12. *Oxaloacetic Acid. Part II.*[1] *The Acid–Base-catalysed Prototropy of Oxaloacetic Acid in Aqueous Solution.*

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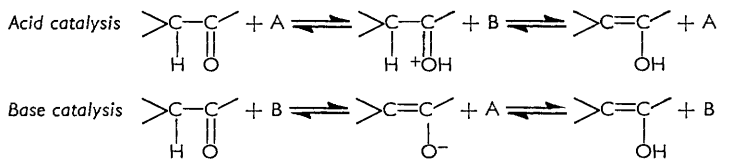
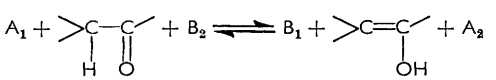
Catalysis of the ketonisation of oxaloacetic acid by acids and bases has been studied by a simple spectrophotometric method. The tautomerisation has been shown to be general acid–base-catalysed; the catalytic constants associated with a number of ionic and neutral species are recorded. The rate equation describing the acid–base catalysis in some buffers has been shown to involve a product term, of the form k_p[Acid][Base], whose significance is considered in relation to concerted and consecutive mechanisms for acid–base-catalysed prototropic rearrangements in aqueous solution.

The mechanism of prototropic isomerisation has been the subject of many kinetic investigations which have been summarised by Bell.[2] Most studies of the kinetics of reactions involving two proton transfers have been made indirectly, *e.g.*, by racemisation, isotope exchange, or halogenation, the last method being of particular importance for keto–enol tautomerisation of acetone [3] and acetoacetic acid and its esters.[4] There are two possible mechanisms for general acid–base catalysis of prototropic rearrangements. In the first, different paths are postulated for the acid- and the base-catalysed reaction but, in each case, two consecutive steps are involved:



The alternative is a concerted reaction in which two catalyst molecules are involved in the transition state, both an acid, A, and a base, B, though these need not be conjugate acid–base pairs:



[1] Part I, *J.*, 1961, 5043.

[2] Bell, "Acid–Base Catalysis," Oxford Univ. Press, 1941; "The Proton in Chemistry," Methuen and Co. Ltd., London, 1959.

[3] Dawson and Spivey, *J.*, 1930, 2180; Bell and Lidwell, *Proc. Roy. Soc.*, 1940, *A*, **176**, 88; Bell and Jones, *J.*, 1953, 88.

[4] Pedersen, *J. Phys. Chem.*, 1933, **37**, 751; 1934, **38**, 601.

Distinction between these two mechanisms, for reactions in aqueous solution, has been the subject of much controversy. It is clear that, in principle, the consecutive and the concerted mechanism may be distinguished by the dependence of reaction rate on buffer concentration; for the former a linear relation should obtain while for the latter an appreciable product term, of the form $k_p[A][B]$, should be observed. However, the solvent may act either as an acid or as a base, and product terms involving the concentration $[H_2O]$ will be kinetically indistinguishable. Dawson and Spivey's results [3] on the iodination of acetone have been analysed in detail in attempts to show the significance of the product term which must be included in the rate equation in order to describe accurately the observed velocities. Pedersen [4] and Swain [5] drew different conclusions from their analyses, the former believing that the evidence for a concerted mechanism was unconvincing while the latter pointed out that Pedersen's analysis was invalid. Bell and Jones [3] repeated the experimental work, on the same reaction, under more carefully controlled conditions and found that the magnitude of the product term was, in fact, greater than that reported by Dawson and Spivey.[3] In the same paper, a detailed analysis is given of the consequences of the concerted mechanism for reactions, in aqueous solution, which show general catalysis by acids and bases. Bell and Jones concluded that the primary reaction path in the iodination of acetone is not, in fact, described by a concerted mechanism.

One reason for preferring the consecutive mechanism, for prototropy in a carbon–oxygen system, is the facility with which oxygen assumes either a positive or a negative charge ($=OH^+$ or $-O^-$). In the isomerisation of methyleneazomethines, where the prototropic shift is between carbon and carbon, the formation of an anionic intermediate is unlikely. The base-catalysed isomerisation would, therefore, be expected to occur by a concerted rather than a consecutive mechanism. The concerted mechanism has been confirmed [6] by a comparison of the rates of racemisation and hydrogen exchange in optically active methyleneazomethines.

