of water is

$$K = \frac{(\text{NR-DHA})}{(\text{DHA})(\text{PE-NH})} \qquad (\text{Eq. 4})$$

and the apparent rate constants for the forward and the reverse reactions, k_1 and k_2, are calculated by means of the following equations:

$$k_1 = \frac{1}{t} \frac{2.303 \, x_e}{a^2 - x_e^2} \log \frac{x_e (a^2 - xx_e)}{a^2 (x_e - x)} \quad (\text{Eq. 5})$$

$$k_2 = \frac{1}{K} k_1 = \frac{(a - x_e)^2}{x_e} k_1 \qquad (\text{Eq. 6})$$

where x_e is the concentration of consumed DHA and PE-NH at equilibrium. The values of k_1 are calculated from the slope of $2.303 \, x_e/(a^2 - x_e^2) \log x_e(a^2 - xx_e)/a^2(x_e - x)$ versus t.

It was observed that the rate constant for forward reaction, k_1, decreased with a decrease of concentration of ethanol in solvent. This fact may be due to an increase in ionizing power of solvent by the addition of water to solvent. The plot of log k_1 versus the reciprocal of dielectric constants,[2] $1/D$, is an approximate straight line (Fig. 2). This phenomenon may be ascribed to the degree of ionization of both DHA and PE-NH at initial stage, namely, the relative concentration of free DHA and free PE-NH.
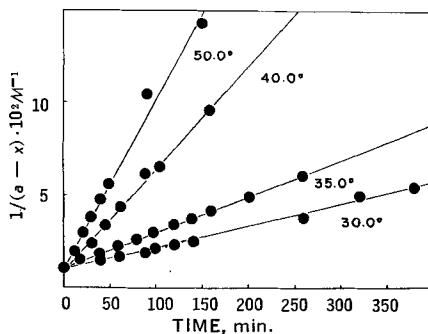
[1] Measured by titrimetric (Karl Fischer) method.
[2] Dielectric constants of ethanol–water mixtures were obtained from Akerlöf, G., *J. Am. Chem. Soc.*, **54**, 4125(1932).

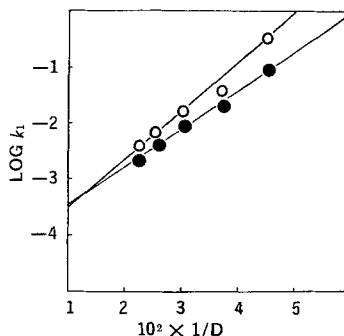*Fig. 1—Second-order plots for the reaction of dehydroacetic acid with β-phenethylamine in 99.73% ethanol.*

Fig. 2—Influence of the dielectric constant on the reaction rate constants for the reaction of dehydroacetic acid with the primary amines. Key: ●, β-phenethylamine; ○, benzylamine.
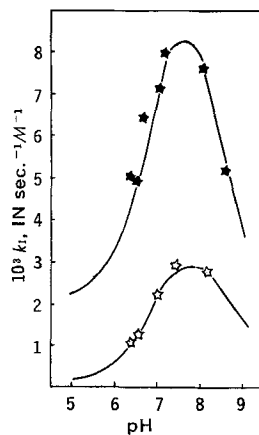

Fig. 3—The k_1-pH profiles for the reaction of dehydroacetic acid with β-phenethylamine. Key: ☆, experimental values at 40°; ★, experimental values at 60°; ———, theoretical lines.

Effect of pH—In Fig. 3, the apparent second-order rate constants in 80% ethanol at 40 and 60° are plotted against the observed pH values. All points on the pH-rate profile have been obtained in the acetate buffer solution or no buffer solution. When
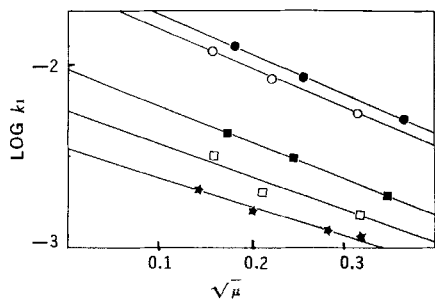
Fig. 4—Effect of the ionic strength on the reaction rate constants for the reaction of dehydroacetic acid with β-phenethylamine. Key: ●, pH 6.84 at 60°; ○, pH 6.58 at 60°; ■, pH 6.98 at 40°; □, pH 6.57 at 40°; ★, pH 6.37 at 40°.

