Application of a Hammett–Taft Relation to Kinetics of Strecker Degradation of α-Amino-acids with Phenalene-1,2,3-trione Hydrate

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The reaction rates of α-amino-acids with phenalene-1,2,3-trione hydrate have been studied as a function of pH of the reaction medium, pK_2 of the amino-group, temperature, and structure of the amino-compounds. From the pH–rate profile, it was possible to postulate the rate-determining step, *i.e.*, attack of amino-acid as a free base on a carbonyl group of the trione. A quantitative estimate of the influence of the steric and polar parameters on the rates was obtained from a Hammett–Taft free energy relationship.

PHENALENE-1,2,3-TRIONE HYDRATE decomposes α-amino-acids quantitatively to the corresponding aldehydes with one carbon atom less with the formation of ammonia, carbon dioxide, and dihydroxyphenalenone.[1-4] The rate of the reaction was followed by determination of the dihydroxy-compound by a titrimetric method[5] using *N*-bromosuccinimide solution.

EXPERIMENTAL

Materials.—Phenalene-1,2,3-trione hydrate, m.p. 270°, was prepared and purified as described previously.[1,6]

α-Amino-acids.—The α-amino-acids used were biochemical reagent grade of purity ⩾99%.

Buffer Solutions.—Buffer solutions with ionic strength μ = 0.5M and covering the pH range 1.5—5.5 were prepared from AnalaR sodium dihydrogen phosphate, disodium hydrogen phosphate, phosphoric acid, trisodium citrate, and citric acid. The calculated amount of AnalaR NaCl or KCl was added for each buffer solution to maintain the ionic strength.

Kinetic Procedure.—α-Amino-acid solution (25 ml; 0.002M) in the appropriate buffer, was introduced to the reaction vessel, a Pyrex round bottom flask with side arm fitted with a glass stopper, filled with nitrogen. This flask and another containing a 0.08M ethanolic solution of trione hydrate (25 ml) were placed in a thermostatted bath (±0.1°). After *ca.* 20 min, the trione hydrate solution was poured into the reaction vessel which was shaken vigorously. The rate of reaction was followed by withdrawing aliquot portions (5 ml), and quenching them in cold 1% acetic acid solution (10 ml). 4% KI solution (5 ml) and starch solution (1 ml) were then added and the contents of the flask were titrated against standardised *N*-bromosuccinimide solution (0.005N). A blank experiment was carried out using the buffer solution instead of the amino-acid solution.

Since the concentration of trione hydrate in the reaction mixture is in large excess of that of the amino-acid, a graph of $\log(a - x)$ against time gave a straight line, establishing that the reaction followed pseudo-first-order kinetics.

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 (glycine and trione hydrate) at 30° in citrate buffer of pH 3.83. Applying the first-order kinetic equation, straight lines were obtained from which k_1 was calculated. The second-order rate constant k_2 was then found from $k_2 = k_1/[\text{trione hydrate}]$. Table 1 summarizes the kinetic data and

TABLE 1

Rates of reaction of glycine with phenalene-1,2,3-trione hydrate at 30° in citrate buffer of pH 3.83 (μ = 0.5M)

[Glycine]/M	[Trione hydrate]/M	10^3k_1/min^{-1}	$10k_2$/l mol^{-1} min^{-1}
0.002	0.08	5.70	1.43
0.002	0.06	4.24	1.41
0.002	0.04	2.72	1.36

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.

[1] R. Moubasher and W. I. Awad, *J. Biol. Chem.*, 1949, **179**, 915.
[2] R. Moubasher, *J. Biol. Chem.*, 1948, **175**, 187.
[3] R. Moubasher, W. I. Awad, and A. Othman, *J. Biol. Chem.*, 1950, **184**, 693.
[4] R. Moubasher and A. Sina, *J. Biol. Chem.*, 1949, **180**, 681.
[5] W. I. Awad, S. Nashed, S. S. M. Hassan, and R. F. Zakhary, *Talanta*, 1972, **19**, 31.
[6] G. Errera, *Gazzetta*, 1913, **43**, 593; 1914, **44**, 18.

