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The Binding Affinity of Amphetamine to 4-Pyridinecarboxaldehyde and the Coenzymes Pyridoxal and Pyridoxal-5-phosphate

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Equilibrium and rate constants have been determined for the reactions of amphetamine with pyridine-4-carboxaldehyde, pyridoxal and pyridoxal-5-phosphate to give the corresponding Schiff bases. The reactions were found to be dependent on amphetamine concentration and pH of the solution.

The overall equilibrium constants are 5.97×10^2, 13.7×10^3 and 8.2×10^3 for amphetamine with pyridine-4-carboxaldehyde, pyridoxal and pyridoxal-5-phosphate, respectively.

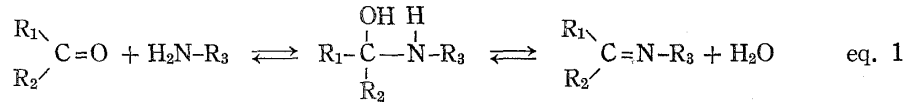
Keywords——pyridoxal; pyridoxal-5-phosphate; 4-pyridine-carboxaldehyde; Schiff's base formation; equilibrium constant; kinetic of reaction

Introduction

During the past two decades increasing attention has been focused upon pyridoxal-5-phosphate as a coenzyme that catalyzes transamination for a wide scope of amino acids.

Kynurenine aminotransferase (EC 2.6.1.7) and kynurenine hydrase (EC 3.7.1.3) catalyze the conversion of kynurenine to kynurenic acid and anthranilic acid, respectively. These two enzymes require the participation of pyridoxal-5-phosphate as a coenzyme. It was shown by Amer, *et al.*[2,3] that potassium antimonyl tartarate (tartaremitic) inhibited these two enzymes, in mouse kidney and liver homogenate, respectively. It was suggested by the authors that the inhibition was mainly due to a reduction in the level of pyridoxal-5-phosphate which resulted in change in the relative concentration of metabolites of kynurenine, which are known to be bladder carcinogens[4–6]

Amphetamine, well known central stimulant which acts on peripheral sympathetic nervous system, may act as an inhibitor to some enzymes involving the coenzyme pyridoxal-5-phosphate.[7] This may be attributed to the formation of a Schiff's base through a tetrahedral intermediate,[8] as follows.



eq. 1

A prior investigation of the interaction of amphetamine with 4-pyridinecarboxaldehyde and the coenzymes pyridoxal and pyridoxal-5-phosphate, was found essential before studying its effect upon the above mentioned enzyme systems.

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Experimental

4-Pyridinecarboxaldehyde (P) was purified under a reduced pressure in nitrogen atmosphere as previously reported.[9] The concentration of the equilibrated aldehyde aqueous stock solution was checked spectrophotometrically at λ 285 nm (ε=1530 L mol^{-1} cm^{-1}). The stock solution was stored in a dark container at 0°. Pyridoxal HCl (PL) and pyridoxal-5-phosphate (PLP) of high purity (>97%) were obtained from Fluka and were used without further purification. Their stock solutions were checked potentiometrically. Amphetamine sulfate, (A), was Smith and Kline product and was used without further purification.

Spectrophotometric measurements were carried out by Pye-Unicam spectrophotometers models SP-8000 and SP-500. The latter is singlebeam provided with a recorder. The cell compartments were thermostated at 25°. The measurements of pH were carried out using a Radiometer digital pH meter model 63 equipped with a combined glass electrole type GK 2301C. The spectrophotometers and the pH meter were calibrated as previously described.[10]

The kinetic studies were carried out using thermostated stock solutions which were adjusted to desired pH byusing dilute NaOH or HCl at constant ionic strength (0.10 M phosphate in case of A-P system and 0.10 M KCl in case of A-PL and A-PLP system). In a thermostated cuvette (1 cm path length in case of A-P and A-PLP systems and 2 or 4 cm pathlengths in case of A-PL system) containing a known concentration of the aldehyde, a proper amount of amphetamine of a known volume and concentration was added by a Hamilton syringe. The resulting solutions were then stirred well in a time of approximately 15 seconds before being ready for the kinetic runs. The reactions were followed with time in the pH range of 8.0—11.0. It is worth mentioning that no reaction has been observed with time in acidic solutions. Moreover, it is found that the spectra of the equilibrated reaction products at higher pH's (>10) can be transformed to that of the reactants by just lowering the pH of the solutions to values <7.0.

The kinetic runs were carried out at wavelengths 276 nm, 275 and 390 nm in case of 4-pyridinecarboxaldehyde, pyridoxal-5-phosphate and pyridoxal, respectively. The wavelengths used were chosen in order that a maximum change in absorbance with time could be observed.

It is important to mention that molar absorptivity of amphetamine is zero at $\lambda \geqslant 275$ nm in the pH range (1.0—12.0). The molar absorptivity of the equilibrated aqueous solution of 4-pyridinecarboxaldehyde at λ 276 nm was 1130 L mole^{-1} cm^{-1} at pH's higher than 7.0, On the other hand variable average molar absorptivities were observed at λ 275 and 390 nm for PLP and PL at pH's higher than 7.0.

In all the kinetic runs, the amphetamine concentration was at least ten times the concentration of the aldehydes under consideration.

