terms by simply decreasing the agitation rate.

It is proposed that the problem we face in developing *in vivo-in vitro* correlations could involve the mechanism of dissolution as well as the intensity of agitation. The nature of the dissolution mechanism in the gastrointestinal tract is not at all clear. In view of the type of agitation a dissolving solid encounters in the tract as a result of gastrointestinal motility, it is quite conceivable that dissolution will not follow pure diffusion layer theory. The process may be obscured by periodic and irregular diffusion currents which could result in a highly complex dissolution mechanism. Perhaps a combination of diffusion layer and Danckwerts' mechanisms may be operative. A great deal of further experimentation on dissolution is required to resolve these questions.

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Keyphrases

Benzoic acid dissolution rates
Surfactant solution—benzoic acid solubility
Stirred conditions—dissolution rates
Static conditions—dissolution rates

Kinetics of Reaction of Dehydroacetic Acid II

Reaction With Primary Amines

By S. GOTO, A. KONO, and S. IGUCHI

It was concluded that the conversion of 2,6-bis-(phenethylamino)-2,5-heptadiene-4-one (BPH) into *N*-phenethyl-lutidone (PL) in 80 percent ethanol is accounted for in terms of aminolysis reaction. In general, the aminolysis reaction proceeds *via* a two-step reaction path involving a *gem*-diamine as an intermediate. The first step in the above reaction of BPH becomes reversible by the addition of excess β-phenethylamine (PE-NH). The mechanistic pathway was discussed. Moreover, it was supported with kinetic investigation using an analog computer that BPH is the most important intermediate in the conversion of 3-(1-phenethylamino)ethylidene-6-methyl-3*H*-pyran-2,4-dione (Schiff's base) to PL in the presence of large excess PE-NH.

IN A PREVIOUS paper (1), the kinetic study of Schiff's base formation between dehydroacetic acid (DHA) and β-phenethylamine (PE-NH) has been shown. A further kinetic investigation on the transformation of Schiff's base to *N*-phenethyllutidone (PL)[1] is described, and also an attempt was made to confirm kinetically the reaction mechanism that was proposed by Iguchi *et al.* (2).

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.

Preparation of 3-(1-Phenethylamino)ethylidene-6-methyl-3H-pyran-2,4-dione (Schiff's Base)—DHA (10 Gm.) was dissolved in 15 ml. ethanol solution containing an equivalent mole of PE-NH and the solution was warmed on a steam bath. After 30 min. the solvent of reaction mixture was distilled *in vacuo*, the residue was washed once with ethyl ether, and recrystallized from ethyl ether–benzene, m.p. 89–91°.

Preparation of 2,6-Bis-(phenethylamino)-2,5-heptadiene-4-one (BPH)—To 5 ml. of ethanol solution of DHA (3.4 Gm.) was added an excess of

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[1] The final product of this series of the reaction.

PE-NH and kept at room temperature for 24 hr. After the solvent was evaporated, the residue was washed once with ethyl ether and then recrystallized, m.p. 115–116°.

Preparation of *N*-Phenethyl-lutidone (PL)—One gram of BPH was dissolved in 20 ml. of water, and a few drops of hydrochloric acid or acetic acid were added. After 10 hr. heating, the reaction solution was dried *in vacuo*. The residue was recrystallized from aqueous methanol, m.p. 167–168°.

Kinetic Studies—BPH was dissolved in 80% v/v ethanol which had previously been adjusted to the desired pH with the addition of acetate buffer solution. The pH values were determined with a Hitachi-Horiba pH meter using glass-saturated calomel electrodes. The ionic strength of the solution was adjusted to 0.1. The solution was transferred to a glass 100-ml. volumetric flask and placed in a constant temperature bath maintained at 60°. Aliquots (1 ml.) were withdrawn at regular time intervals, and the reaction was quenched by cooling in ice water and diluted with distilled water. The progress of the reaction was determined by measuring the samples for residual BPH spectrophotometrically. The products, intermediate (X) and PL, were also determined spectrophotometrically. In the case of conversion reaction of BPH or Schiff's base in the presence of excess PE-NH, the sample solutions were prepared by dissolving substrates in various concentrations of PE-NH (0.0–1.0 *M*).