Effect of pH *on Reaction Rate.*—Rates of reaction of the trione hydrate (0.08M) with each of glycine (0.002M) and DL-phenylalanine (0.002M) at 30° were determined as a function of pH. In order to obtain accurate pH measurements in the 1 : 1 ethanol–water solvent it was decided to employ the Hammett acidity function.[7] Lamothe and McCormick [8] carried out pH measurements for several aqueous buffers in the pH range 2—9 and also determined the Hammett acidity values in the same buffers. Activity corrections were made using the Davies modification of the Debye–Hückel relation [9] and it was found that the Hammett values coincided exactly with the pH measurements, as expected. Thus, plotting the Hammett acidity values against the measured pH gave a straight line. In the present work, the pH values of a series of aqueous citrate buffers in the pH range 2.2—5 were determined by means of a glass electrode and plotted against the measured pH values of the corresponding partially aqueous buffers (50% ethanol), the results fitting a straight line. For the kinetic runs performed in 50% ethanolic buffer it is then justifiable to consider the pH values of the corresponding fully aqueous buffers which are customarily used to assign a quantitative meaning to pH values. The velocity constants for the reaction of the trione hydrate with each of glycine and DL-phenylalanine in a series of 50% ethanolic buffers are summarized in Table 2.

TABLE 2

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

pH of aqueous buffer	Glycine		DL-Phenylalanine	
	10^3k_1/min^{-1}	log k_1 + 3	10^3k_1/min^{-1}	log k_1 + 3
1.45	1.22	0.0864	1.07	0.0294
2.20	2.64	0.4216	2.03	0.3075
2.39	3.50	0.5441	2.66	0.4249
2.83	4.83	0.6839	3.64	0.5605
3.32	5.54	0.7435	4.12	0.6149
3.83	5.70	0.7559	4.09	0.6177
4.35	5.83	0.7657	4.20	0.6235
4.88	5.60	0.7482	4.16	0.6191

For each of the two amino-acids, logarithms of the velocity constant k_1 are plotted as a function of pH in Figure 1. The plot shows clearly that the velocity constant increases rapidly with pH and approaches a limiting value at pH *ca.* 3.4. However, at pH values $\geqslant$5.4, the blank experiments gave positive measurements. Also, in the presence of amino-acid the reaction indicated an infinity value which was nearly double the calculated value. Such anomalous behaviour may be accounted for on the basis that the ammonia produced in the reaction probably reduces an equivalent amount of the excess of trione at pH $\geqslant$ 5.4. This interpretation was confirmed by carrying out the reaction between the trione hydrate (0.08M) and ammonia (0.002M) at various pH at 30° and the results are similar to those obtained by Moore and Stein [10] for the reaction between ninhydrin and ammonia at pH 5.5.

Mechanism of Reaction.—Figure 1 shows that in the reactions of the trione hydrate with glycine and DL-phenylalanine, the velocity constant increases with

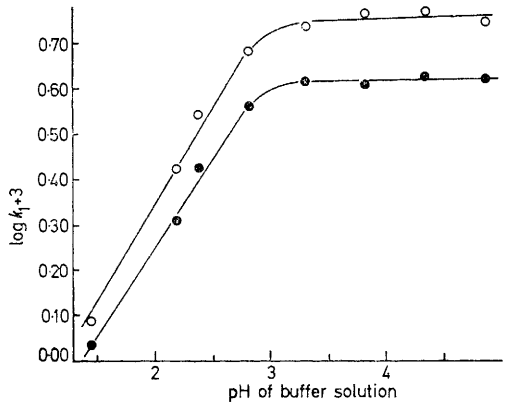


FIGURE 1 Rates of reaction of amino-acids and phenalene-1,2,3-trione hydrate as a function of pH at 30°: ○, glycine; ●, DL-phenylalanine