Results and Discussion

Schiff Base from Amphetamine and 4-Pyridinecarboxaldehyde

Figure 1 shows the spectra of amphetamine at various pH values. The spectra retain the same pattern with slight increase in absorption as pH increases. Figure 2 shows the variation in absorbance as function of pH at λ 267 nm. The pK_a value of the deprotonation of amino group was calculated using the method of successive approximation[11]. A value of 10.23±0.11 was obtained for pK_a at μ=0.10 M. The pK values of 4-pyridinecarboxaldehyde were previously reported[12] for the deprotonation of heterocyclic nitrogen and the hydrated aldehydic group as follows,

$$HP.H_2O^+ \rightleftarrows P.H_2O + H^+ ; pK_1 = 4.77 \qquad \text{eq. 2}$$

$$P.H_2O \rightleftarrows POH^- + H^+ ; pK_2 = 12.22 \qquad \text{eq. 3}$$

The formation constant of the hydrated form of 4-pyridinecarboxaldehyde was reported by several authors[9,13]

$$P + H_2O \rightleftarrows P.H_2O ; K_h = 1.28 \qquad \text{eq. 4}$$

Spectral change, when 4-pyridinecarboxaldehyde and amphetamine were mixed in aqueous solution at pH values greater than 7.0, is shown in Fig. 3. The spectral band at λ 285 nm of 4-pyridinecarboxaldehyde was blue shifted when the solutions were equilibrated at various

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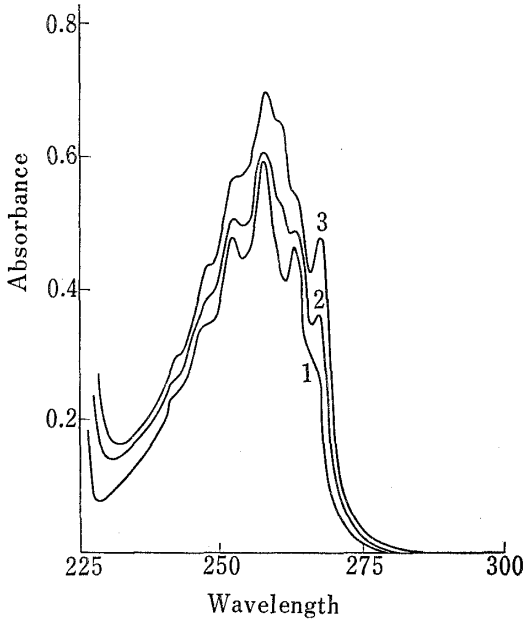

Fig. 1. The Spectra of Amphetamine (3×10^{-3}M) at Various pH Values (l= 1 cm, μ=0.10M, T=25°)

$T_A=3.0\times10^{-3}$M
1: pH= 7.17, 2: pH=10.00, 3: pH=12.00

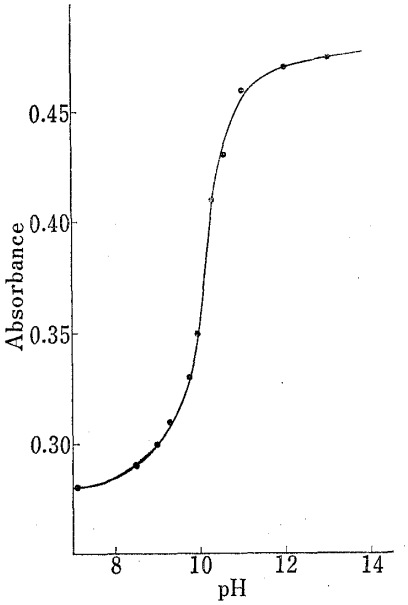

Fig. 2. Absorbance of Amphetamine (3×10^{-3}M) at λ 267.5 nm (l=1 cm) as Function of pH Values

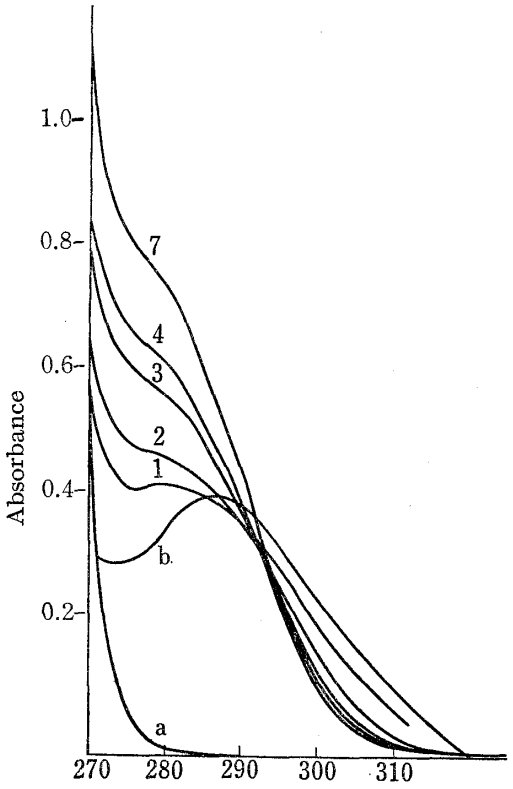

Fig. 3. Spectra of A-P System at Different pH Values under Equilibrium Conditions ($T_P=2\times10^{-4}$M, $T_A=6\times10^{-3}$M, μ=0.10M, T=25°)

a-(A) pH(1—12), b-(P) pH(6—11), c-pH_1 8.53, pH_2 9.0, pH_3 9.5, pH_4 9.73, pH_7 10.5

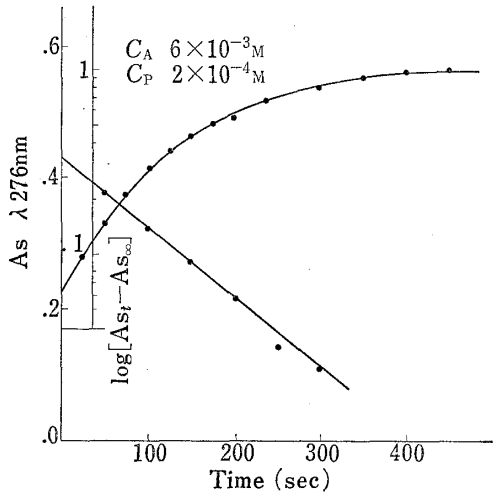

Fig. 4 a) Typical Absorbance-time Curve in A-P System ($T_P=2\times10^{-4}$M, $T_A=6\times10^{-3}$M, pH=10.5, λ= 276 nm, μ=0.10M, T=25°)
b) log (As_∞-As_t) as Function of Time