The present work is concerned with the kinetics of the general acid–base catalysis of the keto–enol tautomerisation of oxaloacetic acid. The tautomeric equilibrium, in aqueous solutions, at neutral pH, is not so unfavourable to the enol form as is the equilibrium for acetone or even acetoacetic acid. The solid form of oxaloacetic acid is enolic and the ketonisation which occurs when the solid is dissolved in aqueous solution can readily be observed by a direct, spectrophotometric method.

Experimental

Oxaloacetic acid was prepared by Wohl and Oesterlin's method.[7] Carbonate–bicarbonate and acetate buffers were prepared from " AnalaR " reagents. Commercial samples of imidazole (pK_a^{20} 7·04; ref. 8) triethanolamine (pK_a^{20} 8·0; ref. 9), and *N*-methyldiethanolamine (pK_a^{20} 8·61; ref. 9) were used in the preparation of the remaining buffers. All buffer solutions were adjusted to constant ionic strength (μ 0·1; but for carbonate–bicarbonate 0·2) with added sodium chloride.

The ketonisation of oxaloacetic acid was followed spectrophotometrically by the method described in Part I.[1] Buffer solutions were kept in Unicam cells at 1·5° ± 0·1° for 30 min. before addition of oxaloacetic acid dissolved in cold ethanol. Linear plots of log $(D_t - D_{eq})$ against t, where D_t, D_{eq} are the optical densities (280 mμ) at t sec. and at equilibrium, respectively, were obtained over more than four half-lives. The sums of the first-order rate coefficients for ketonisation (k_1) and enolisation (k_{-1}) were calculated from the equation:

$$k_1 + k_{-1} = \frac{2{\cdot}303}{t} \log \frac{D_0 - D_{eq}}{D_t - D_{eq}},$$

[5] Swain, *J. Amer. Chem. Soc.*, 1950, **72**, 4578.
[6] Hughes and Ossorio, *J.*, 1952, 426.
[7] Wohl and Oesterlin, *Ber.*, 1901, **34**, 1139.
[8] Hofmann, " Imidazole and its Derivatives," Part I, Interscience Publ. Inc., New York, 1953, p. 23.
[9] Banks, Diamantis, and Vernon, *J.*, 1961, 4235.

where D_0 is the optical density (280 mμ) at zero time and all other symbols are as defined above.

pH Measurements below pH 9·2 were made, at 0°, by using a conventional glass electrode, standardised against 0·05M-potassium hydrogen phthalate (pH 4·01).[10] At more alkaline pH's, a Cambridge " alki " glass electrode was used, after standardisation against 0·01M-borax (pH 9·46).[10]

Results

The kinetics of ketonisation of oxaloacetic acid have been studied in the pH range 5·0—10·6. The experimental method does not permit accurate measurement of the high rates of reaction outside this range. The appropriate rate equation for the system buffered by a base, B, and its conjugate acid, A, is

$$(k_1 + k_{-1}) = (k_1 + k_{-1})_0 + k_{OH_3^+}[OH_3^+] + k_{OH^-}[OH^-] + k_A[A] + k_B[B], \quad (1)$$

where all concentrations are expressed in mole $l.^{-1}$, A and B are the stoicheiometric concentrations of acid and base, and $(k_1 + k_{-1})_0$ is the residual value of $(k_1 + k_{-1})$ due to catalysis of the tautomerisation by water alone.

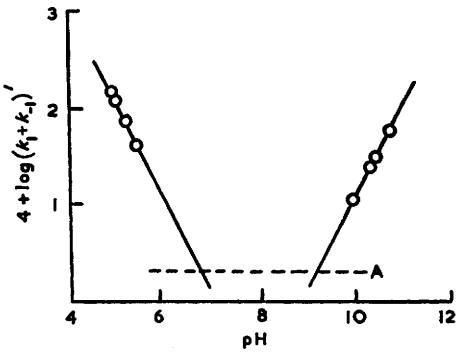


FIG. 1. The dependence of log $(k_1 + k_{-1})'$ (reaction rate at zero buffer concentration) on pH.

