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Kinetics and Mechanism of Decarboxylation of α-Amino Acids by Ninhydrin

ZAHEER KHAN and A. AZIZ KHAN*

Department of Chemistry, Aligarh Muslim University, Aligarh-202 002

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The kinetic study of decarboxylation of α-amino acids has been carried out at various concentrations of ninhydrin at different temperatures (50 – 80°) and hydrogen ion concentration from 1.0×10^{-3} to 1.0×10^{-6} mol dm^{-3}. The reaction follows an irreversible pseudo first order reaction in presence of excess ninhydrin. The rate of the reaction increases with increase in pH and temperature. On the basis of the observed data a possible mechanism has been proposed.

RUHEMANN[1] who discovered the reaction of amino acids with ninhydrin, showed the formation of purple coloured product, ammonia, carbon dioxide and aldehyde in the pH range 2.0 – 5.0 qualitatively. Stable purple colour (azine-bis-indandione) was not formed at pH below 4.0, but evolution of carbon dioxide was observed under these conditions. Measurement of the evolved carbon dioxide was made by various workers[2,3], and others studied the decarboxylation of amino acid with glyoxal and ninhydrin in acetate buffer solution[4]. These workers simply discussed the substituents effect on decarboxylation and had not discussed the mechanism and kinetics. The work reported here is related with the study of the kinetics and mechanism of decarboxylation of amino acids by ninhydrin.

Experimental

Glycine, L-alanine, L-phenylalanine, L-asparagine and serine (all B.D.H.) were used as such. Solutions of amino acids and ninhydrin (B.D.H.) were prepared in McIlvaine buffer[5] of pH 3.0, 4.0, 5.0 and 6 0. pH measurements were made using an Elico LI-10 pH meter.

The requisite solutions of the reactants except ninhydrin were thermostated in an oil-bath (±0.1°). The reaction was started by adding the requisite volume of ninhydrin solution. The ionic strength was maintained with potassium nitrate solution. The concentration of amino acid was 2.0×10^{-3} mol dm^{-3} and that of ninhydrin was varied from 0.04 to 0.16 mol dm^{-3}. The carbon dioxide evolved was swept out by a constant current of pure nitrogen gas and was absorbed in standard barium hydroxide solution at different time intervals. The concentration of absorbed CO_2 was estimated by titrating excess barium hydroxide solution with standard hydrochloric acid[3]. In all the cases, ninhydrin was used without adding any modified reagent.

Results and Discussion

The decarboxylation of amino acids was found to follow irreversible pseudo-first order reaction in excess of ninhydrin concentration. The rate constants (k_{obs}) were calculated by integration method[6].

The reaction was studied at different concentration of ninhydrin keeping other parameters constant. The plots of k_{obs} vs [ninhydrin] are shown in Fig. 1. The temperature dependence of decarboxylation was studied at 0.16 *M* ninhydrin in the temperature range 50 – 80°. The observed data were found to fit well in the Arrhenius and Eyring equations.