pH and assumes a limiting value at pH *ca.* 3.4 and remains constant up to pH *ca.* 5.4. Roberts and Caserio [11] pointed out 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, and therefore the occurrence of a maximum rate for the reaction is obtained at the particular pH where not all the amine has been protonated, and, at the same time, enough of the carbonyl compound exists as its conjugate acid to afford a reasonable rate. Thus the limiting reaction rate is obtained when the product of concentrations of the two reacting species, the protonated trione (Htrione$^+$), and the nonprotonated amino-acid (RNH_2), is a maximum [equation (1)]. In the trione hydrate, the hydrated

$$\text{Rate} = k[\text{Htrione}^+][RNH_2] \qquad (1)$$

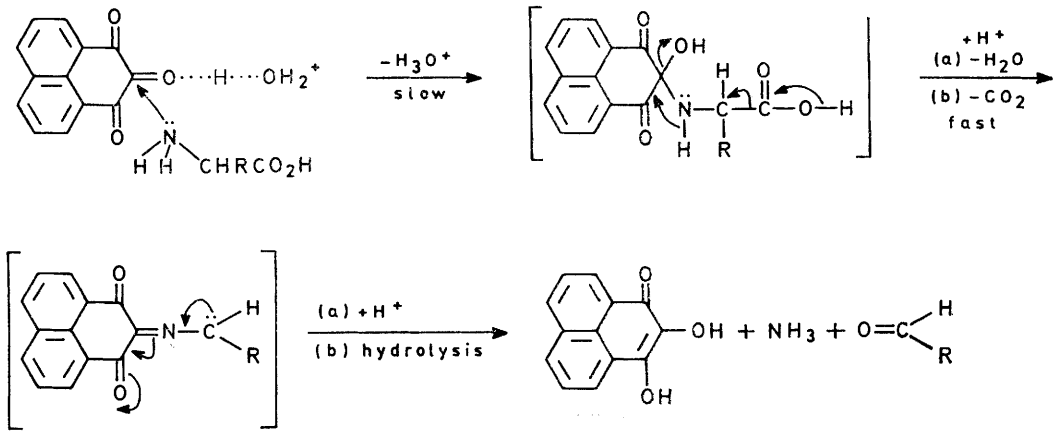
carbonyl has no partial positive charge, as proposed for ninhydrin in a kinetic study of the equivalent reaction of

SCHEME 1

ninhydrin.[12] An equilibrium between the trione and its hydrated form may be present, in acid medium as for the hydration of carbonyl compounds investigated

[7] L. P. Hammett, 'Physical Organic Chemistry,' McGraw-Hill, New York, 1940, ch. 9.
[8] P. J. Lamothe and P. G. McCormick, *Analyt. Chem.*, 1972, **44**, 821.
[9] C. W. Davies, 'Ion Association,' Butterworths, London, 1962.
[10] S. Moore and W. H. Stein, *J. Biol. Chem.*, 1948, **176**, 367.
[11] J. D. Roberts and M. C. Caserio, 'Basic Principles of Organic Chemistry,' Benjamin, New York, 1965, p. 449.
[12] M. Friedman and C. W. Sigel, *Biochemistry*, 1966, **5(2)**, 478.

by Bell (Scheme 1).[13] The reaction pathway may be interpreted as shown in Scheme 2. Since the observed pseudo-first-order rate constant was found to increase with pH over the range 1.45—3.4 (*cf.* Table 2 and Figure 1), this indicates that the overall reaction in this pH range is second order and the rate-limiting step is the



SCHEME 2

attack of amino-acid as a free base on the carbonyl group while the rate of dehydration of carbinol–amine addition compound is fast. If the rate of decomposition of the addition compound is the rate-determining step, the reaction rate would increase with decrease of pH which is not the case. As the pH is increased above 3.4, the overall rate of reaction is nearly constant and the reaction seems to be pH independent. Since the reaction between glycine and the trione hydrate at pH 3.83 was proved to be second-order (Table 1), where the order of reaction is unchanged and the increase in the concentration of free amino-group in the amino-acid is compensated by the decrease in the concentration of the reactive conjugate acid of the trione the rate-limiting step is still the same as that in the pH range 1.45—3.4.