Line A represents the maximum possible value of log $(k_1 + k_1)_0$, *i.e.*, the reaction due to catalysis by water alone.

Extrapolation, to zero buffer concentration, of the results obtained in acetate (Table 1) and carbonate–bicarbonate solutions (Table 2), gives a series of values of $(k_1 + k_{-1})' = (k_1 + k_{-1})_0 + k_{OH_3^+}[OH_3^+] + k_{OH^-}[OH^-]$. A plot of log $(k_1 + k_{-1})'$ against pH is shown in Fig. 1. The residual rate constant, $(k_1 + k_{-1})_0$, is effectively zero and is neglected in all subsequent calculations. The regions in which catalysis by OH_3^+ and OH^- are significant are well separated, hence $k_{OH_3^+}$ and k_{OH^-} can be determined from plots of $(k_1 + k_{-1})'$ against $[OH_3^+]$ and $[OH^-]$, respectively.* The values of $[OH_3^+]$ were calculated from the measured pH's, by assuming an activity coefficient of 0·83 for OH_3^+, at μ 0·1 and 0·80 at μ 0·2. The ionic product of water, K_w, appropriate to a temperature of 1·5° and μ 0·2 (carbonate–bicarbonate) or 0·1 (other buffers) was used in calculating the values of $[OH^-]$.

For acetate, carbonate–bicarbonate, and *N*-methyldiethanolamine buffers (Tables 1—3), the values of k_A and k_B [equation (1)] were found by the method described by Bell and Lidwell.[3] Equation (1) can be rearranged into the form:

$$(k_1 + k_{-1}) = (k_1 + k_{-1})_0 + k_{OH_3^+}K_a[A]/[B] + k_{OH^-}K_w[B]/K_a[A] + k_A[A] + k_B[B], \quad (2)$$

where $K_w = [OH_3^+][OH^-]$ and $K_a = [B][OH_3^+]/[A]$. For a given, constant ratio of $[A]/[B] = x$ (*i.e.*, at constant pH, in the same buffer), $(k_1 + k_{-1}) = k_x + [A]\{k_A + k_B/x\}$, where k_x depends only on x. The slope of the linear plot of $(k_1 + k_{-1})$ against [A] is then $(k_A + k_B/x) = y$. A

* The possibility that the increase in reaction rate at zero buffer concentration in the pH range 5·6—5·0 is due to an increase in concentration of the monoanion, (SH^-), which might be more reactive than the predominant dianion (S^{2-}), has been considered ($pK^{25}_{a_2} = 3·83$ for oxaloacetic acid [11]). Terms of the form $[OH_3^+][S^{2-}]$ and $[H_2O][SH^-]$ would be kinetically indistinguishable but calculation shows that, if reaction is *via* the monoanion, the rate coefficient associated with catalysis by water is some 10^4 times larger than that associated with catalysis, by water, of the reaction of the dianion. It is, therefore, concluded that there is no significant contribution, to the overall rate of reaction, from a reaction involving the monoanion.

[10] Bates, " Electrometric pH Determinations," John Wiley and Sons, Inc., New York, 1954, p. 74.
[11] Pedersen, *Acta Chem. Scand.*, 1952, **6**, 243.

series of values of y is found for different, constant values of x, and a plot of y against $1/x$ is a straight line of slope k_B and intercept k_A. Theoretical values of $(k_1 + k_{-1})$, calculated from the measured values of k_A, k_B, $k_{OH_3^+}$ and k_{OH^-}, are shown in Tables 1—3.

The results for imidazole and triethanolamine buffers fit equation (3):

$$(k_1 + k_{-1}) = (k_1 + k_{-1})' + k_A[A] + k_B[B] + k_p[A][B]. \quad (3)$$

TABLE 1.

Acetate buffer.