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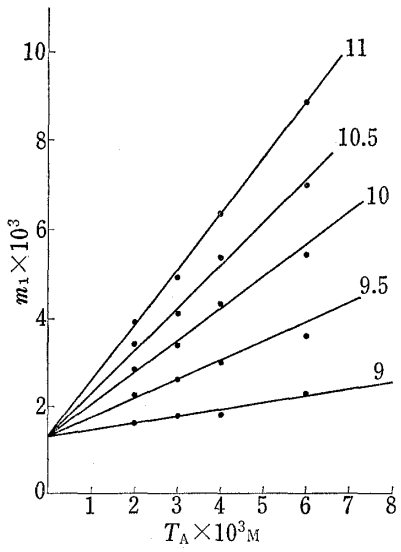

Fig. 5. Macroscopic Rate Constants as Function of the Analytic Concentration of Amphetamine at Various pH Values ($T_P=2\times10^{-4}$M)

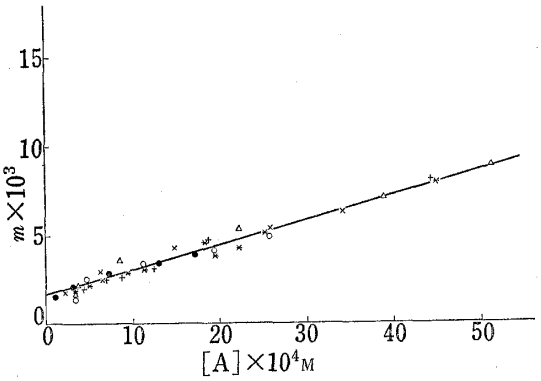

Fig. 6. Macroscopic Rate Constant as Function of the Free Concentration of nonprotonated Species of Amphetamine in A-P System (2×10^{-4}M$\leqslant T_P\leqslant4\times10^{-4}$M, 2×10^{-3}M$\leqslant T_A\leqslant8\times10^{-3}$M, $\mu=0.10$M, $T=25°$)

$C_A\times10^3$M		$C_P\times10^4$
	●:	2.0
	○:	3.0
	×:	4.0
	△:	6.0
	✳:	6.0
	+:	3.0

Table I-a. Macroscopic Rate constants[a] for the A-P system

pH	$C_A\times10^3$M	$C_P\times10^4$M	$m\times10^3$ L mol^{-1}sec^{-1}	$C_P\times10^4$M	$m\times10^3$ L mol^{-1}sec^{-1}	$C_P\times10^4$M	$m\times10^3$ L mol^{-1}sec^{-1}
9.00	2.0	2.0	1.59	3.0	—	4.5	—
	3.0		1.75		1.50		—
	4.0		1.76		—		1.45
	6.0		2.10		1.90		—
	8.0		—		—		1.90
	12.0		—		2.60		2.50
9.50	2.0		2.10		—		—
	3.0		2.60		2.20		—
	4.0		3.00		—		1.70
	6.0		3.60		2.90		—
	8.0		—		—		3.10
	12.0		—		4.70		4.70
10.00	2.0		2.80		—		—
	3.0		3.40		3.10		—
	4.0		4.30		—		2.40
	6.0		5.40		4.20		—
	8.0		—		—		4.70
	12.0		—		7.90		8.00
10.50	2.0		3.40		—		—
	3.0		4.10		3.90		—
	4.0		5.40		—		3.00
	6.0		7.00		5.70		—
	8.0		—		—		6.50
	12.0		—		12.00		12.10
11.00	2.0		3.90		—		—
	3.0		4.90		5.00		—
	4.0		6.30		—		3.90
	6.0		8.80		7.20		—
	8.0		—		—		8.50
	12.0		—		15.60		15.80

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Table I-b

pH	$C_A \times 10^3$M	$C_{PLP} \times 10^4$M	$m_1 \times 10^2$ mol^{-1} sec^{-1}
8.50	2.0	2.0	0.23—0.28
	3.0		0.40
	4.0		0.45
	5.0		0.57
	6.0		0.62
9.00	2.0		0.37
	3.0		0.57
	4.0		0.75
	5.0		0.76—0.96
	6.0		0.99—1.16
9.50	2.0		0.46—0.49
	3.0		0.89—1.06
	4.0		1.55
	5.0		1.59
	6.0		1.84
10.00	2.0		1.84
	3.0		2.40
	4.0		2.86
	5.0		3.65
	6.0		4.95

Table I-c

pH	$C_A \times 10^3$M	$C_{PL} \times 10^4$M	$m_1 \times 10^2$ mol^{-1} sec^{-1}
9.00	3.00	1.50	0.68
	3.75	1.50	0.75—0.88
	2.00	2.00	0.62
	3.00	2.00	0.67—0.83
9.50	3.00	1.50	1.66—1.92
	3.75	1.50	1.70—1.97
	2.00	2.00	1.30—1.40
	3.00	2.00	1.60—1.97
10.00	3.00	1.50	2.90
	3.75	1.50	4.40
	2.00	2.00	2.90
	3.00	2.00	3.50
10.50	3.00	1.50	6.50
	3.75	1.50	7.20—8.10
	2.00	2.00	4.20
	2.00	3.00	7.08

a) The average of 3 determinations.

Table II. The Values of a and b for A-P, A-PL and A-PLP Systems

System	a) 10^3 sec^{-1}	b) mol^{-1} sec^{-1}
A-P	1.70±0.13	1.30±0.03
A-PL	2.36±0.60	19.41±0.70
A-PLP	2.31±0.20	31.70±3.20

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pH values. The rate of the reaction of A with P is presented in Fig. 4 (a). A linear relationship has been obtained for more than 75% of the reaction when log (As_∞—As_t) was plotted versus time, where As_∞ and As_t are the absorbances at infinite time and at time, t, respectively, Fig. 4(b). In addition, a linear relation between the macroscopic rate constant, m_p, Table Ia, and T_A (the analytic concentration of A) has been obtained at various pH values, Fig. 5. On the other hand when m_p was plotted versus the free concentration of the nonprotonated species of amphetamine, A, a linear relation was also obtained, Fig. 6. This relationship may be expressed explicitly as follows,

$$m = a + b[\mathrm{A}] \qquad \text{eq. 5}$$

where a and b are constants. The free concentration of amphetamine was obtained from the following equation:

$$[\mathrm{A}] = T_A \left(1 + \frac{[\mathrm{H}^+]}{K_a}\right)^{-1}$$

where T_A is the initial concentration of amphetamine, $[H^+]$ is the hydrogen ion concentration, and K_A is the deprotonation constant of amphetamine. The constants a and b were independent of the initial concentration of 4-pyridinecarboxaldehyde in the range of $(2.0—4.0)\cdot10^{-4}$ M. Table II depicts their values.

