Tetrahedron. Vol. 34, pp. 339 to 344. Pergamon Press 1978. Printed in Great Britain

KINETICS OF THE REACTION OF α-AMINO ACIDS WITH PHENALENE-1,2,3-TRIONE HYDRATE

R. F. ZAKHARY* and M. L. ISKANDER
Chemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

(*Received in UK 27 May 1977; Accepted for publication 8 August 1977*)

Abstract—The rate of the title reaction was studied as a function of pH of the reaction medium, temperature, basicities and steric environment of amino groups. Kinetic constants were calculated from a linear free-energy equation permits calculation of separate polar and steric parameters associated with the amino acids which influence rates. The mechanism deduced from the study of the pH-rate profile involving the attack of the unprotonated amino group as a basic amine nucleophile at the CO carbon. The activation parameters of the reaction were determined and $\Delta S^{\neq}$ was found to be a linear function of $\Delta H^{\neq}$.

Hammett, Taft, and collaborators[1-3] demonstrated that the rates of reaction of individual members of a series of related reagents with certain compounds are a function of polar and steric parameters of the reagents. The present investigation of the effect of variables on reactivities of amino groups in a number of amino acids with phenalene-1,2,3-trione hydrate, established that the observed rates are also determined by polar and steric substituent factors which are related to readily measured physical constants. These observations support a postulated reaction mechanism and allow predictions of reaction rates of new amino acids with trione hydrate under various conditions of pH, temperature and concentration.

Phenalene-1,2,3-trione hydrate decomposes α-amino acids quantitatively to the corresponding aldehydes with one C atom less with the formation of ammonia, carbon dioxide, and dihydroxyphenalenone.[4-6] The rate of the reaction was followed by determination of the dihydroxy compound by a titrimetric method[8] using N-bromosuccinimide solution.

RESULTS AND DISCUSSION

Order of reaction. The order proved to be second-order by carrying out the reaction using different initial concentrations of both reactants (DL-phenylalanine and phenalene-1,2,3-trione hydrate) at 30° in citrate buffer of pH 4.25. When $\log [a(b-x)/b(a-x)]$ was plotted vs time a straight line was obtained. The amount of the product formed, x, was calculated in moles/l, since 1.0 ml of 0.005 N N-bromosuccinimide = 0.53 mg of dihydroxyphenalenone. Table 1 summarizes the kinetic data and k_2 is essentially invariant over the indicated range of concentration confirming that the reaction between α-amino acids and the trione hydrate is of the second-order.

Effect of pH *on reaction rate.* Rates of reaction of the trione hydrate (0.004) with DL-phenylalanine (0.004) at 30° were determined as a function of pH on the acidic side and the results are summarized in Table 2.

At pH values ≥ 5.4, the blank experiments gave positive measurements. Also, it was found that ammonia produced in the reaction can reduce an equivalent amount of the trione at pH values ≥ 5.4 when the reaction carried out in excess of trione with ammonia, instead of amino-acid, at various pH at 30°[9] and the results are similar to those obtained by Moore and Stein[10] for the reaction between ninhydrin and ammonia at pH 5.5.

In Fig. 1 the logarithm of the second-order rate constant rises with increasing pH until it levels off at about 3.2 and higher. The variation of the logarithms of the rate constants with pH can be explained as pointed out by Roberts and Caserio,[11] that protonation of an amino group decreases its nucleophilic character, while protonating a carbonyl compound enhances the reaction. These two effects are oppositely influenced by pH, as the pH increased from 1.7 to 3.2 the concentration of the protonated trione is slightly decreased, at the same time, the concentration of nonprotonated amino acid is moderately increased; and the controlling factor in this pH range is the concentration of the free amino acid since the rate increases with pH. Therefore the maximum rate will be found at that pH where not all of the

Table 1. Rates of reaction of DL-phenylalanine with phenalene-1,2,3-trione hydrate at 30° in citrate buffer of pH 4.25 (μ = 0.5 M)

Trione hydrate (mol/l)	DL-Phenylalanine (mol/l)	$10^4 k_2$ ($l\,mol^{-1}\,sec^{-1}$)
0.004	0.004	2.97
0.004	0.008	3.02
0.004	0.012	3.10
0.004	0.016	2.93