$k_{OH_3^+} = 1{\cdot}16 \times 10^3$ l. mole^{-1} sec.$^{-1}$; $k_A = 1{\cdot}44$ l. mole^{-1} sec.$^{-1}$; $k_B = 0{\cdot}043$ l. mole^{-1} sec.$^{-1}$.

(i) [A]/[B] = 0·429; $10^2(k_1 + k_{-1})' = 1{\cdot}49$ sec.$^{-1}$.					
[A] + [B] (mole l.$^{-1}$)	0·143	0·1072	0·0715	0·0358	0·0143
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	7·99	6·59	4·69	3·03	1·85
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	8·10	6·45	4·80	3·14	2·15
(ii) [A]/[B] = 0·333; $10^2(k_1 + k_{-1})' = 1{\cdot}20$ sec.$^{-1}$.					
[A] + [B] (mole l.$^{-1}$)	0·10	0·075	0·050	0·025	
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	5·41	4·26	3·26	2·17	
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	5·11	4·13	3·15	2·17	
(iii) [A]/[B] = 0·180; $10^2(k_1 + k_{-1})' = 0{\cdot}73$ sec.$^{-1}$.					
[A] + [B] (mole l.$^{-1}$)	0·118	0·0885	0·0590	0·0295	0·0118
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	3·67	3·01	2·33	1·44	0·97
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	3·77	3·02	2·26	1·51	1·05
(iv) [A]/[B] = 0·111; $10^2(k_1 + k_{-1})' = 0{\cdot}44$ sec.$^{-1}$					
[A] + [B] (mole l.$^{-1}$)	0·10	0·075	0·050	0·025	
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	2·31	1·79	1·38	0·93	
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	2·27	1·81	1·36	0·90	

TABLE 2.

Carbonate–bicarbonate buffer.

$k_{OH^-} = 2{\cdot}14 \times 10^2$ l. mole^{-1} sec.$^{-1}$; $k_A = 0{\cdot}01$ l. mole^{-1} sec.$^{-1}$; $k_B = 0{\cdot}108$ l. mole^{-1} sec.$^{-1}$.

(i) [A]/[B] = 0·82; $10^3(k_1 + k_{-1})' = 1{\cdot}07$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	2·5
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	7·20	5·50	4·05	2·52
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	7·44	5·85	4·04	2·55
(ii) [A]/[B] = 0·408; $10^3(k_1 + k_{-1})' = 2{\cdot}35$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	2·5
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	10·20	8·20	6·50	4·35
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	10·31	8·22	6·33	4·34
(iii) [A]/[B] = 0·111; $10^3(k_1 + k_{-1})' = 5{\cdot}24$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	2·5
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	14·80	12·75	10·0	7·70
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	15·00	12·60	10·14	7·60

TABLE 3.

N-Methyldiethanolamine buffer.

k_A negligible; $k_B = 7{\cdot}45$ l. mole^{-1} sec.$^{-1}$.

(i) [A]/[B] = 0·813; $10^2(k_1 + k_{-1})' = 0{\cdot}007$ sec.$^{-1}$.					
10^2([A] + [B]) (mole l.$^{-1}$)	2·5	2·0	1·5	1·0	
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	10·42	8·55	6·21	3·93	
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	10·28	8·22	6·16	4·11	
(ii) [A]/[B] = 1·634; $10^2(k_1 + k_{-1})' = 0{\cdot}004$ sec.$^{-1}$.					
10^2([A] + [B]) (mole l.$^{-1}$)	3·0	2·5	2·0	1·5	1·0
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	8·38	7·17	5·38	4·12	2·73
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	8·48	7·07	5·66	4·24	2·83
(iii) [A]/[B] = 4·27; $10^2(k_1 + k_{-1})' = 0{\cdot}004$ sec.$^{-1}$.					
10^2([A] + [B]) (mole l.$^{-1}$)	5·0	4·0	3·0	2·0	1·0
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	7·17	5·50	4·20	2·67	1·20
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	7·07	5·64	4·23	2·82	1·41

TABLE 4.

Imidazole buffer.