The interaction of 4-pyridinecarboxaldehyde with amphetamine in alkaline solution may take place according to the following scheme[14]

$$\begin{array}{lllll}
(a) & P & + HA^+ & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & S + H^+ + H_2O \\
 & \updownarrow H_2O & & & \\
(b) & P.H_2O & + HA^+ & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & S + H^+ + H_2O \\
 & \updownarrow + H^+ & & & \\
(c) & POH^- & + HA^+ & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & S + H_2O \\
 & & \updownarrow - H^+ & & \\
(d) & P & + A & \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} & S + H_2O \\
 & \updownarrow & & & \\
(e) & P.H_2O & + A & \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} & S + H_2O \\
 & \updownarrow & & & \\
(f) & POH^- & + A + H^+ & \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} & S + H_2O
\end{array} \qquad \text{eq. 6}$$

where S stands for the Schiff base species. This is in analogy to what has been reported by Sander and Jencks.[13] Protonated species of the Schiff base such as HS^+ was ignored since the basicity of azomel-hine nitrogen is lower than that of the amine and the solution was alkaline. The rate equation describing the formation of S can be written as follows:

$$\frac{d(\mathrm{S})}{dt} = (\mathrm{A})(\mathrm{P})\left[\frac{k_1(\mathrm{H}^+)}{K_A} + \frac{k_2K_h(\mathrm{H}^+)}{K_A} + \frac{k_3K_2K_h}{K_A} + k_4 + k_5K_h + k_6K_2K_h\right]$$
$$- (\mathrm{S})[k_{-1}(\mathrm{H}^+) + k_{-2}(\mathrm{H}^+) + k_{-3} + k_{-4} + k_{-5} + k_{-6}] \qquad \text{eq. 7}$$

If we assume that pH is not altered during the reaction progress, the following integrated expression for eq. 7 can be obtained,

$$\ln((\mathrm{S}_\infty) - (\mathrm{S}_t)) = \ln(\mathrm{S}_\infty) - m_p t \qquad \text{eq. 8}$$

where S_∞ and S_t are the concentrations of the Schiff base at equilibrium and at time t. The

14) The vertical protolytic and hydration reactions are assumed to take place faster than the Schiff base reactions.

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macroscopic rate constant, m_p will have the following form,

$$m_p = (k_{-1}(H^+) + k_{-2}(H^+) + k_{-3} + k_{-4} + k_{-5} + k_{-6}) + (A)\left[\frac{k_1(H^+)}{K_A} + \frac{k_2K_h(H^+)}{K_A} + \frac{k_3K_2K_h}{K_A} + k_4 + k_5K_h + k_6K_2K_h\right] \quad \text{eq. 9}$$

The observed macroscopic rate constant was obtained experimentally from the slope of the plot ln (As_∞—As_t) *vs.t* when $T_A \gg T_p$. The derived rate constant (eq. 9) can be correlated with that obtained empirically (eq. 5), *i.e.*,

$$a = (H^+)(k_{-1} + k_{-2}) + (k_{-3} + k_{-4} + k_{-5} + k_{-6}) \quad \text{eq. 10}$$

$$b = \frac{(H^+)}{K_a}(k_1 + k_2K_h) + \left(\frac{k_3K_2K_h}{K_A} + k_4 + k_5K_h + k_6K_2K_h\right) \quad \text{eq. 11}$$

It is obvious from eq. 10 and 11 that a and b are function of hydrogen ion concentration. This concentradicts the facts that the constants a and b are independent of hydrogen ion concentration, and that reactions (a) and (b) do not contribute significantly to the formation of the Schiff base, *i.e.*,

$$a = k_{-3} + {}_{-4} + k_{-5} + k_{-6} \quad \text{eq. 12}$$

$$b = \frac{k_3K_2K_h}{K_A} + k_4 + k_5K_h + k_6K_2K_h \quad \text{eq. 13}$$

Since the POH^- do not actually exist under the conditions used in this study (8.0>pH> 11), one can suggest that reactions (c) and (f) are of minor importance in the formation of the Schiff base. On the other hand, since the reactions take place in aqueous solution, it is expected that P will be mostly hydrated and the most significant reaction in Schiff base formation is reaction (e). The predetermined values of a and b actually correspond to k_{-5} and k_5K_h. The overall equilibrium constant of the formation of Schiff base is

$$K_p = \frac{[S]}{[P.H_2O][A]} = \frac{k_5}{k_{-5}} = \frac{b}{aK_h} = 5.97 \times 10^2 \quad \text{eq. 14}$$

Schiff Base from Amphetamine and Pyridoxal-5-phosphate

Spectral changes were observed when amphetamine solution was mixed with pyridoxal-5-phosphate solution in the pH range 7.0—11.0. The PLP bands are slightly red shifted. An appreciable increase in absorbance was obtained in the wave length range 275—290 nm, similar to that obtained in case of A-P system, Fig. 7. A linear relationship was obtained when log (As_∞—As_t) was plotted versus time for more than 70% of the reaction, Fig. 8. Table Ib lists the values of m_{PLP}. In addition, a linear relation between the macroscopic rate constant and A, the free concentration of the nonprotonated amphetamine species, was obtained, Table Ib and Fig. 9. This relationship can be expressed similar to eq. 5. The constants a and b are listed in Table II.