Table 2. Rate constants of reaction of phenalene-1,2,3-trione hydrate with DL-phenylalanine as a function of pH in buffers (μ = 0.5 M) at 30°

pH	$10^4 k_2$ ($l\,mol^{-1}\,sec^{-1}$)	($\log k_2 + 4$)
1.75	1.19	0.076
2.11	1.52	0.182
2.33	1.82	0.260
2.74	2.39	0.378
3.20	2.88	0.459
3.74	2.91	0.464
4.25	2.97	0.473
4.77	2.85	0.455

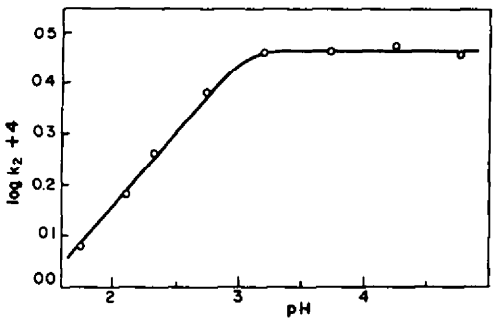


Fig. 1. Rates of reaction of DL-phenylalanine and phenalene-1,2,3-trione hydrate as a function of pH at 30°.

amine has been protonated, and, at the same time, enough of the carbonyl compound exists as its conjugate acid to afford a reasonable reaction rate. It is obvious that the rate of such a reaction must pass through a maximum as a function of pH where the product of the concentrations of reactive species, protonated trione (H trione$^+$), and non-protonated amino acid (RNH_2), is a maximum eqn (3). For trione

$$K_{trione} = [trione][H^+]/[H\ trione^+]. \quad (1)$$

For the amino acid (RNH_2)

$$K_2 = [RNH_2][H^+]/[RNH_3^+] \quad (2)$$

$$Rate = k[H\ trione^+][RNH_2]. \quad (3)$$

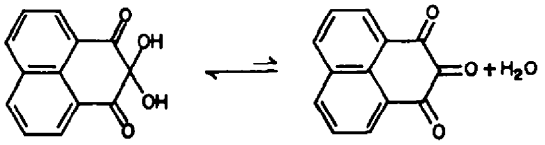
As the pH increased above 3.2, the concentration of the various ionized species of the amino acids and their relative reactivities as nucleophiles are effected.

The measurements of the reaction are made under conditions where only a minute fraction of the amino acid exists with its amino group in the free-base form. It is of interest to calculate how fast the active form of the amino acid (having its amino group free) is reacting with the trione hydrate. For this purpose there are two molecular species to be considered, the anion $H_2NCHRCOO^-$ and the uncharged form $H_2NCHRCOOH$. The fraction of the former species present in our citrate buffer of known pH is $10^{(pH-pK_2)}$, and the fraction of the latter species is 10^{pK_z} where K_2 is the ionisation constant of the amino group of the amino acid, and K_z is the equilibrium constant between the zwitterion and the uncharged amino acid.[12] Bartlett and Jones[13] found that the fraction of neutral glycine present at pH 4.74 is 1/1,500,000 and the fraction of the anion form is 1/620,000; i.e. about two-thirds of the free amino group which responsible for the reaction with anhydride of N-carboxy-α-amino acids at pH 4.74 is situated on the glycine anion, and the other third is on neutral glycine. As expected, a negative charge on the carboxyl group might enhance the nucleophilic reactivity of the amino group, so that the anion would be inherently more reactive than the neutral form. Thus, as the pH increased above 3.2, the concentration of the amino acid anion increases and since the relative reactivity as nucleophile compared to neutral form is high, one can expect, the rise in pH values above 3.2 should further increase the rate, which is not the case.

In a series of experiments of reaction between DL-phenylalanine and trione hydrate at 30° in citrate buffer of pH 4.25 in presence of varying citric acid concentration (0.02–0.1 M), it was noted that the rate of reaction is slightly increased in presence of 0.07 M citric acid and higher. This provides evidence that although the free amino acid concentration is lowered by citric acid added, yet the rate of reaction is slightly increased, indicating that the concentration of the reactive conjugate acid of the trione (which is highly decreased above pH 3.2) is the controlling factor in the pH range 3.2–5.4.

Also, the order of reaction between DL-phenylalanine and trione hydrate at pH 4.25 proved to be second-order (Table 1), confirming that the rate-limiting step is the first step (Scheme 2).