$k_B = 0.291$ l. mole^{-1} sec.$^{-1}$; $k_A = 0.03$ l. mole^{-1} sec.$^{-1}$; $k_p = 1.22$ l.^{2} mole^{-2} sec.$^{-2}$.

(i) [A]/[B] = 3·165; $10^3(k_1 + k_{-1})' = 0.28$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	2·5
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	11·70	8·52	5·60	2·56
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	11·70	8·48	5·46	2·74
(ii) [A]/[B] = 1·00; $10^3(k_1 + k_{-1})' = 0.078$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	20·0	15·0	10·0	7·5
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	44·3	31·5	19·9	14·7
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	44·4	31·1	19·2	13·9
10^2([A] + [B]) (mole l.$^{-1}$)	5·0	2·5	1·5	
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	9·33	4·35	2·51	
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	8·86	4·29	2·56	
(iii) [A]/[B] = 0·563; $10^3(k_1 + k_{-1})' = 0.042$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	20·0	15·0	10·0	7·5
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	49·7	35·4	22·3	15·9
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	50·7	35·9	22·6	16·4
10^2([A] + [B]) (mole l.$^{-1}$)	5·0	2·5	1·5	1·0
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	11·0	5·11	2·93	1·96
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	10·6	5·15	3·05	2·04
(iv) [A]/[B] = 0·198; $10^3(k_1 + k_{-1})' = 0.028$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	20·0	15·0	10·0	7·5
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	56·2	41·7	27·2	20·1
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	56·3	41·0	26·5	19·6
10^2([A] + [B]) (mole l.$^{-1}$)	5·0	2·5	1·5	1·0
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	13·3	6·33	3·77	2·37
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	12·9	6·34	3·76	2·52

TABLE 5.

Triethanolamine buffer.

$k_A < 0.002$ l. mole^{-1} sec.$^{-1}$; $k_B = 0.610$ l. mole^{-1} sec.$^{-1}$; $k_p = 2.24$ l.^{2} mole^{-2} sec.$^{-2}$.

(i) [A]/[B] = 4·0; $10^3(k_1 + k_{-1})' = 0.044$ sec.$^{-1}$.					
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	2·5	
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	16·4	11·5	7·37	3·24	
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	15·8	11·4	7·04	3·30	
(ii) [A]/[B] = 1·0; $10^3(k_1 + k_{-1})' = 0.034$ sec.$^{-1}$.					
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	3·5	1·0
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	36·7	26·8	17·5	8·31	3·10
$10^3(k_1 + k_{-1})$ (sec.$^{-1}$) calc.	36·1	26·1	16·7	7·80	3·11

TABLE 6.

Succinate buffer.

[A]/[B] = 0·35; $10^2(k_1 + k_{-1})' = 0.237$ sec.$^{-1}$.					
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	2·5	1·0
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	2·05	1·53	1·01	0·535	0·330

Tri(hydroxymethylamino)methane.

(i) [A]/[B] = 4·88; $10^2(k_1 + k_{-1})' = 0.005$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	10·0	7·5	5·0	2·5
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	1·05	0·742	0·449	0·144
(ii) [A]/[B] = 1·05; $10^2(k_1 + k_{-1})' = 0.003$ sec.$^{-1}$.				
10^2([A] + [B]) (mole l.$^{-1}$)	9·1	6·825	4·6	2·3	0·91
$10^2(k_1 + k_{-1})$ (sec.$^{-1}$) obs.	3·02	2·18	1·33	5·99	1·84

Values of k_A and k_B have been determined, as described above, from results obtained at very low buffer concentrations, where the dependence of $(k_1 + k_{-1})$ on [A] is approximately linear. Results obtained at higher buffer concentrations have been analysed by the method applied by Bell and Jones [3] to the iodination of acetone. Equation (3) may be written in the form

$$(k_1 + k_{-1}) = (k_1 + k_{-1})' + [B]\{k_A x + k_B + k_p[A]\}, \quad (4)$$

where x is the stoicheiometric buffer ratio, [A]/[B]. For different, constant values of x, the approximately linear plots of $[(k_1 + k_{-1}) - (k_1 + k_{-1})']/[B]$ against [A] are parallel, and the common slope gives the value of k_p. Theoretical values of $(k_1 + k_{-1})$ calculated from the measured values of k_A, k_B, and k_p are shown in Tables 4 and 5.