Reactions of PLP with amphetamine in alkaline solution may be represented by the following scheme,[14]

$$\begin{array}{lcl}
(a) & H\ PLP.H_2O^{2-} + HA^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & SH^{2-} + H^+ + H_2O \\
 & \Big\updownarrow + H^+ & \Big\updownarrow \\
(b) & PLP.H_2O^{3-} + HA^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & S^{3-} + H^+ + H_2O \\
 & + H^+ \Big\updownarrow \quad\quad + H^+ \Big\updownarrow & \\
(c) & H\ PLP.H_2O^{2-} + A \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & SH^{2-} + H_2O \\
 & \Big\updownarrow + H^+ & + H^+ \Big\updownarrow \\
(d) & PLP.H_2O^{3-} + A \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} & S^{3-} + H_2O
\end{array} \quad \text{eq. 15}$$

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where H PLP·H_2O^{2-} and PLP·H_2O^{3-} (pK_4=8.14[13]) are the hydrated PLP species mainly existing in the pH range consideration. H_4PLP^+, H_3 PLP and H_2 PLP^- species of PLP were ignored since the pK values of their equilibria are 1.60, 3.63 and 5.98, respectively[15]. Nonhydrated species were also neglected under the experimental conditions used (equeous solution). The rate equation describing meachanism 15 can be written as follows:

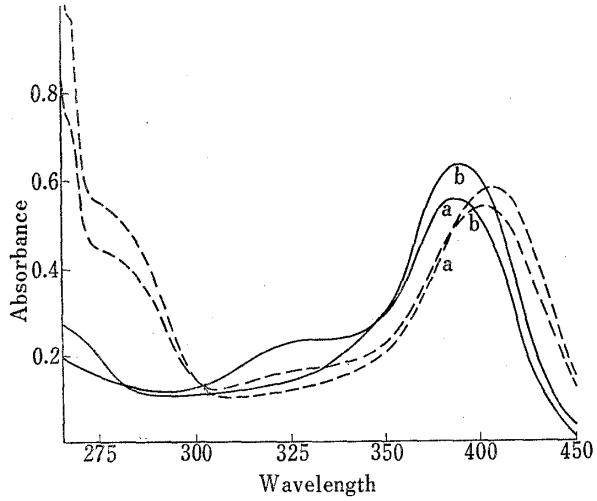

Fig. 7. The spectra of PLP and Equilibrated mixture of A and PLP at Various pH Values ($T_{PLP}=1\times10^{-4}$M, $T_A=5\times10^{-3}$A, $\mu=0.1$M, $T=25°$)

------: A-PLP system
——: PLP
T_{PLP} $=1\times10^{-4}$M
T_A $=5\times10^{-3}$M
a- pH=8.00
b- pH=9.00

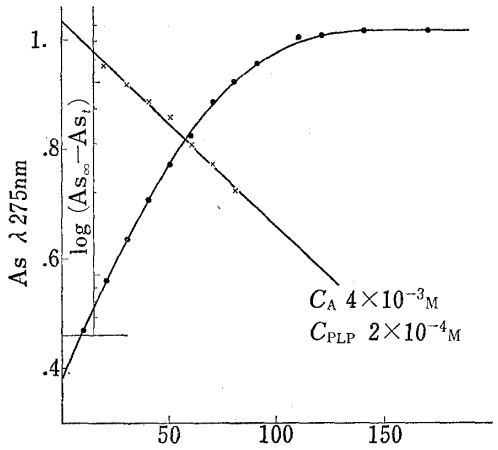

Fig. 8 a). Typical Absorbance-time Curve in A-PLP System ($T_{PLP}=2\times10^{-4}$M, $T_A=4\times10^{-3}$M, $\lambda=275$ nm, pH=9.50, $\mu=0.10$M, $T=25°$)
b). log (As_∞-As_t) as Function of Time

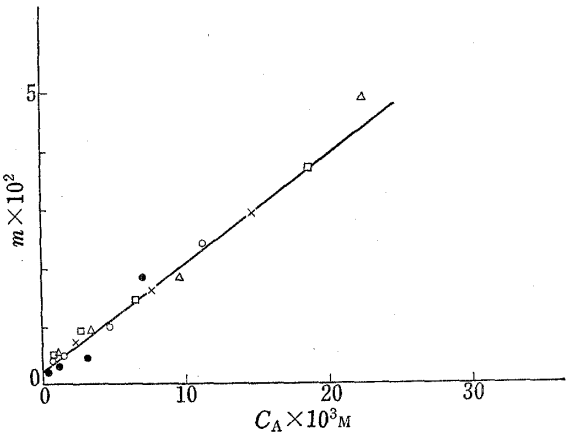

Fig. 9. Macroscopic Rate Constant as Function of the Free Concentration of nonprotonated Species of Amphetamine in A-PLP System ($T_{PLP}=2\times10^{-4}$M, 2×10^{-3}M, $T_A=6\times10^{-3}$M, $\mu=0.10$M, $T=25°$)

$C_A\times10^3$M ●: 2.0 $C_{PLP}\times10^4$M 2.0
○: 3.0 2.0
×: 4.0 2.0
□: 5.0 2.0
△: 6.0 2.0

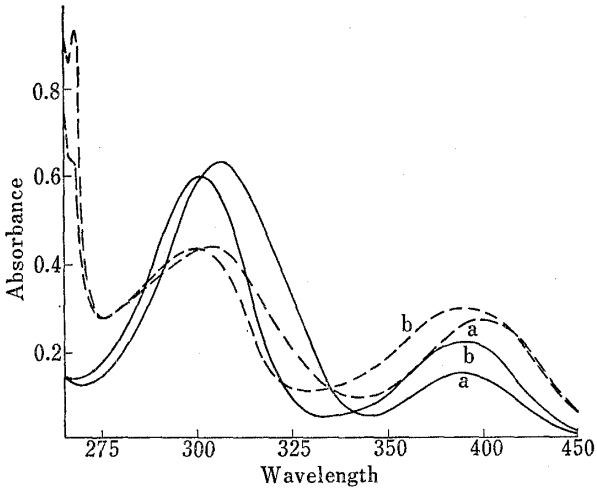

Fig. 10. The Spectra of PL and equilibrated Mixture of A and PL at Various pH Values ($T_{PL}=1.0\times10^{-4}$M, $T_A=4.0\times10^{-3}$M, $\mu=0.10$M, $T=25°$)

------: A-PL system
——: PL
T_{PL} $=1\times10^{-4}$M
T_A $=5\times10^{-3}$M
a- pH=9.00
b- pH=10.00

15) F.J. Anderson and A.E. Martell, *J. Am. Chem. Soc.*, **80**, 99 (1964).