Calculations of Amounts of Products, BPH, and Schiff's Base—The absorbances of the sample solution were measured against a blank solution at 266 mμ, 322 mμ, and 370 mμ. The calculations are based on the relations;

$$A_{266} = \epsilon^{PL}_{266}(PL)$$

$$A_{322} = \epsilon^{X}_{322}(X) + \epsilon^{BPH}_{322}(BPH)$$

$$A_{370} = \epsilon^{BPH}_{370}(BPH) + \epsilon^{X}_{370}(X)$$

Molar absorptivity values are $\epsilon^{PL}_{266} = 19{,}900$, $\epsilon^{X}_{322} = 20{,}000$, $\epsilon^{BPH}_{322} = 3{,}600$, $\epsilon^{BPH}_{370} = 41{,}600$, $\epsilon^{X}_{370} = 2{,}500$,

where $A\lambda$ is the absorbance at a wavelength λ in mμ for the sample solution, and (PL), (X), and (BPH) are molar concentrations of PL, intermediate, and BPH, respectively. The molar absorptivity value of intermediate could be estimated from the initial concentration of BPH, the molar absorptivity value of BPH, and the absorbances of the sample solution at 322 mμ and 370 mμ in the initial reaction time when the formation of PL was not observed.

In the case of conversion reaction of Schiff's base in the presence of excess PE-NH, the absorbances of the sample solution were measured at 266 mμ, 310 mμ, and 370 mμ. The calculations for each compound follow:

$$A_{266} = \epsilon^{PL}_{266}(PL) + \epsilon^{S}_{266}(S)$$

$$A_{310} = \epsilon^{S}_{310}(S) + \epsilon^{BPH}_{310}(BPH)$$

$$A_{370} = \epsilon^{BPH}_{370}(BPH)$$

Molar absorptivity values are: $\epsilon^{S}_{266} = 1{,}800$, $\epsilon^{S}_{310} = 16{,}100$, $\epsilon^{BPH}_{310} = 2{,}600$. Other values were already described, where (S) is the concentration of Schiff's base in the aliquot. The percentage deviation for above analytical procedure was ±4%. The existence of PE-NH did not interfere with their determinations.

Thin-Layer Chromatographic Analysis—The thin-layer plates (5 × 20 cm.) were coated with a 0.5-mm. layer of Silica Gel B-5 (Wako pure reagent). After the sample solution was spotted on the plates, the plates were developed with ethyl ether. The solvent front was allowed to advance 13 cm. When the developed plates were exposed to iodine vapor in a sealed glass box, the visible spots with R_f values corresponding to BPH (0.47) and PL (0.0) were recognized. When acetone was used, the R_f value of PL ascended 0.15. In the case of the reaction between Schiff's base and PE-NH, the R_f value for Schiff's base was 0.22. Unfortunately, it was impossible to get an R_f value for the intermediate on the developed plates.

Computer Analysis—A Beckman Instrument Inc., Ease analog computer was utilized for this investigation.

RESULTS AND DISCUSSION

Conversion of 2,6-Bis-(phenethylamino)-2,5-heptadiene-4-one (BPH) in Acetate Buffer Region—An attempt was made at first to investigate the conversion of BPH to PL in acetate buffer region. The apparent first-order rate constants for the loss of BPH decreased rapidly with the increase of experimental pH as shown in Table I.

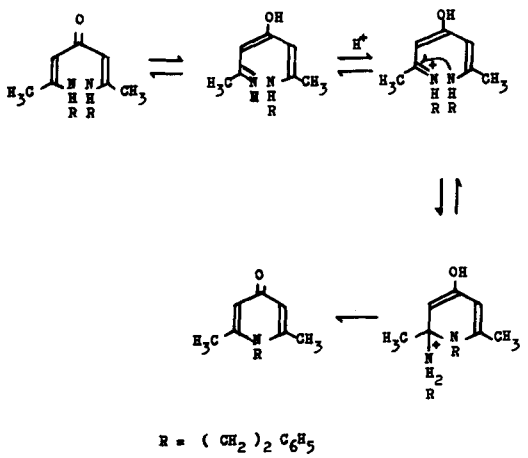
The conversion of BPH was followed by the loss of spectrophotometric absorbance. Because absorbance at 370 mμ decreased with time concurrent with increase in absorbance over the region 322 to 266 mμ, the following route was postulated:

$$\text{BPH} \xrightarrow{k_1} \text{intermediate (X)} \xrightarrow{k_2} \text{PL}$$

TABLE I—APPARENT FIRST-ORDER RATE CONSTANT FOR THE CONVERSION OF BPH IN ACETATE BUFFER REGION AT 60°[a]

Observed pH	k_1	k_2
4.82	...	7820
6.41	...	226
7.11	630, 620[b]	46.0, 46.0[b]
7.53	219	22.0
8.18	51.3, 54.5[c]	8.80, 8.80[c]
8.50	25.1, 27.0[d]	2.00, 2.00[d]
9.85[e]	6.97	1.13
10.1[f]	7.00	3.37