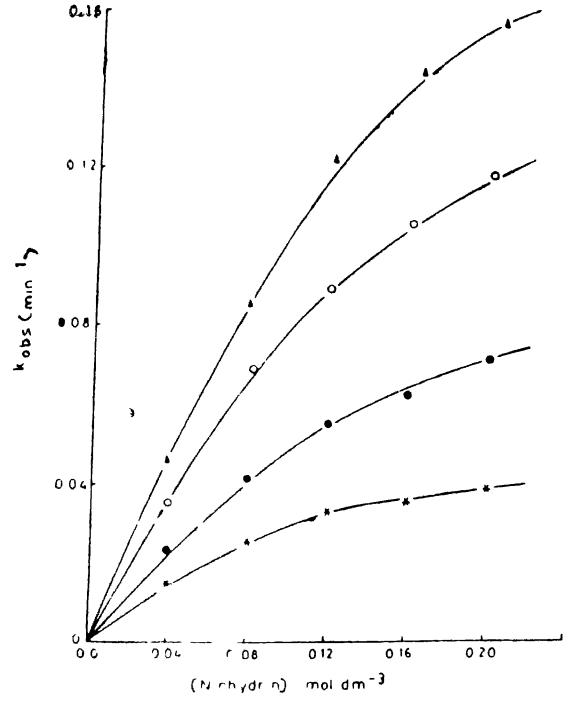
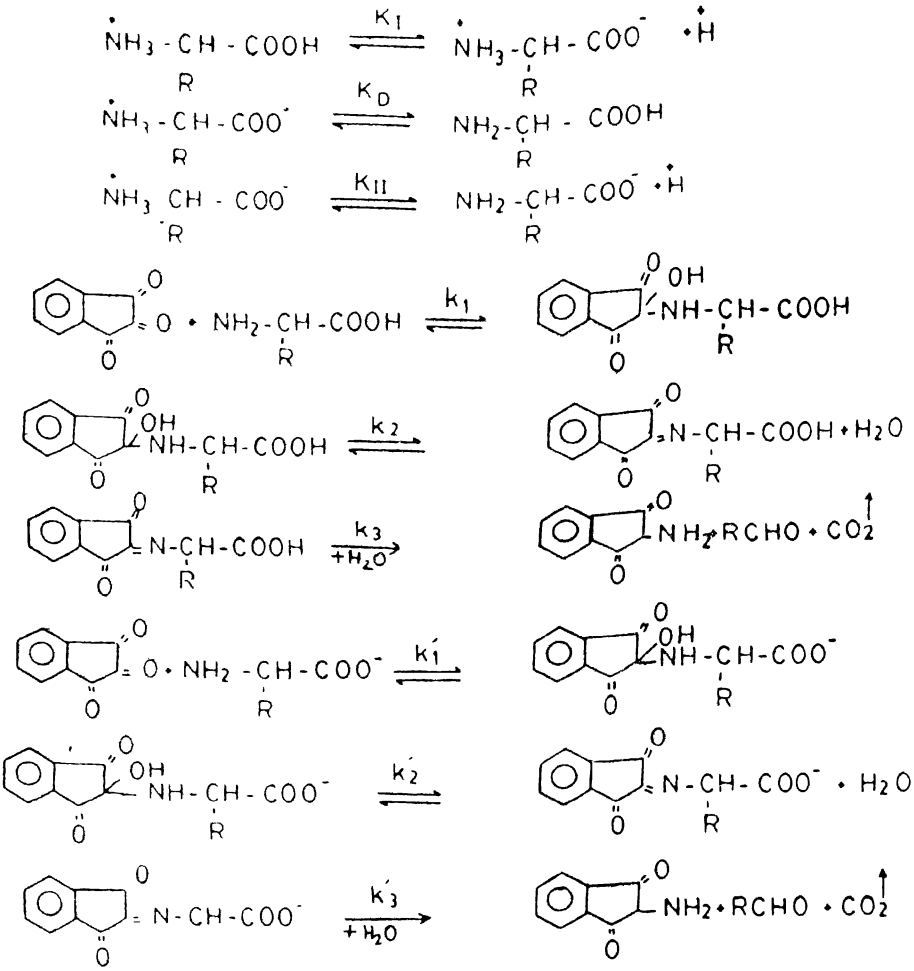


Fig. 1. Dependence of k_{obs} on [ninhydrin] for the reaction of glycine at 70° and [glycine] = 2.0×10^{-3} mol dm^{-3} and [H^+] = (*) 1.0×10^{-3}, (●) 1.0×10^{-4}, (○) 1.0×10^{-5} and (▲) 1.0×10^{-6}.

The rate of decarboxylation was found to increase with increase in pH of the reaction medium. In the



Scheme 1

hydrogen ion concentration range studied, the four species exist as $\overset{+}{N}H_3CH(R).COOH$, $\overset{+}{N}H_3CH(R).COO^-$, $NH_2CH(R).COOH$ and $NH_2CH(R).COO^-$. The species $\overset{+}{N}H_3CH(R).COOH$ and $\overset{+}{N}H_3CH(R).COO^-$ cannot attack on the carbonyl carbon of ninhydrin because the unshared electron pair of the nitrogen are combined with a proton. Therefore, $NH_2CH(R).COOH$ and $NH_2CH(R).COO^-$ are responsible for the reaction.