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$$\frac{d(S^{3-}+SH^{2-})}{dt} = k_1(H\ PLP.H_2O^{2-})(HA^+) + k_2(PLP\cdot H_2O^{3-})(HA^+) + k_3(H\ PLP.H_2O^{2-})(A) + k_4(PLP.H_2O^{3-})(A) - [k_{-1}(SH^{2-})(H^+) + k_{-2}(S^{3-})(H^+) + k_{-3}(SH^{2-}) + k_{-4}(S^{3-})] \quad \text{ep. 16}$$

From the knowledge of K_4 of PLP and K_{SH} ($[S^{3-}][H^+]/[SH^{2-}]$) eq. 16 is expressed in the following form,

$$\left(1+\frac{(H^+)}{K_{SH}}\right)\frac{d(S^{3-})}{dt} = (PLP)(A)[k_1(H^+)^2/K_AK_4) + k_2(H^+)/K_A) + (k_3(H^+)/K_4) + k_4] - (S)(k_{-1}(H^+)/K_{SH}) + k_{-2}(H^+) + (k_{-3}(H^+)/K_{SH}) + k_{-4} \quad \text{eq. 17}$$

If it is assumed that pH is not altered during the reaction progress, the following integrated expression for eq. 17 is obtained,

$$\ln((S_\infty^{3-}) - (S_t^{3-})) = \ln(S_\infty^{3-}) - m_{PLP}\,t \quad \text{eq. 18}$$

where m_{PLP} is;

$$m_{PLP} = (k_{-1}(H^+)/K_{SH} + k_{-2}(H^+) + k_{-3}(H^+)/K_{SH} + k_{-4})/\left(1+\frac{(H^+)}{K_{SH}}\right) + (A)\left(\frac{k_1(H^+)^2/K_4K_A + k_2(H^+)/K_A + k_3(H^+)/K_4 + k_4)}{\left(1+\frac{(H^+)}{K_4}\right)}\right)$$

Equation 19 is similar to eq. 5. The constants a and b are

$$a = \frac{(H^+)(k_{-1}/K_{SH^{2-}} + k_{-2} + k_{-3}/K_{SH^{2-}})\,k_{-4}}{(1+(H^+)/K_{SH})} \quad \text{eq. 19}$$

$$b = \frac{k_1(H^+)^2/K_4K_A + k_2(H^+)/K_A + k_3(H^+)/K_4 + k_4}{1+(H^+)/K_4} \quad \text{eq. 20}$$

Although the derived constants a and b are functions of hydrogen ion concentrations, yet this was not verified experimentally. Thus, one may conclude that the hydrogen ion dependent terms in eq. 19 and 20 should drop out, *i.e.*, $a=k_{-4}$ and $b=k_4$. Reactions (a), (b), and (c) in scheme 15 should be of minor importance and reaction (d) must be quite significant in the formation of the Schiff base. The equilibrium constant of the reaction (d) is.

$$K_{PLP} = \frac{k_4}{k_{-4}} = 8.22\times10^3 \quad \text{eq. 21}$$

Schiff Base from Amphetamine and Pyridoxal

Figure 10 shows the spectra of pyridoxal and-the equilibrated mixture of pyridoxal and amphetamine at various pH's. The bands are slightly blue shifted with increase in absorbance in the wavelength range 350—450 nm and desrease in absorbance in the wavelength range 275—350 nm. The plot of log (As_∞—As_t)*vs*·*t* was linear for more than 70% of the reactions of PL with A, Fig. 11. A linear relation between the macroscopic rate constant, m_{PL} (Table Ic), and A was obtained; Fig. 12. An equation similar to that obtained in case of A-P system (eq. 5) can describe the linear relationship shows in Fig. 12. Table II lists the values of a and b.

In aqueous solutions, numerous species of pyridoxal may coexist. The pK values of hydrated pyridoxal are 4.15, 8.67, and 13.05[16] In alkaline solutions, eight species of PL may be present. These species are shown in Fig. 13. In the pH range 8.0—11.0, the most predominant species are: HPL, HPL·H_2O, HPL·AC (protonated hemiacetal species), PL^-, PL·H_2O^-, and PL. AC^-. Since the reactions of PL with amphetamine took place in aqueous solution

16) M. Samir El-Ezaby, *J. Univ. Kuwait* (Sci), **1**, 33 (1974).