[a] $10^4\ k_1$ and $10^6\ k_2$ in sec.$^{-1}$. Initial concentration: BPH, 0.8 to 3.0 × $10^{-3}M$. The ionic strength was adjusted to 0.1 by the addition of sodium chloride. [b] When the concentration of acetate buffer solution was at twofold ranges, no significant differences in the apparent rate constants were observed. [c] When the concentration of acetate buffer solution was at a half range, no significant differences in the apparent rate constants were observed. [d] When the concentration of substrates was twofold ranges, no significant differences in the apparent rate constants were observed. [e] No acetate buffer solution was used. [f] Sodium acetate was used.



R = $(CH_2)_2C_6H_5$

Scheme I—Proposed route for the conversion of BPH into PL.

The first-order rate constants for the conversion of BPH at pH values below 6.4 were quite large. The reason for this behavior is considered as follows. Under more acidic conditions, all BPH is predominantly protonated and the intramolecular amine attached to the protonated carbon atom becomes an important reaction pathway, and it is expected that the conversion rate of BPH is related to the existence of protonated BPH. The pH-independent region for k_1 is observed under a weakly basic condition and the rate becomes small in this region. It is probably the consequence of the minimum protonated BPH existence. These results may be accounted for in terms of intramolecular aminolysis reaction that is the replacement of the amine component by another amine in the same molecule. Numerous papers (3–5) have mentioned that the imines, particularly when existing in cationic form $\left(\diagup C{=}\overset{+}{N}\diagdown\right)$, readily react with various nucleophilic reagents involving water and amines, and that the reaction is accelerated with an addition of hydrogen ions or an increase in negative charge of nucleophilic reagents. The conversion mechanism of BPH to PL was proposed as shown in Scheme I.

The reaction proceeds *via* a two-step reaction path involving a *gem*-diamine as an intermediate. Such an intermediate is analogous to the carbinolamine for hydrolysis and formation of Schiff's base. Moreover, the formation and decomposition of the *gem*-diamine are expected to be a function of pH. Koehler *et al.* (3) have stated "although the existence of an intermediate in the aminolysis reaction of Schiff's base has yet to be demonstrated, the nature of the reaction almost certainly required that they proceed via a two-step reaction path involving a gem-diamine as intermediate." In our experiment, the existence of the intermediate was not shown by the thin-layer chromatogram. But it is expected that the absorbance at 322 mμ may show the existence of the intermediate.

Conversion of 2,6-Bis-(phenethylamino)-2,5-heptadiene-4-one (BPH) in the Presence of Excess β-Phenethylamine (PE-NH)—The first step in BPH conversion reaction became reversible in the presence of excess PE-NH. When a great excess of PE-NH was added, the equilibrium constant (K) governing the formation of the intermediate (X) from BPH was smaller than in the presence of small excess PE-NH. It may be explained by the acceleration of reverse reaction (X → BPH) in more basic conditions because it was observed that the reverse reaction rate became quite large in a 0.04 N NaOH solution. The conversion rate of the intermediate (X) into PL was also accelerated proportionally to the increasing PE-NH. This fact may be rationalized in terms of the general acid catalytic reaction by the protonated PE-NH in this step. Therefore, the process of the conversion of BPH to PL can be described as follows:

$$\text{BPH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{intermediate (X)} \overset{k_2}{\longrightarrow} \text{PL}$$

Based on the assumption, $k_1 + k_{-1} \gg k_2$, the molar concentrations of BPH, intermediate (X), and PL over the whole reaction period can be described by approximate equations (6) as shown in the following:

$$(\text{BPH}) = \frac{k_{-1}}{(k_1 + k_{-1})} \cdot e^{-\frac{k_1}{(k_1 + k_{-1})} \cdot k_2 t} \quad \text{(Eq. 1)}$$

$$(\text{X}) = \frac{k_1}{(k_1 + k_{-1})} \cdot e^{-\frac{k_1}{(k_1 + k_{-1})} \cdot k_2 t} \quad \text{(Eq. 2)}$$

$$(\text{PL}) = 1 - e^{-\frac{k_1}{(k_1 + k_{-1})} \cdot k_2 t} \quad \text{(Eq. 3)}$$

Where (BPH), (X), and (PL) are the molar concentrations of BPH, intermediate, and PL, respectively. The k_1 and k_{-1} are the rate constants for forward and reverse reactions, respectively, and k_2 is the rate constant for formation of PL from the intermediate. The equilibrium constant ($K = k_1/k_{-1}$) shown in Table II, can be estimated from experimental results.