Effect of Polar and Steric Factors on Rates.—At pH 3.83, equation (1) 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. To establish whether rates in the trione reaction are a function of basicities, as a measure of nucleophilicities[14] of the amino-groups, k_1 and also k_2 were determined for 11 α-amino-acids with varying pK_2 values and the results are summarized in Table 3 and Figure 2. The anionic and neutral forms of an amino-acid rather than the zwitterion would be expected to react with the trione because the zwitterion has a positive charge on the nitrogen atom and thus could not participate. At any given pH the concentration of the amino-acid is governed by equation (2) where [A] and [$HA^{\pm}$] represent the con-

$$pH = pK_2 + \log([A]/[HA^{\pm}]) \qquad (2)$$

centrations of unprotonated amino-acid and the zwitter-

ion species respectively. The observed rates, at any pH, should decrease with increasing pK_2 (as shown in Table 3) since [A] is lowered. To correlate the observed rate constant k_2 with basicities of the amino-group, second-order rate constants k_A, based on unprotonated amino-acid as the reactive species, were computed from equation (3), originally derived by Friedman and Wall,[15] where [H^+] is the hydrogen ion concentration,

$$k_{A^-} = k_2(1 + [H^+]/K_2) \qquad (3)$$

K_2 is the ionization constant of the amino-group, and k_{A^-} is the second-order anion rate constant. Since the term representing the concentration of uncharged

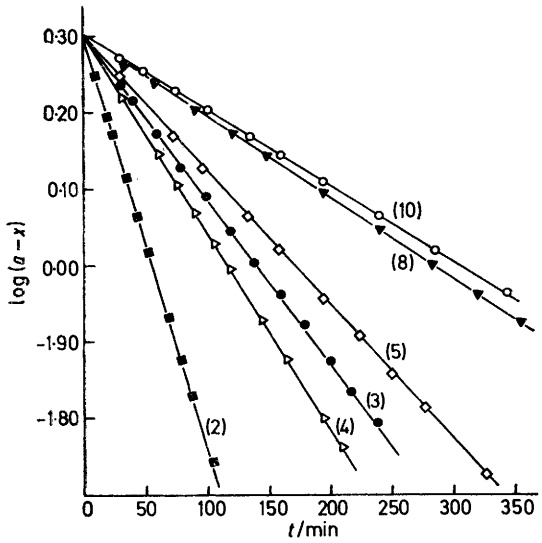


FIGURE 2 Plot of log $(a - x)$ against time for the reaction of some α-amino-acids (0.002M) and phenalene-1,2,3-trione hydrate (0.08M) at pH 3.83 and 30°. The numbers correspond to the amino-acids listed in Table 3

nitrogen (RNH_2) [equation (1)] includes the concentration of amino-acid anion,[8] the term k_{A^-} in equation (3) was substituted by k_A in the present work.

[13] R. P. Bell, *Dan Kemi*, 1966, **47**(**9**), 138.

[14] J. O. Edwards and R. G. Pearson, *J. Amer. Chem. Soc.*, 1962, **84**, 16.

[15] M. Friedman and J. S. Wall, *J. Amer. Chem. Soc.*, 1964, **86**, 3735.

It can be seen from Table 3 that the α-amino-acids (2)—(11) have in common the amino-group attached to a secondary carbon atom (similar steric environment) whereas in glycine (1) the amino-group is attached to a primary carbon atom. The differences in rate constants k_A for the sterically similar amino-acids (2)—(11) (Table 3) may be due to the differences in basicities of the amino-group as measured by the pK_2 values since the concentration of amino-acids is the same. Brønsted-type plots of the logarithms of the second-order rate constants k_A (Table 3), against the pK_2 values [12,16] of the amino-groups at 30° gave, with the exception of histidine, a straight line (Figure 3). This linear relationship demonstrates clearly that the calculated rate constant k_A is a function of the basicity of the amino-group as expected from its pK_2 value. As pointed out by Friedman and Sigel,[12] the reason for the faster rate in the case of L-histidine appears to be the presence of the NH group in the imidazole ring, since L-1-methylhistidine gives the expected rate constant.