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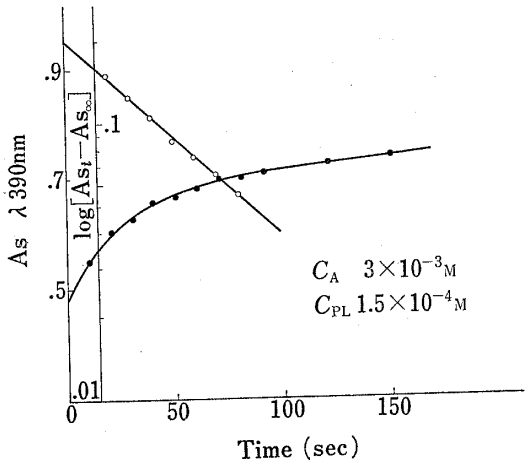

Fig. 11 a). Typical Absorbance-Time Curve in A-PL System ($T_{PL}=3\times10^{-3}$ M, $T_A=1.5\times10^{-4}$M, $\lambda=390$ nm, pH=9.50, $\mu=0.10$M, $T=25°$)
b). log (As_∞-As_t) as Function of Time

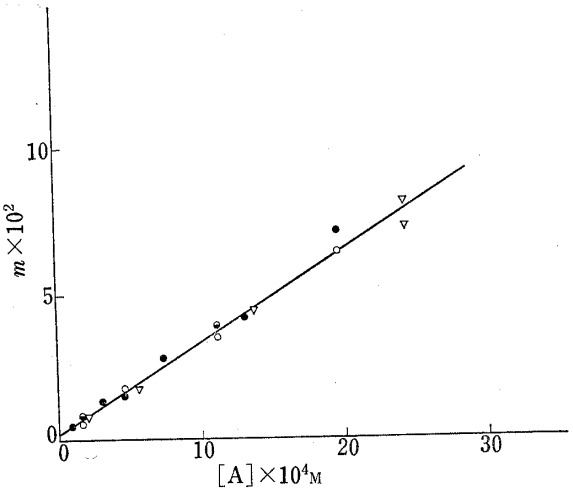

Fig. 12. Macroscopic Rate constant as Function of the Free Concentration of the Non-protonated Species $T_{PL}=(1.5—2.0)10^{-4}$M, $T_A=(3—3.75)10^{-3}$M, $\mu=0.10$M, $T=25°$

$C_A\times10^3$M		
△	3.75–$C_{PL}\times10^4$M	1.5
○	3.0 –	1.5
◒	3.0 –	2.0
●	3.0 –	2.0

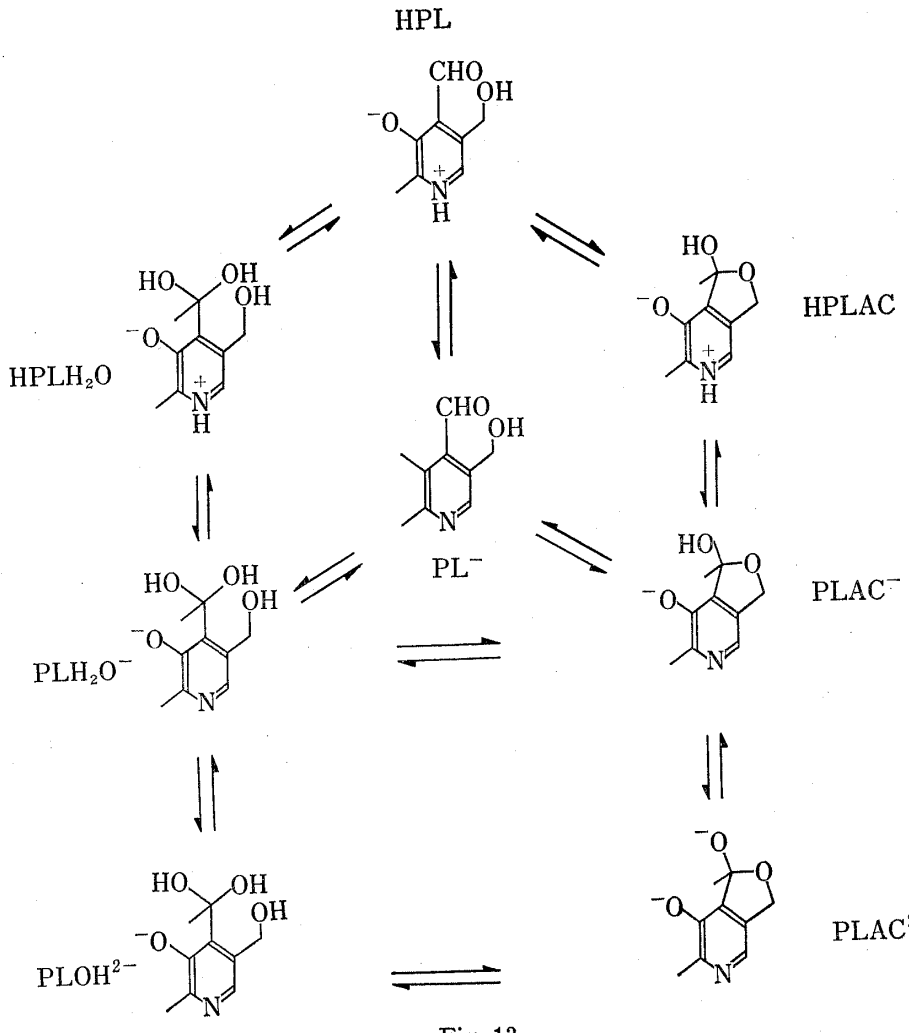

Fig. 13

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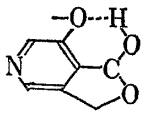
the concentrations of the hydrated and hemiacetal species of PL will be only considered. Thus, the interaction of PL with amphetamine, in alkaline solutions, may follow scheme 22,

$$
\begin{array}{lcccc}
\text{(a)} & \mathrm{HPL.H_2O} + \mathrm{HA^+} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & \mathrm{SH} + \mathrm{H^+} + \mathrm{H_2O} \\
 & \Updownarrow -\mathrm{H^+} & & \Updownarrow \\
\text{(b)} & \mathrm{PL.H_2O} + \mathrm{HA^+} & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & \mathrm{S} + \mathrm{H^+} + \mathrm{H_2O} \\
 & & & \Updownarrow \\
\text{(c)} & \mathrm{HPL\ AC} + \mathrm{HA^+} & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & \mathrm{SH} + \mathrm{H^+} + \mathrm{H_2O} \\
 & \Updownarrow -\mathrm{H^+} & & \Updownarrow \\
\text{(d)} & \mathrm{PL\ AC} + \mathrm{HA^+} & \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} & \mathrm{S} + \mathrm{H^+} + \mathrm{H_2O} \\
 & \Updownarrow & & \Updownarrow \\
\text{(e)} & \mathrm{HPL.H_2O} + \mathrm{A} & \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} & \mathrm{SH} + \mathrm{H_2O} \\
 & \Updownarrow -\mathrm{H^+} & & \Updownarrow \\
\text{(f)} & \mathrm{PL.H_3O} + \mathrm{A} & \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} & \mathrm{S} + \mathrm{H_2O} \\
 & & & \Updownarrow \\
\text{(g)} & \mathrm{HPL\ AC} + \mathrm{A} & \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} & \mathrm{SH} + \mathrm{H_2O} \\
 & \Updownarrow -\mathrm{H^+} & & \Updownarrow \\
\text{(h)} & \mathrm{PL\ AC} + \mathrm{A} & \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} & \mathrm{S} + \mathrm{H_2O}
\end{array}
\qquad \text{eq. 22}
$$