Under the experimental condition (BPH: $1 \times 10^{-2}M$, PE-NH: 1.0 M) in which PE-NH is

TABLE II—SUMMARY OF RATE CONSTANT[a] AND EQUILIBRIUM CONSTANT ON THE REACTION OF BPH IN THE PRESENCE OF EXCESS β-PHENETHYLAMINE AT 60°

REACTION: $\text{BPH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{X} \overset{k_2}{\longrightarrow} \text{PL}$

(BPH) M	(PE-NH) M	$\frac{(\text{PE-NH})}{(\text{BPH})}$	$K\left(=\frac{k_1}{k_{-1}}\right)$	k_1	k_{-1}	k_2
0.00146	0.0	0.0	...	6.97	0.0	0.0113
0.00139	0.017	12.2	9.7	6.53	0.673	0.0219
0.00155	0.024	15.5	6.5	6.22	0.957	0.0246
0.00105	0.100	95.4	1.8	6.40	3.56	0.140

[a] 10^4k in sec.$^{-1}$.

TABLE III—SUMMARY OF RATE CONSTANT[a] AND EQUILIBRIUM CONSTANT ON THE REACTION OF BPH IN THE PRESENCE OF EXCESS β-PHENETHYLAMINE AT 60°

REACTION: $BPH \underset{}{\overset{K}{\rightleftharpoons}} X \xrightarrow{k_2} PL$, with $BPH \xrightarrow{k_3} C$

(BPH) M	(PE-NH) M	(PE-NH)/(BPH)	K	k_2	k_3
0.010	0.20	20.0	0.33	0.120	0.008
0.010	0.50	50.0	0.18	0.190	0.008
0.010	1.00	100.0	...	0.019	0.007
				$\widetilde{k_{app.}}$[b]	

[a] k in hr.$^{-1}$. [b] $k_{app.}$ represents the apparent first-order rate constant for the conversion of BPH into PL.

greatly in excess of substrate, the reaction appearance has been changed, that is, the concentration of the intermediate (X) was maintained quite small or almost zero over the entire experimental period of kinetic run. On the other hand, it was determined experimentally that the amount of PL formed was not equal to the amount of BPH decreased. Based on the above result, another route must be established. Three pathways are considered:

$BPH \rightleftharpoons X \rightarrow PL$, with $BPH \rightarrow C$ (Route 1)

$BPH \rightleftharpoons X \rightarrow PL$, with $X \rightarrow C$ (Route 2)

$BPH \rightleftharpoons X \rightarrow PL \rightarrow C$ (Route 3)

But Route 3 should be neglected because no degradation was observed for PL over the entire experimental time. The selection of a suitable route was conducted by using an electronic analog computer. Since the equilibrium between BPH and the intermediate (X) is quickly formed at the initial stage of conversion reaction, the rate constant in each reaction step can be easily estimated. When their validity was checked by the analog computer, a good agreement was found between the computer calculated concentration-time curves and the experimental results in the case of Route 1. And the Route 2 may be omitted. The sign C shows the overall products except PL and they are assumed to be mainly various condensation products. The obtained values for rate constants and equilibrium constants are shown in Table III.

Conversion of Schiff's Base in the Presence of Excess β-Phenethylamine (PE-NH)—The experimental condition of treating a great excess of PE-NH (1.0 M) over the Schiff's base (1×10^{-2} M) was selected for the purpose of deciding on a major reaction route. The route for the conversion of Schiff's base to BPH would be attacked by an amine molecule on 6 position of carbon atom, opening the pyronl ring and continuous decarboxylation. But, unfortunately, no intermediates in the above reaction route could be isolated synthetically and spectrophotometrically. Based on the assumption that the concentrations of intermediates are almost zero, the apparent first-order rate constant on the conversion of Schiff's base into BPH was calculated. Typical changes of the spectral curves as a function of time are given in Fig. 1.

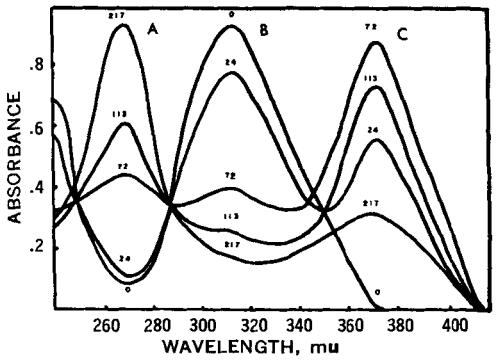


Fig. 1—The spectral changes of the reaction between Schiff's base and excessive β-phenethylamine in 80% ethanol at 60°. Initial concentration: Schiff's base, 1×10^{-2}M; β-phenethylamine 1.0 M. Key: A, PL; B, Schiff's base; C, BPH. The time of the readings after the start of the reaction is given in hours.