In the trione hydrate, the hydrated carbonyl has no partial positive charge, as represented by ninhydrin in a kinetic study of the ninhydrin reaction.[14] An equilibrium between the trione and its hydrated form can be present specially in acid medium in a similar manner to the reversible hydration of carbonyl compounds investigated by Bell (Scheme 1).[15]



and also

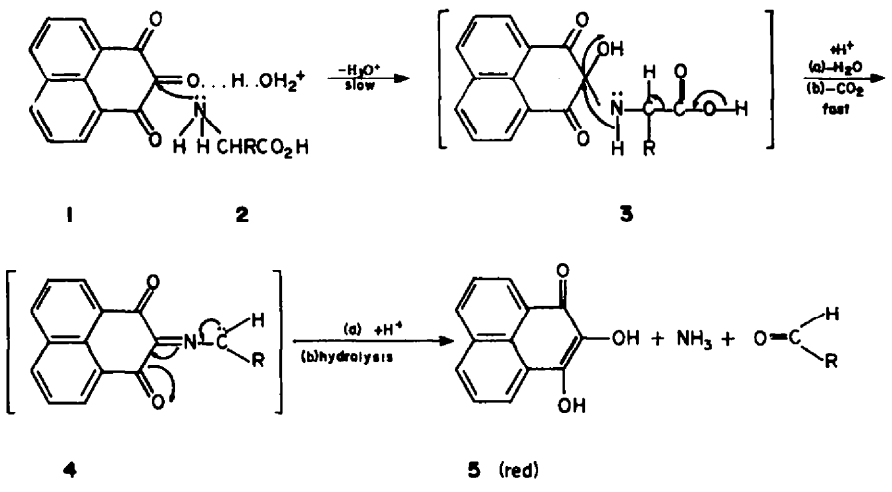
H trione$^+$ ⇌ trione + $H^+(H_3O^+)$.

Scheme 1.

The reaction pathway may be interpreted as shown in Scheme 2.

Since the observed second-order rate constant was found to increase with pH over the range 1.7–3.2 (see Table 2 and Fig. 1), this indicates that the overall reaction in this pH range is second-order and the rate-limiting step is the attack of amino acid as a free base on the CO group while the dehydration of carbinol-amine addition compound (3) is fast. If the rate of decomposition of the last compound is the rate-determining step, the reaction rate would increase with decrease of pH which is not the case.

Effect of polar and steric factors on rates. At pH 4.25, eqn (3) shows that the rate of reaction depends only on pK_2 of the amino group of the amino acids since the amount of protonated trione is presumably fixed. The dependence of reaction rates on differences in inherent basicities, and thus nucleophilicities,[16] of the amino groups, as indicated by their pK_2's, can be deduced by comparing the rates of reaction of the several amino acids at the same amino acid anion concentrations.[13] The second-order rate constant was determined for a series of α-amino acids with varying pK_2 values and the results are summarized in Table 3. The anionic form of an amino acid rather than the zwitterion would be expected to react with the trione because the latter form has a positive charge on the N atom and could not participate. At any given pH the concentration of the amino acid is governed by eqn (4); (see text). Where A^- represents the amino acid anion and HA^* the zwitterion. If the anion is the reactive species, eqn (4) predicts that at any given pH the observed rates should decrease with increasing pK_2 values (as shown in Table 3) because the greater the pK_2 the lower the anion concentration. To correlate the observed rate constant k_2 with basicities of the amino



Scheme 2.

group, second-order rate constants k_{A^-}, based on amino acid anion as the reactive species, were computed from eqn (5a) given by Burchfield and Storrs,[17] which is the same eqn (5b) derived by Friedman and Wall,[18] where $[H^+]$ is the hydrogen ion concentration, K_2 or K_a is the ionisation constant of the amino group, k_2, observed overall second-order rate constants, and k_{A^-} is the second-order anion rate constant.

$$pH = pK_2 + \log([A^-]/[HA^+]) \quad (4)$$

$$k_2 = \frac{K_a k_{A^-}}{K_a + [H^+]} \quad (5a)$$

$$k_{A^-} = k_2(1 + [H^+]/K_2) \quad (5b)$$

It can be seen from Table 3 that the α-amino acids (2–15), the amino groups are attached to secondary C atoms (similar steric environment) whereas in glycine (1) the amino group is attached to a primary C atom. Since k_{A^-} rate constants measure rates at the same amino acid anion concentration, differences in these rate constants for a sterically similar series of amino acids (2–15) should then be due to inherent differences in basicities of amino groups as measured by pK_2 values. This point is strikingly illustrated by the observed k_{A^-} values of L-tyrosine and L-3,5-di-iodotyrosine (Table 3) which k_{A^-} for L-tyrosine is about 4.5 times greater than the corresponding value for the di-iodo derivative, although the steric environment of the amino groups in these two amino acids is nearly identical. This difference in rate constants must be due to the difference in pK_2 values of the amino groups in the two compounds.