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 3.83 and 30°

No.	Amino-acid	10^3k_1/ min^{-1}	10^2k_2/ l mol^{-1} min^{-1}	$10^{-2}k_A$/ l mol^{-1} min^{-1}	log k_A	pK_2 amino-group
(1)	Glycine	5.70	14.25	88.63	3.9475	9.47
(2)	L-3,5-Di-iodotyrosine	12.18	30.44	7.19	2.8569	7.72
(3)	L-1-Methylhistidine	4.88	12.21	11.55	3.0626	8.72
(4)	L-Histidine	5.87	14.66	22.51	3.3524	8.85
(5)	DL-Tyrosine	3.98	9.95	14.64	3.1656	9.00
(6)	DL-Phenylalanine	4.10	10.25	15.53	3.1914	9.00
(7)	DL-Methionine	3.50	8.78	13.71	3.137	9.08
(8)	DL-Leucine	2.40	6.00	15.01	3.1764	9.45
(9)	DL-Aspartic acid	2.39	5.99	15.66	3.1948	9.47
(10)	DL-Glutamic acid	2.26	5.64	16.31	3.2125	9.54
(11)	DL-Norleucine	2.16	5.41	18.47	3.2664	9.63

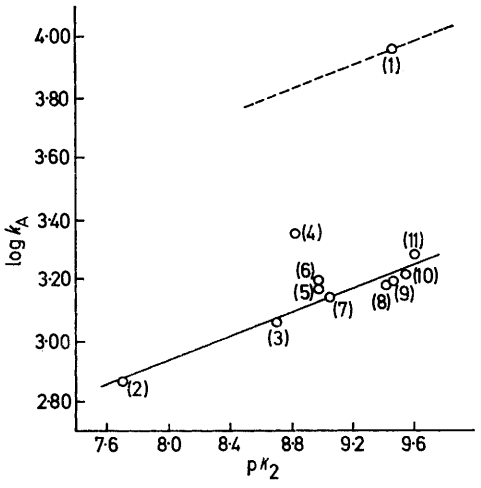


FIGURE 3 Variation of log k_A with pK_2 values for the reaction of α-amino-acids with trione hydrate at pH 3.83 and 30°. The numbers correspond to the amino-acids listed in Table 3

Treatment of the kinetic data obtained for our trione and pK_2 values (Figure 3) may be described by equation (4) which is an extension of the Brønsted catalysis law.

$$\log k_A = \rho \text{ (slope) } pK_2 + b \text{ (intercept)} \quad (4)$$

Equation (4) may be expressed as a Hammett–Taft free energy relationship [5] which relates the logarithm of the ratio of the second-order rate constants of any α-amino-acid denoted as $k_{A(II)}$ and of glycine denoted as $k_{A(I)}$, to differences in steric and polar factors; ρ, the slope, is the

$$\log [k_{A(II)}/k_{A(I)}] = \rho[pK_2(II) - pK_2(I)] + [b(II) - b(I)] \quad (5)$$
$$= \rho\,\sigma^A + E_s$$

polar reaction parameter that measures the sensitivities of rates to basicities of amino-groups; σ^A is the parameter that represents the polar effects of substituents and is simply the difference in the pK_2 values of any α-amino-acid and glycine, and gives a quantitative measurement of the change in the basicity of an amino-group due to the introduction of additional substituents into the α-carbon of glycine; E_s is the steric substituent constant that depends on the size and steric requirements of the substituents in the reaction. It represents the difference in the intercepts of two parallel lines, one experimentally obtained for the series of amino-acids containing a secondary carbon atom and the other (Figure 3) which includes only glycine. The validity of this approach finds precedent in similar reactions [12,15] involving amino-acids of varying structures which indicate that the linear plots of sterically similar series are parallel.