The catalytic constants have not been determined in succinate, tri(hydroxymethylamino)-methane, or phosphate buffers, but the results in Table 6 and that given in Table 2 of Part I, show that an appreciable product term is required in the rate equation to describe the catalysis of tautomerisation in these buffers.

Discussion

The constancy of the equilibrium molar extinction coefficient of oxaloacetic acid in the pH range 5·0—10·6 (cf. Part I) leads to the conclusion that the tautomeric equilibrium constant is unchanged over this range. Since the sum of the first-order rate coefficients, $(k_1 + k_{-1})$, is most easily determined from the optical data, the catalytic effects of acids and bases on the isomerisation have been considered in relation to this quantity rather than to the initial velocity of ketonisation. The separate values of k_1 and k_{-1} may be calculated by using a value of 5·45 for the tautomeric equilibrium constant, k_1/k_{-1} (Part I).

The rate equation describing a general acid–base-catalysed reaction can normally be expressed as the sum of the individual terms which account for catalysis by the different acids and bases present in solution [equation (1)]. The catalytic constants associated with the ions OH_3^+ and OH^- have been determined by extrapolation, to zero buffer concentration, of the results obtained in acetate and carbonate buffers, respectively (Tables 1 and 2). The pH regions in which specific catalysis by hydrogen and hydroxyl ions are significant are well separated (see Figure), as for the mutarotation of glucose.[12] The system is further simplified by the virtual absence of catalysis by water. The catalytic constants associated with the acidic and basic species studied are summarised in Table 7.

Table 7.

Acid	k_A (l. mole^{-1} sec.$^{-1}$)	Base	k_B (l. mole^{-1} sec.$^{-1}$)
OH_3^+	$1{\cdot}16 \times 10^3$	OH^-	$2{\cdot}14 \times 10^2$
HOAc	1·44	OAc^-	0·043
Imidazolium	0·03	Imidazole	0·291
$^+NH(CH_2{\cdot}CH_2{\cdot}OH)_3$	$<0{\cdot}002$	$N(CH_2{\cdot}CH_2{\cdot}OH)_3$	0·610
$^+NHMe(CH_2{\cdot}CH_2{\cdot}OH)_2$	Negligible	$NMe(CH_2{\cdot}CH_2{\cdot}OH)_2$	7·45
HCO_3^-	0·01	CO_3^{2-}	0·108

The data are too limited to permit calculation of the Brönsted exponents, particularly since three of the five buffers studied in detail are nitrogen bases. For mutarotation of glucose,[1,2] the results obtained with pyridine, histidine, and α-picoline deviate more markedly from the Brönsted relation than do those for other buffer systems. In the present case, the variation of k_A and k_B with acidic dissociation constant is qualitatively as required by the Brönsted relation although the increase in k_B on change of the buffer from tri-ethanolamine to *N*-methyldiethanolamine is much larger than would be expected for a change in pK of 0·6. The Brönsted relation does not appear to apply to the carbonate–bicarbonate system.

The experimental results so far available show that the tautomerisation of oxaloacetic acid is catalysed by OH_3^+, OH^-, uncharged, anionic, and cationic acids, and neutral and anionic bases. The results for acetate, *N*-methyldiethanolamine, and carbonate–bicarbonate buffers (Tables 1—3) are apparently consistent with the commonly accepted mechanistic theory for two-proton transfer, *i.e.*, consecutive attack by an acid and a base or *vice versa*. For the same three buffers, there is good agreement between the measured tautomerisation constants and those calculated from experimentally determined catalytic constants. The overall rate constant is, therefore, apparently described accurately by equation (1). The results for phosphate, succinate, triethanolamine, imidazole, and

[12] Osaka, *Z. phys. Chem.*, 1900, **35**, 702.

tri(hydroxymethylamino)methane (Tables 4—6) are not consistent with equation (1). The plot of rate constant against buffer concentration is, in each case, convex to the concentration axis. Consequently, a product term, of the form k_p[A][B] must be introduced into the rate equation in order to describe accurately the experimentally determined reaction rates. The agreement between the measured rate constants and those calculated on the basis of equation (3) is good.