Brønsted-type plots of $\log k_{A^-}$ (Table 3) against pK_2 values[14,19–22] of the amino groups at 30° gave, with the exception of histidine, a straight line (Fig. 2). This linear relationship demonstrate that the calculated rate constant k_{A^-} is a function of the basicities of the amino groups. The reason for the faster rate in case of L-histidine, than predicted from the pK_2 value of its amino group, appears to be the presence of the NH group in the imidazole ring,[14] since L-1-methylhistidine gives the expected rate constant.

Treatment of the kinetic data obtained for our trione

Table 3. Rates of reaction of amino acids with phenalene-1,2,3-trione hydrate as a function of pK_2 of the amino groups at pH 4.25 and 30°

No.	Amino acid	$10^4 k_2$ (l mol^{-1} sec^{-1})	k_{A^-}	pK_2 values of amino groups
1	Glycine	3.98	66.07	9.47
2	L-3,5-Di-iodotyrosine	12.59	3.72	7.72
3	L-1-Methylhistidine	3.90	11.51	8.72
4	*m*-Chloro-DL-phenylalanine	3.80	12.59	8.77
5	*o*-Chloro-DL-phenylalanine	3.89	13.80	8.80
6	*p*-Chloro-DL-phenylalanine	3.39	12.59	8.82
7	L-Histidine	5.50	21.90	8.85
8	L-Tyrosine	3.02	16.98	9.00
9	DL-Threonine	3.02	16.98	9.00
10	DL-Phenylalanine	2.97	16.69	9.00
11	DL-Methionine	2.57	17.38	9.08
12	DL-Leucine	1.78	28.18	9.45
13	DL-Aspartic acid	1.83	30.34	9.47
14	DL-Glutamic acid	1.66	32.36	9.54
15	DL-Norleucine	1.48	35.48	9.63

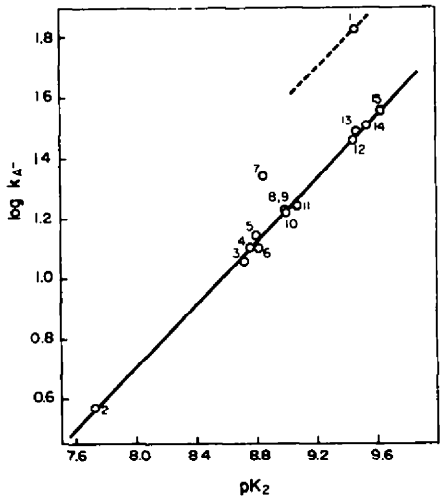


Fig. 2. Variation of $\log k_{A^-}$ with pK_2 values for the reaction of α-amino acids with trione hydrate at pH 4.25 and 30°. The numbers correspond to the amino acids listed in Table 3.

reaction, in a manner analogous to that described by Taft[2,23] for other reactions, makes it possible to separate the polar and steric factors which influence the reaction rates. The linear relationship between $\log k_{A^-}$ and pK_2 values (Fig. 2) may be described by eqn (6) which is an extension of the Brønsted catalysis law.

$$\log k_{A^-} = \rho(\text{slope})pK_2 + b(\text{intercept}). \quad (6)$$

It was found from the study of other reactions[13,14,18] involving amino acids of varying structures, that the linear plots of sterically similar series are parallel, i.e. the slopes of lines are nearly identical. Taking in consideration the above approach, eqn (6) may be expressed as a Hammett–Taft free energy relationship (eqn 7) which correlates the logarithm of the ratio of the k_{A^-} rate constants of any α-amino acid and that of glycine, the simplest amino acid, to differences in polar and steric factors.

$$\log \frac{k_{A^-}\ (\text{any } \alpha\text{-amino acid})}{k_{A^-}\ (\text{glycine})} = \rho\sigma^{A} + E_s. \quad (7)$$