The term σ^A is written with superscript A to denote 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 Figure 3, and

[16] C. Schmidt, P. Kirk, and W. Appleman, *J. Biol. Chem.*, 1930, **88**, 285.

[17] R. Taft, jun., in 'Steric Effects in Organic Chemistry,' ed. M. S. Newman, Wiley, New York, 1956, ch. 13.

[18] W. Pavelich and R. Taft, jun., *J. Amer. Chem. Soc.*, 1957, **79**, 4935.

found to be 0.193. Substituting in the values of $k_{A(II)}$, $k_{A(I)}$, ρ, and σ^A, the parameter E_s can be readily computed and the results are summarized in Table 4. The steric substituent constants E_s (-0.730 ± 0.026), calculated from equation (5), are free energy parameters which give a direct measure of the steric factor associated with the amino-component. The larger E_s values 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.

TABLE 4

Polar and steric substituent constants for the rates of reaction of amino-acids with phenalene-1,2,3-trione hydrate at pH 3.83 and 30°

Amino acid	σ^A	$\rho\,\sigma^A$	E_s	Relative rates
Glycine	0	0	0	1
L-3,5-Di-iodotyrosine	−1.75	−0.338	−0.753	2.136
L-1-Methylhistidine	−0.75	−0.145	−0.740	0.857
L-Histidine	−0.62	−0.120	−0.476	1.029
DL-Tyrosine	−0.47	−0.091	−0.691	0.698
DL-Phenylalanine	−0.47	−0.091	−0.666	0.719
DL-Methionine	−0.39	−0.075	−0.735	0.616
DL-Leucine	−0.02	−0.004	−0.767	0.421
DL-Aspartic acid	0.00	0.00	−0.753	0.421
DL-Glutamic acid	+0.07	+0.014	−0.749	0.396
DL-Norleucine	+0.16	+0.031	−0.712	0.380
		Average -0.730 ± 0.026		

Activation Parameters.—The rates of reaction of α-amino-acids with the trione hydrate were examined at different temperatures at pH 3.83 and the activation parameters $\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$, and $\Delta S^{\ddagger}$ were calculated at 30° and the results are summarized in Table 5.

The data in Table 5 establish that with an increase in the structural and steric complexity of the amino-compounds the enthalpy of activation of the reaction increases to some extent while the free energy remains unchanged. The activation entropy thus becomes more positive as molecular size and α-carbon substitution increases but not in a systematic manner definitely relating to the steric series. This trend in the entropies would appear to contrast with the rule of Price and Hammett.[19] The role of entropy in determining the order of reactivity within a reaction series can be demonstrated *via* the differential thermodynamic parameters of activation taking glycine as the standard as shown in Table 6. According to the rule of Price and Hammett [19] phenylalanine, di-iodotyrosine, and leucine have much less entropy to lose than methionine on going to the transition state and hence show smaller negative entropy changes.

TABLE 5

Temperature dependence of the reaction between α-amino-acids and phenalene-1,2,3-trione hydrate at pH 3.83 and activation parameters at 30°

	$10^3 k_2$/l mol^{-1} s^{-1}				$\Delta H^{\ddagger}$/	$\Delta G^{\ddagger}$/	$-\Delta S^{\ddagger}$/
Amino-acid	25°	30°	35°	40°	kcal mol^{-1}	kcal mol^{-1}	cal mol^{-1} K^{-1}
Glycine	1.42	2.38	3.94	6.33	18.06	21.38	10.98
DL-Methionine	0.88	1.46	2.34		17.11	21.68	15.10
DL-Tyrosine	1.03	1.66	2.69	4.26	17.08	21.59	14.89
DL-Glutamic acid	0.56	0.94	1.54	2.50	17.95	21.94	13.18
DL-Aspartic acid	0.58	1.00	1.68	2.69	18.28	21.91	12.00
DL-Phenylalanine	1.00	1.71	2.80	4.61	18.28	21.60	10.95
L-3,5-Di-iodotyrosine	2.99	5.07	8.21		17.83	20.93	10.24
DL-Leucine	0.56	1.00	1.72		19.81	21.91	6.93