The apparent discrepancy between the results in these two sets of buffers may be explained by the limitations of the experimental method. In 0·1M-acetate buffer, the half-life of the reaction lies between 10 and 30 sec., depending on the buffer ratio, even at 1·5°. Accurate values cannot be obtained for faster reactions, *i.e.*, at higher buffer concentrations. The catalytic constant for the neutral base in *N*-methyldiethanolamine is so large that the maximum buffer concentration which can be used lies between 0·03M and 0·05M. The absence of a product term from the available results does not, therefore, imply that a product term is not involved at higher buffer concentrations. The experimental method limits the range of buffer concentrations which may be studied. For carbonate–bicarbonate buffers, pH measurements and control of pH are difficult and the accuracy of the results obtained when using this buffer system is correspondingly reduced.

The above results must now be considered within the framework of the controversy concerning distinction between consecutive and concerted mechanisms in prototropic rearrangements in aqueous solution. The earlier arguments presented by Pedersen and Swain have been summarised by Bell.[2] The present work has been analysed by the method first proposed by Swain and applied more rigorously by Bell and Jones [3] to the iodination of acetone. This analysis involves the assumption that, if a concerted mechanism obtains, the reactivity of a given acid, A, is independent of the nature of the base, B, involved in a concerted attack on the substrate. The first-order rate coefficient for the prototropic rearrangement can then be expressed in the form

$$k_T = k_c \sum_A r_A[A] \sum_B r_B[B], \qquad (5)$$

where $k_T = k_1 + k_{-1}$, and r_A and r_B are the reactivities of acids, A, and bases, B. If we assume that the relative reactivity of water is unity, the equation may be written more explicity for a solution containing a base, B, and its conjugate acid, BH^+, in the form:

$$k_T = k_c\{[H_2O] + a_1[OH_3^+] + a_2[BH^+]\}\{(H_2O) + b_1[OH^-] + b_2[B]\}. \qquad (6)$$

If equation (6) is expanded, coefficients of terms in $[OH_3^+]$, $[OH^-]$, $[BH^+]$, [B] and [B][BH^+] can be equated to the experimentally determined catalytic constants of equation (3). The residual velocity constant, k_0, is equated to the sum of the two remaining terms. Hence,

$$k_0/k_c = [H_2O]^2 + a_1b_1K_w; \qquad k_{BH^+}/k_c = a_1[H_2O] + a_1b_2K_a;$$
$$k_{OH_3^+}/k_c = a_1[H_2O]; \qquad k_B/k_c = b_2[H_2O] + a_2b_1K_wK_a;$$
$$k_{OH^-}/k_c = b_1[H_2O]; \qquad k_p/k_c = a_2b_2.$$

Values of k_c, a_1, b_1, a_2, and b_2 have been calculated by Swain for the iodination of acetone, the mutarotation of glucose, and the enolisation of acetoacetate, such that the agreement between the experimentally determined values of k_0, $k_{HO_3^+}$, k_{OH^-}, k_{BH^+}, k_B, and k_p and those calculated from the above six simultaneous equations is within a factor of two. However, Bell and Jones point out that there is some ambiguity in this superficial fit of the experimental data with catalytic constants predicted on the basis of a concerted mechanism. For example, for the iodination of acetone, " the coefficient a_2 makes only a small contribution to the values of k_{BH^+} and k_B, so its value can be adjusted within wide limits in order to fit the observed value of k_p ". They go on to consider the possible consequences of different relative contributions of what they call " direct " and " indirect "

terms * to the quantities k_{BH^+}, k_B, and k_0. They conclude that one of two conditions must be fulfilled if the concerted mechanism makes more than a small contribution to the overall reaction: either the contribution of the product term must be significant at low buffer concentrations or the sum of the exponents of the Brönsted relation must be unity.