The polar reaction parameter ρ gives a measure of sensitivities of rates to basicities of amino groups, and the polar substituent parameter σ^{A}, defined as the difference in pK_2 values between any α-amino acid and glycine, gives a quantitative measurement of the change in basicity of an amino group due to the introduction of additional substituents into the α-carbon of glycine. The steric substituent constant E_s depends on the size and steric requirements of the substituents in the reaction. The E_s values for the individual amino acids were calculated by means of eqn (7) from data in Table 3 and the results, together with the σ^{A} and $\rho\sigma^{A}$ values, are shown in Table 4. The term σ^{A} is written with superscript A to donate its origin and applicability to aliphatic amino compounds. The term ρ was evaluated from the slope of the linear plot of $\log k_{A^-}$ against pK_2 in Fig. 2, and found to be 0.51. The steric substituent constants, E_s (-0.358 ± 0.012; except histidine), calculated from eqn (7), are free-energy parameters that give a direct measure of the steric factor associated with the amino component. This point is illustrated best by comparing the reactivities of glycine and norleucine. One of the hydrogens on the α-C atom in glycine is replaced by a Bu group in norleucine. This Bu group causes an increase in the pK_2 value of the amino group (polar factor) as well as a change in the steric environment near the amino group (steric factor). The effect of polar factor resulting from the change in the pK_2 value on rates is quantitatively given by $\rho\sigma^{A}$ and that of steric factor by E_s. Equation (7) permits the calculation of individual contributions of polar and steric factors to relative reactivities (Table 4).

The larger E_s value associated with the trione reaction is not unexpected because the aromatic ring system of the trione component is relatively rigid and nonflexible; consequently, the amino-component is more limited in the number of orientations it can assume during the formation of the transition state.

On the basis of present results, it may be concluded that both polar and steric factors influence rates in the trione reaction. An examination of the mechanism of this reaction (Scheme 2) reveals that both of these parameters are involved during the attack of amino group on the CO group of the trione in the first step of the depicted mechanism. Our results are therefore consistent with the view that the first step is rate-determining. It may be assumed that decarboxylation of 3 is not the rate-determining step because under the conditions of our reaction decarboxylation would be expected to be unimolecular and not subject to steric hindrance.[24] A slight polar parameter might conceivably exist if the double bonds of the planar transition state are influenced by the R groups via hyperconjugation or π-orbital overlap. Also the hydrolysis of IV to products could not be rate controlling because in the first place, rates should be governed by steric factors alone, and in the second place, all amino acids should have identical rates.

Activation parameters. The rates of reaction of α-amino acids with the trione hydrate were examined at three different temperatures at pH 4.25 and the activation parameters were calculated[25,26] at 30° and the results are summarized in Table 5.

To elucidate the effect of structure in the reaction studied, the results in Table 5 have been used to plot $\Delta H^{\neq}$ vs $\Delta S^{\neq}$ for a series of amino acids undergoing the same reaction. The best line was obtained by applying the principle of least squares as shown in Fig. 3. Equation (8) describes this plot where, $\Delta H_0^{\neq}$ is the value of $\Delta H^{\neq}$

Table 4. Polar and steric substituent constants for the rates of reaction of amino acids with phenalene-1,2,3-trione hydrate at pH 4.25 and 30°

No.	Amino acid	σ^{A}	$\rho\sigma^{A}$	E_s	Rel. Obsd. rates
1	Glycine	0	0	0	1
2	L-3,5-Di-iodotyrosine	−1.75	−0.893	−0.357	3.16
3	L-1-Methylhistidine	−0.75	−0.383	−0.376	0.98
4	*m*-Chloro-DL-phenylalanine	−0.70	−0.357	−0.363	0.96
5	*o*-Chloro-DL-phenylalanine	−0.67	−0.342	−0.338	0.98
6	*p*-Chloro-DL-phenylalanine	−0.65	−0.332	−0.388	0.85
7	L-Histidine	−0.62	−0.316	−0.164	1.38
8	L-Tyrosine	−0.47	−0.240	−0.350	0.76
9	DL-Threonine	−0.47	−0.240	−0.350	0.76
10	DL-Phenylalanine	−0.47	−0.240	−0.358	0.75
11	DL-Methionine	−0.39	−0.199	−0.381	0.65
12	DL-Leucine	−0.02	−0.01	−0.360	0.45
13	DL-Aspartic acid	0.00	0.00	−0.338	0.46
14	DL-Glutamic acid	+0.07	+0.036	−0.346	0.42
15	DL-Norleucine	+0.16	+0.082	−0.352	0.37
			Average	-0.358 ± 0.012	