where SH and S are the protonated and nonprotonated species of the Schiff base. Although reactions (a), (b), (e), and (f) may lead to the formation of Schiff base, yet this may take place slowly with respect to the other reactions. The formation of PL hemiacetal species, actually increases the positive character of the aldehydic carbon, making the formation of a Schiff base with amphetamine more likely. In addition, the, hydrogen bonding with the negatively charged metaoxy group enhance amphetamine interaction with PL.



If reactions (c), (d), (g), and (h) are taken only in consideration, the rate equation describing the formation of Schiff base is expressed as follows:

$$\frac{d(\mathrm{SH+S})}{dt} = (\mathrm{PL.AC^-})(\mathrm{A})[k_3(\mathrm{H^+})^2/K_3K_\mathrm{A} + k_4(\mathrm{H^+})/K_\mathrm{A} + k_7(\mathrm{H^+})/K_3 + k_8] - (\mathrm{S})[k_{-3}(\mathrm{H^+})^2/K_\mathrm{SH} + k_{-4}(\mathrm{H^+}) + k_{-7}(\mathrm{H^+})/K_\mathrm{SH} + k_{-8}] \qquad \text{eq. 23}$$

where K_3 is the dissociation constant of HPL·AC and K_SH is defined as $[\mathrm{S}][\mathrm{H^+}]/[\mathrm{SH}]$. The integrated from of eq. 23, at constant hydrogen ion concentration, is

$$\ln((\mathrm{S}_\infty) - (\mathrm{S}_t)) = \ln(\mathrm{S}_\infty) - m_\mathrm{PL}\, t \qquad \text{eq. 24}$$

In such case m_PL will take the following form;

$$m_\mathrm{PM} = (k_{-3}(\mathrm{H^+})^2/K_\mathrm{SH} + k_{-4}(\mathrm{H^+}) + k_{-7}(\mathrm{H^+})/K_\mathrm{SH} + k_{-8}) + (\mathrm{A})(k_3(\mathrm{H^+})^2/K_3K_\mathrm{A} + k_4(\mathrm{H^+})/K_\mathrm{A} + k_7(\mathrm{H^+})/K_3 + k_8) \qquad \text{eq. 25}$$

The observed m_PL was obtained experimentally from the slope of the plot ln $(\mathrm{As}_\infty - \mathrm{As}_t)$*vs.* time when $T_\mathrm{A} \gg T_\mathrm{PL}$. Thus, eq. 25 can be correlated with that obtained empirically (eq. 5), *i.e.*,

$$a = k_{-3}(\mathrm{H^+})^2/K_\mathrm{SH} + k_{-4}(\mathrm{H^+}) + k_{-7}(\mathrm{H^+})/K_\mathrm{SH} + k_{-8} \qquad \text{eq. 26}$$

$$b = k_3(\mathrm{H^+})^2/K_3K_\mathrm{a} + k_4(\mathrm{H^+})/K_\mathrm{A} + k_7(\mathrm{H^+})/K_3 + k_8 \qquad \text{eq. 27}$$

Equations 26 and 27 indicates that the constants a and b are hydrogen ion dependent.

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This was not confirmed experimentally and turned out that a and b correspond actually to k_{-8} and k_8, respectively. In other words, reaction (h) in scheme 22 contribute significantly in the formation of the Schiff base. The equilibrium constant corresponding to this reaction is

$$K_{PL} = \frac{k_8}{K_{-8}} = \frac{b}{a} = 1.37 \times 10^4 \qquad \text{eq. 28}$$

Conclusion

Sander and Jencks[13] reported that the addition compounds formed from 4-pyridinecarboxaldehyde and the primary amines *e.g.* *n*-propylamine, undergo rapid dehydration to the Schiff base. The addition reaction usually takes place at a rate too fast to be measured with stopped-flow techniques. The reactions of amphetamine, $C_6H_5.CH_2.CH.CH_3.NH_2$, with P, PLP, and PL may behave in a similar manner and what actually obtained is the slower dehydration step. The formation of the intermediate carbinolamine species cannot be detected by the simple technique used in this study. Besides, taking the electronic spectra of the solution of the reactants during the reaction was not feasible and cannot be of much help to detect such intermediate. However, the presence of this intermediate cannot be overlooked. If the steps corresponding to their formation were invoked in the above reaction schemes, then

$$D + A \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} DA \underset{k_{-j}}{\overset{k_j}{\rightleftharpoons}} S + H_2O \qquad \text{eq. 29}$$

where D stands for the aldehyde and DA for the carbinolamine. One expects that the equilibrium constants (eq. 14, 21, and 28) obtained in this study should have the form, $K_D = k_i k_j / k_{-i} k_{-j}$, if a steady state approximation on the species DA is acheived.

It is clear from the kinetic data in the three systems studied that reactivity of aldehyde toward amphetamine increases in the following order PL>PLP>P. This was attributed to the presence of metaoxy group in PL and PLP which facilitate Schiff base formation through increasing the positive character of the carbon atom of the aldehydic group. The presence of the phosphate group in PLP may decrease its reactivity toward amphetamine over that of PL due to the steric hinderance effect of the former.

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