On the basis of these results and previous observation[7] a mechanism (Scheme 1) is proposed for the interaction of ninhydrin and neutral and anionic forms of the amino acid. On the basis of this scheme the following rate equation is derived,

$$k_{obs}=\frac{(k'_1k'_2K_{II}+k_1k_2K_D[H^+])[\text{Ninhydrin}]}{[H^+]^2/K_I+K_D[H^+]+K_{II}+(K_{II}k'_1+K_D[H^+]k_1)[\text{Ninhydrin}]} \quad (1)$$

on rearrangement, equation (1) gives equation (2),

$$1/k_{obs}=\frac{[H^+]^2/K_I+K_D[H^+]+K_{II}}{(k_1k_2K_D[H^+]+k'_1k'_2K_{II})[\text{Ninhydrin}]}+\frac{k_1K_D[H^+]+k'_1K_{II}}{k_1k_2K_D[H^+]+k'_1k'_2K_{II}} \quad (2)$$

The values of K_I, K_{II} and K_D (glycine, alanine, phenylalanine, serine and asparagine) are approximately in the range of 10^{-3}, 10^{-10} and 10^5 respectively[8]. The $[H^+]^2/K_I$ and K_{II} are negligible in comparison to $K_D[H^+]$ in the numerator of the first term of equation (2). Introducting these approximations, equation (2) simplifies to equation (3) which shows linearity,

$$1/k_{obs}=\frac{B_1}{[\text{Ninhydrin}]}+B_2 \quad (3)$$

where, $B_1 = \dfrac{K_D[H^+]}{k_1k_2K_D[H^+]+k'_1k'_2K_{II}}$ and

$$B_2 = \frac{k_1K_D[H^+]+k'_1K_{II}}{k_1k_2K_D[H^+]+k'_1k'_2K_{II}}$$

Fig. 2 shows the dependence of $1/k_{obs}$ on 1/[Ninhydrin] for a given $[H^+]$. B_1 and B_2, the gradients and intercepts of the plots, were calculated (Table 1), which are dependent on $[H^+]$. The reaction (Scheme 1) is consistent with the observed rate law.

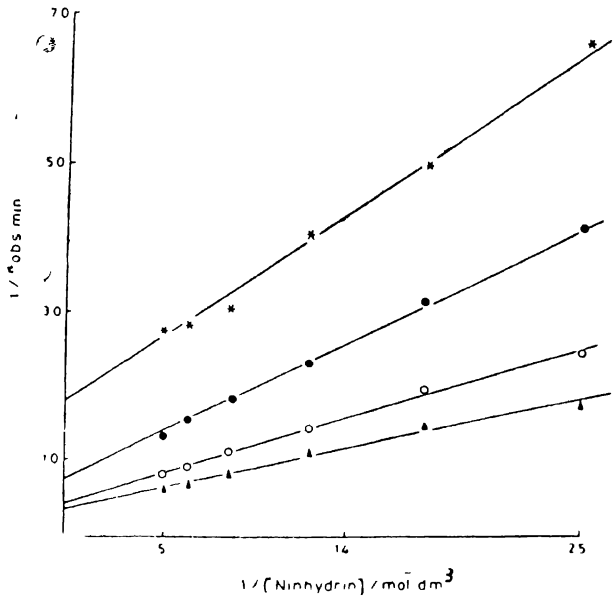


Fig. 2. Linear dependence of $1/k_{obs}$ against 1/[ninhydrin] under the conditions in Fig. 1.

Ingold-Taft equation was used to examine the substituent effect on the decarboxylation rate of the above mentioned amino acids. The results indicate that the logarithms of the relative rates have a linear relationship with the σ values (figure not shown). Therefore, the rate constant is strongly dependent on the different type of the substituents.

TABLE 1—VALUES OF B_1 AND B_2 CORRESPONDING TO EQUATION (3)

[Glycine]=2.0×10⁻² mol dm⁻³, Temp.=70°, μ=1.0 mol dm⁻³

$[H^+]$	B_1 min	B_2 M min
1.0×10⁻³	18.0	2.44
1.0×10⁻⁴	7.5	1.33
1.0×10⁻⁵	4.0	0.88
1.0×10⁻⁶	3.5	0.61

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