TABLE I—OBSERVED RATE CONSTANT ($10^3 k_1$ IN M^{-1} sec.$^{-1}$) FOR THE REACTION OF DHA WITH PE-NH IN 80% ETHANOL[a]

Observed pH	Observed Rate Constant
40° C.	
6.37[b]	0.97
6.57[b,c]	1.44
6.98[b,c]	2.25
7.50	2.90
8.13	2.80
60° C.	
6.58[b,c]	5.01
6.84[b]	6.45
7.07	7.17
7.19[c]	7.80
8.10[c]	7.67
8.63	5.00

[a] Initial concentration: DHA, 1×10^{-2} M; PE-NH, 1×10^{-2} M ($\mu = 0.1$). [b] When the ionic strength was changed, a significant difference in the apparent rate constant was observed (see Fig. 4). [c] When the concentration of acetate buffer solution was at eightfold ranges, no significant differences in the apparent rate constants were observed.

the concentration of acetate buffer solution ($\mu = 0.1$) was changed at eightfold ranges, no significant difference in the apparent rate constants was observed. But a significant ionic strength effect was observed for the reaction at the same pH and buffer (Fig. 4); therefore, the ionic strength was adjusted to 0.1 by the addition of sodium chloride. As the concentration of the protonated PE-NH increases with a decrease in pH, the ability of PE-NH to act as a nucleophilic agent is lost, and thus a decrease in the rate constant is observed. On the other hand, in alkaline media where the concentration of the protonated PE-NH is at a minimum, a reduction in the rate is again observed. This is attributed to a decrease in the concentration of free DHA. Since a maximum of the pH-profile occurred at about a pH of 7.5 (Fig. 3 and Table I), these results suggest that a probable mechanism would be the attack of a nucleophilic reagent such as free undissociated PE-NH onto the protonated carbonyl carbon atom in free undissociated DHA. Assuming that the relative species are free PE-NH and DHA, the forward rate v_f is expressed as:

TABLE II—APPARENT pKa VALUES[a] IN ETHANOL-WATER MIXTURES AT 40°C.

Vol. % of Ethanol[b]	pKa
β-Phenethylamine	
97.5	8.00
88.2	8.37
78.4	8.61
68.6	8.71
49.0	8.80
Dehydroacetic Acid	
98.5	7.55
89.3	7.25
79.2	6.80
69.5	6.40
59.2	6.25

[a] The titrations were conducted on 40-ml. sample solutions with 0.1 N HCl and 0.1 N NaOH aqueous solutions and pKa values were calculated by Shima's method (see Shoji, R., and Yoshimura, H., "Theory and Measurement of pH," Maruzen, Tokyo, Japan, p. 130.). Estimated pKa values for β-phenethylamine and dehydroacetic acid in 80% ethanol were 8.58 and 6.83, respectively. [b] The per cent ethanol at each pKa was calculated from the known volume in the original solution and the known volume of aqueous titer added to achieve the apparent pH corresponding to that pKa value.

$$v_f = k_f (\text{DHA})_f (\text{PE-NH})_f \qquad (\text{Eq. 7})$$

$$= k_f \frac{K_1}{K_1 + (\text{H}^+)} \frac{(\text{H}^+)}{K_2 + (\text{H}^+)} (a - x)^2 \quad (\text{Eq. 8})$$

where k_f is the true rate constant for the forward reaction. The K_1 and K_2 are the dissociation constants of the protonated PE-NH and DHA, respectively. The apparent rate constant, k_1, for the forward reaction can be written:

$$k_1 = k_f \frac{K_1}{K_1 + (\text{H}^+)} \frac{(\text{H}^+)}{K_2 + (\text{H}^+)} \quad (\text{Eq. 9})$$

Moreover, k_f is expressed as:

$$k_f = k_0 + k_{\text{H}+} (\text{H}^+) \qquad (\text{Eq. 10})$$

where k_0 may be the rate constant for the solvent catalyzed, and $k_{\text{H}+}$ is the bimolecular rate constant for hydrogen ion. A plot of k_f against the hydrogen-ion concentration estimates $k_{\text{H}+}$ from the slope and k_0 from the intercept. The values of k_0 and $k_{\text{H}+}$ were calculated to be 2.2×10^{-1} mole^{-1} sec.$^{-1}$ and 5.5×10^4 mole^{-1} sec.$^{-1}$ at 40°, 6.0×10^{-1} mole^{-1} sec.$^{-1}$ and 8.0×10^5 mole^{-1} sec.$^{-1}$ at 60°, respectively. The values of K_1 and K_2 in 80% ethanol were calculated by titration in various ethanol concentrations at 40°. The results are shown in Table II. The values of K_1 and K_2 in 80% ethanol at 40° applied to Eqs. 8 and 9.

In conclusion, it has been suggested that the rate-determining step for the reaction between DHA and PE-NH in 80% ethanol is the attack of free PE-NH on the protonated carbonyl carbon atom in free DHA.

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