Table 5. Temperature dependence of the reaction between α-amino acids and phenalene-1,2,3-trione hydrate at pH 4.25 and activation parameters at 30°

Amino acid	$10^4 k_2$(l mol^{-1} sec^{-1}) 22.5°	30°	37.5°	$\Delta H^{\neq}$ (kcal mol^{-1})	$\Delta G^{\neq}$ (kcal mol^{-1})	$-\Delta S^{\neq}$ (cal mol^{-1} K^{-1})
Glycine	1.62	3.98	9.12	20.39	14.50	6.79
DL-Threonine	1.41	3.02	6.20	17.36	15.34	17.41
DL-Phenylalanine	1.33	2.97	6.27	18.16	15.35	14.79
DL-Aspartic acid	0.78	1.83	3.98	19.08	15.22	12.73
DL-Leucine	0.76	1.78	3.91	19.31	15.04	12.06
DL-Methionine	1.07	2.57	5.82	19.76	15.31	9.77

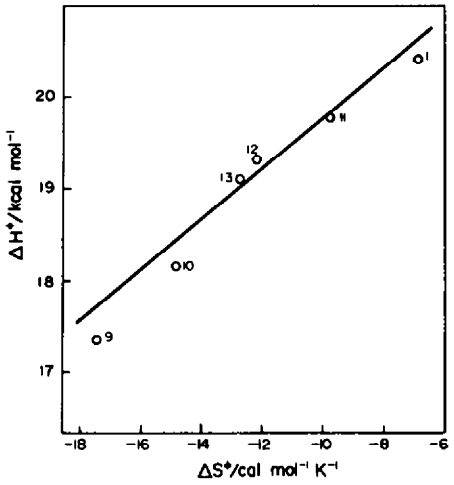


Fig. 3. Isokinetic plot for the reaction of trione hydrate with α-amino acids. The numbers correspond to the amino acids listed in Table 3.

corresponding to $\Delta S^{\neq} = 0$, and β is a constant, which has the dimensions of absolute temperature and is therefore termed the isokinetic temperature. The value of this temperature has been calculated and found to be 274°K (correlation coefficient = 0.997) lower than experimental temperature by about 30°, meaning that the rate seems to be governed by the entropy of activation. Two important conclusions may be drawn from the fact that good correlations are obtained:[26,27] (a) the mechanism of the reaction of trione hydrate and any α-amino acids tested follow one and the same mechanism, and (b) there is no large change in steric hindrance about the reaction center on going through the series of α-amino acids studied.

$$\Delta H^{\neq} = \Delta H_0^{\neq} + \beta \Delta S^{\neq} \qquad (8)$$

EXPERIMENTAL

Materials. Phenalene-1,2,3-trione hydrate, m.p. 270°, was prepared and purified as described previously.[4,28]

α-Amino acids. The α-amino acids used were biochemical reagent of purity not less than 99%.

Buffer solutions. Buffer solns with ionic strength μ = 0.5 M and covering the pH range 1.7–5.5 were prepared from A.R., sodium dihydrogen phosphate, disodium hydrogen phosphate, phosphoric acid, trisodium citrate and citric acid. The calculated amount of A.R., NaCl or KCl was added for each buffer solution ot maintain the ionic strength.

Kinetic procedure. The general procedure has been described.[29] α-Amino acid soln 10 ml; 0.02 M and phenalene-1,2,3-trione hydrate 40 ml; 0.005 M, both dissolved in the appropriate buffer (ionic strength 0.5 M) were mixed at 30° in a Pyrex round bottom flask with side arm fitted with a glass stopper, filled with N_2. The rate of reaction was followed by determining the formed dihydroxyphenalenone, which is a red compound; completely insoluble in cold water; solubility not more than 0.2% in cold water.[8] Thus, after about 10 min, a number of 5 ml aliquots were quickly introduced into separate glass-stoppered Pyrex test tubes, filled with N_2 and were placed in the same thermostat. At increasing intervals of time, one of the test tubes was removed, 3–10 ml of glacial AcOH was added to stop the reaction and to dissolve the red product, the contents were then transferred to a conical flask and was diluted to 25 ml with distilled water. 4% KI soln (5 ml) and starch soln (1 ml) were then added, the contents were titrated against standardised N-bromosuccinimide soln[8] (0.005 N). A blank experiment was carried out using the buffer soln instead of amino acid soln. The pH's of the mixture were measured at the beginning and at the end of the reaction. No significant change was observed. The rate constant k_2 was calculated from the kinetic equation of a second-order reaction with the same initial concentration of both reactants.