The changes of maximum absorbance at 310 mμ are characteristic of the first-order conversion of Schiff's base, because the intermediate (X) ($\lambda_{max.}$ = 322 mμ) formed remains almost zero during the solution conversion. The obtained first-order rate constant for loss of Schiff's base is adapted to Eqs. 4–7 together with the rate constants which were calculated in the previous section for BPH conversion in the presence of a great excess of PE-NH.

Schiff's base (S) $\xrightarrow{k}$ (0.0145 hr.$^{-1}$) BPH $\xrightarrow{k'}$ (0.019 hr.$^{-1}$) PL; BPH $\xrightarrow{k''}$ (0.007 hr.$^{-1}$) other products (C)

Therefore, the differential equations for the above route are:

$$d(S)/dt = -k(S) \quad \text{(Eq. 4)}$$

$$d(BPH)/dt = k(S) - k'(BPH) - k''(BPH) \quad \text{(Eq. 5)}$$

$$d(PL)/dt = k'(BPH) \quad \text{(Eq. 6)}$$

$$d(C)/dt = k''(BPH) \quad \text{(Eq. 7)}$$

The circuit diagram used in an electronic analog computation for the above equations is shown in Fig. 2.

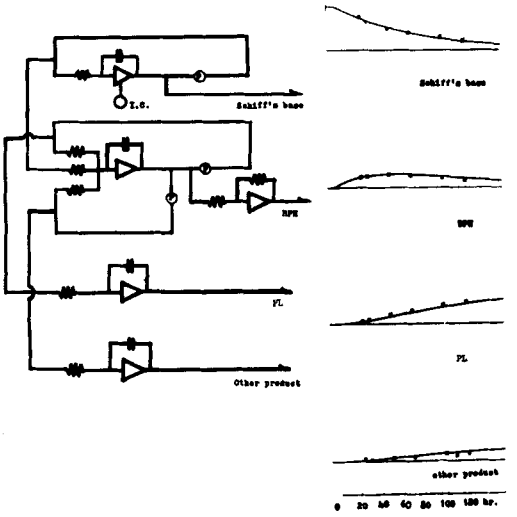


Fig. 2—Block diagram and computer curves obtained by computer analysis. Key: ——, obtained curve; •, experimental value

The agreement between the obtained and experimental values supports the idea that the reaction proceeds on the strength of the above mechanism represented by the simplified scheme and that BPH is the most important intermediate in the conversion route of Schiff's base into PL in the presence of a great excess of PE-NH.

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Keyphrases

Dehydroacetic acid
Schiff's base transformation *N*-phenethyllutidone
Aminolysis reaction
UV spectrophotometry—kinetic analysis
TLC—analysis

Thiry-Vella Dog as a Biologic Model for Evaluation of Drug Absorption from the Intestinal Mucosa

By R. G. SAMPLE, G. V. ROSSI, and E. W. PACKMAN

The Thiry-Vella fistula dog was found to provide a quantitative and reproducible pharmacometric system for the evaluation of drug absorption from the intestinal mucosa. Instillation of buffered solutions of *N*-acetyl-*p*-aminophenol (acetaminophen) into the *in situ* jejunal loop resulted in rapid and essentially complete absorption. Within the range examined (75, 150, 300, and 450 mg.), an increase in dose was reflected by a commensurate increase in the plasma concentration of *N*-acetyl-*p*-aminophenol. Plasma concentration curves for each dosage level were parallel over a 2-hr. postadministration period. The Thiry-Vella fistula provides a stable, readily accessible segment of intestinal mucosa with blood, nerve, and lymph supplies intact. The chronicity of the fistula preparation permits each animal to serve as its own control and to be used repeatedly to compare the absorption characteristics of the same drug at different dosage levels, as well as different drugs at the same or alternate dosage levels.

A WIDE VARIETY of *in vivo* and *in vitro* techniques have been employed in the study of intestinal absorption depending fundamentally on the objectives and preferences of the investigator. Although many methods have been introduced, there has not been developed a relevant and reproducible biologic model for the study of factors relating to drug absorption from the intestinal mucosa, and for evaluation of concepts established principally in *in vitro* systems.

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