Attempts to calculate the values of the reactivities a_1, b_1, a_2, b_2, and the constant, k_c, for the tautomerisation of oxaloacetic acid in solutions buffered with imidazole or triethanolamine lead to the conclusion that the analysis is inapplicable in this case. The positive values of the coefficients which are consistent with the measured catalytic constants lead to the prediction of a residual velocity, due to catalysis by water alone, which is some 100 times greater than the maximum possible experimental residual velocity. Table 8 summarises the results obtained, for imidazole, by assuming that $[H_2O] = 55$, $K_w = 7{\cdot}9 \times 10^{-16}$, and $K_a = 10^{-7}$ and putting $k_c = 10^{-6}$, $a_1 = 2{\cdot}1 \times 10^7$, $b_1 = 3{\cdot}9 \times 10^6$, $a_2 = 3{\cdot}4 \times 10^2$, and $b_2 = 5{\cdot}3 \times 10^3$.

TABLE 8.

	$k_{OH_2^+}$	k_{OH^-}	k_{BH^+}	k_B	k_p	k_0
Experimental	$1{\cdot}16 \times 10^3$	$2{\cdot}14 \times 10^2$	0·03	0·291	1·22	10^{-5}
Calculated	$1{\cdot}16 \times 10^3$	$2{\cdot}14 \times 10^2$	0·03	0·292	1·93	3×10^{-3}

If a value of k_c is chosen such that the predicted residual velocity constant of $\sim 10^{-5}$ sec.$^{-1}$, then the coefficients a_2 and b_2 are negative and the analysis has no physical significance.

Although Swain's analysis fails for the acid–base-catalysed prototropy of oxaloacetic acid, it is certainly not possible to eliminate the possible occurrence of a concerted mechanism in this system. The actual magnitude of the product term, although of little significance on Bell's view, is appreciably greater than for the halogenation of acetone. For example, the product term contributes 25—30% of the total rate constant in 0·2M-glyoxaline and 15—23% in 0·1M-triethanolamine. For halogenation of acetone, in systems buffered by acetate, the contribution of the product term is of the order of 4% in 0·1M-buffer and only rises to 15—20% in buffer solutions which are over 0·35M. Bell's conclusion that the occurrence of a product term is consistent with there being a small contribution from a concerted mechanism can, therefore, certainly be extended to the present system.

Swain pointed out that product terms will be observed only in systems for which the solvent (water) is a very inefficient catalyst. This is clearly true for the tautomerisation of oxaloacetic acid but the reason for this is not obvious. A possible explanation lies in the fact that, in this particular case, the substrate carries two negative charges since both carboxyl groups are fully ionised in the pH range studied [11] ($pK_1^{25} = 2{\cdot}32$; $pK_2^{25} = 3{\cdot}83$). Orientation of water molecules by the two anionic groups adjacent to the reactive sites in the molecule, while favouring nucleophilic attack on the enolic group, may hinder electrophilic attack at the second site. Catalysis by water alone would, on these terms, be expected to be less efficient for a concerted than for a consecutive mechanism.

One factor which would favour a concerted rather than a consecutive mechanism, in this case, is the ease with which a charge would be spread through the molecule owing to the high degree of conjugation. Synchronous attack by an acid and a base would, therefore, be easier than in a less highly conjugated system.

Although a substrate was used for which a concerted mechanism would be favoured, the only evidence for such a mechanism still lies in the observation of a relatively large contribution to the overall rate of reaction by a product term. It is not possible, on the available evidence, to decide whether reaction proceeds exclusively by a concerted mechanism, but it is certain that a consecutive mechanism cannot account for all the

* Direct terms: $[H_2O]^2$, $[BH^+][H_2O]$, $[B][H_2O]$. Indirect terms: $[H_2O^+][OH^-]$, $[BH^+][OH^-]$, $[B][OH_3^+]$.

results. Studies on the acid–base-catalysed tautomerisation of diethyl oxaloacetate are in progress.

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