TABLE 6

Relative entropies and energies of activation for the reaction of amino-acid and phenalene-1,2,3-trione hydrate at pH 3.83 and 30°

Amino-acid	$\Delta\Delta H^{\ddagger}$/ kcal mol^{-1}	$\Delta\Delta S^{\ddagger}$/ cal mol^{-1} K^{-1}	$\Delta\Delta G^{\ddagger}$/ kcal mol^{-1}
Glycine	0	0	0
DL-Methionine	−0.95	−4.12	0.30
DL-Tyrosine	−0.98	−3.91	0.21
DL-Glutamic acid	−0.11	−2.20	0.56
DL-Aspartic acid	0.22	−1.02	0.53
DL-Phenylalanine	0.22	0.03	0.22
L-3,5-Di-iodotyrosine	−0.23	0.74	−0.45
DL-Leucine	1.75	4.05	0.53

A plot of $\Delta H^{\ddagger}$ against $\Delta S^{\ddagger}$ of activation for a series of structurally similar amino-acids undergoing the same reaction is shown in Figure 4. It is clear that not only

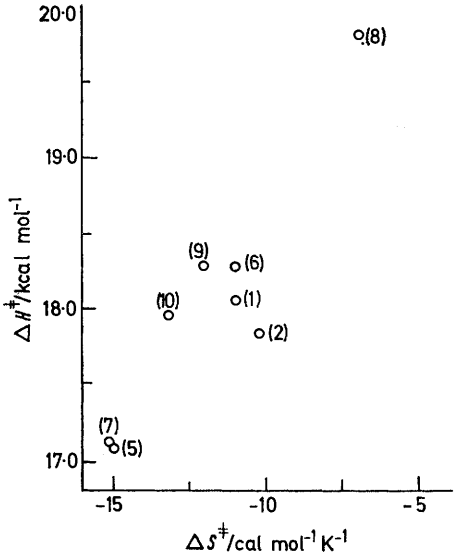


FIGURE 4 Enthalpy and entropy effects for the reaction of trione hydrate with α-amino-acids. The numbers correspond to the amino-acids listed in Table 3

[19] F. Price and L. Hammett, *J. Amer. Chem. Soc.*, 1941, **63**, 2387.

is there no correlation of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ but that the absence of correlation cannot be attributed to any reasonable estimate of experimental uncertainty. Similar results were obtained from data on the formation of oximes,[20] on the formation of thiosemicarbazones,[21] and on the formation of guanylhydrazones [22] of the same or nearly the same series of carbonyl compounds. It is often considered that a large change in the entropy or enthalpy of activation indicates a change in mechanism. This is not necessarily true, a more reliable criterion being whether the change in structure leads to a significant deviation from the 'isokinetic' line.[23] Since the isokinetic relationship is not usually followed with the precision of a linear free energy relationship, judgments concerning the significance of a divergent point must be tempered with caution.

Moderate changes in steric hindrance apparently do not displace a compound from the isokinetic line. Large increases in steric hindrance in the transition state can be expected to cause an increase in $\Delta H^{\ddagger}$ and a decrease in $\Delta S^{\ddagger}$,[17] the result being a predictable departure from the isokinetic line which is the case in the present work.

[5/350 *Received*, 19*th February*, 1975]

[20] F. Fitzpatrick and J. Gettler, *J. Amer. Chem. Soc.*, 1956, **78**, 530.
[21] I. Fiarman and J. Gettler, *J. Amer. Chem. Soc.*, 1962, **84**, 961.
[22] D. Brooks and J. Gettler, *J. Org. Chem.*, 1962, **27**, 4469.
[23] J. Leffler, *J. Org. Chem.*, 1955, **20**, 1202.