REFERENCES

[1]L. P. Hammett, *Physical Organic Chemistry*, Chap. 7. McGraw-Hill, New York (1940).
[2]R. Taft, Jr., *Steric Effects in Organic Chemistry* (Edited by M. S. Newman), Chap. 13. Wiley, New York (1956).
[3]H. H. Jaffé, *Chem. Rev.* **53**, 214 (1953).
[4]R. Moubasher and W. I. Awad, *J. Biol. Chem.* **179**, 915 (1949).
[5]R. Moubasher, *Ibid.* **175**, 187 (1948).
[6]R. Moubasher, W. I. Awad and A. Othman, *Ibid.* **184**, 693 (1950).
[7]R. Moubasher and A. Sina, *Ibid.* **180**, 681 (1949).
[8]W. I. Awad, S. Nashed, S. S. M. Hassan and R. F. Zakhary, *Talanta* **19**, 31 (1972).
[9]W. I. Awad, S. Nashed, S. S. M. Hassan and R. F. Zakhary, *J. Chem. Soc.* Perkin II 128 (1976).
[10]S. Moore and W. H. Stein, *J. Biol. Chem.* **176**, 367 (1948).
[11]J. D. Roberts and M. C. Caserio, *Basic Principles of Organic Chemistry*, p. 449. Benjamin, New York (1965).
[12]J. T. Edsall and M. H. Blanchard, *J. Am. Chem. Soc.* **55**, 2344 (1933).
[13]P. D. Bartlett and R. H. Jones, *Ibid.* **79**, 2153 (1957); P. D. Bartlett and D. C. Dittmer, *Ibid.* **79**, 2159 (1957).
[14]M. Friedman and C. W. Sigel, *Biochemistry* **5**(2), 478 (1966).
[15]R. P. Bell, *Dan Kemi* **47**(9), 138 (1966); R. P. Bell, *Advances in Physical Organic Chemistry* (Edited by V. Gold), Vol. 4, pp. 1–29. Academic Press, London (1966).
[16]J. Edwards and R. Pearson, *J. Am. Chem. Soc.* **84**, 16 (1962).
[17]H. Burchfield and E. Storrs, *Contribs. Boyce Thompson Inst.* **19**, 169 (1957).
[18]M. Friedman and J. Wall, *J. Am. Chem. Soc.* **86**, 3735 (1964).
[19]C. Schmidt, P. Kirk and W. Appleman, *J. Biol. Chem.* **88**, 285 (1930).
[20]J. Nevenzel, W. Shelberg and C. Niemann, *J. Am. Chem. Soc.* **71**, 3024 (1949).
[21]J. P. Greenstein and M. Minitz, *Chemistry of the Amino Acids*, Vol. 1, Chap. 4. Wiley, New York (1961).

[22]D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*. Butterworths, London (1965).
[23]W. Pavelich and R. Taft, Jr., *J. Am. Chem. Soc.* **79**, 4935 (1957).
[24]E. S. Gould, *Mechanism and Structure in Organic Chemistry*, Chap. 9 and footnote 78. Holt, Reinhart & Winston, New York (1959).
[25]J. E. Bunnett, *Technique of Organic Chemistry* (Edited by A. Weissberger), Vol. 8, Part 1 (2nd Edn), p. 176. Interscience, New York (1961).
[26]L. L. Schaleger and F. A. Long, *Advances in Physical Organic Chemistry* (Edited by V. Gold), Vol. 1. Academic Press, London (1963).
[27]J. E. Leffler, *J. Org. Chem.* **20**, 1202 (1955).
[28]G. Errera, *Gazzetta* **43**, 593 (1913); **44**, 18 (1914).
[29]W. I. Awad, S. Nashed, S. S. M. Hassan and R. F. Zakhary, *Egypt J. Chem.* **17**(3), 235 (1974).