[CONTRIBUTION FROM THE INSTITUTE OF EXPERIMENTAL BIOLOGY, UNIVERSITY OF CALIFORNIA]

Catalytic Effect of Acetate and Phosphate Buffers on the Iodination of Tyrosine

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In the reactions between iodine and tyrosine[1] it was observed that the rate is accelerated by the phosphate buffer. It was further noted that such an effect is not due to the increase of ionic strength. It is now possible to show that the specific rate, k_2, in phosphate buffer corresponds to the sum of a series of catalytic effects represented by the equation

$$k_2 = k_{\mathrm{OH}}(\mathrm{OH}^-) + k_{\mathrm{HA}}(\mathrm{HPO_4^=}) + k_{\mathrm{H_2A}}(\mathrm{H_2PO_4^-}) \quad (1)$$

where k_{OH}, k_{HA} and $k_{\mathrm{H_2A}}$ are the catalytic coefficients of hydroxide, dibasic and monobasic phosphate ions, respectively. In view of the equilibria

$$\mathrm{H_2PO_4^-} \rightleftarrows \mathrm{H^+} + \mathrm{HPO_4^=}$$

$$\mathrm{H_2O} \rightleftarrows \mathrm{H^+} + \mathrm{OH^-}$$

equation (1) may be written

$$k_2 = k_{\mathrm{OH}}\,K_{\mathrm{W}}\frac{1}{(\mathrm{H^+})} + (\mathrm{HPO_4^=})\left[k_{\mathrm{HA}} + \frac{k_{\mathrm{H_2A}}}{K_{\mathrm{H_2A}}}(\mathrm{H^+})\right] \quad (2)$$

where $K_{\mathrm{H_2A}}$ is the dissociation constant of $\mathrm{H_2PO_4^-}$.

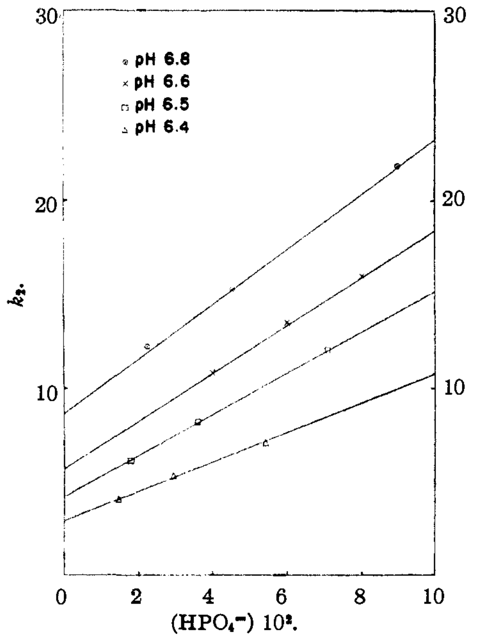

Fig. 1.—Specific rate, k_2, as the function of $(\mathrm{HPO_4^=})$ at 25°.

Catalytic Activity of Phosphate Buffer.—Table I presents a summary of the specific rates obtained in different pH of different concentrations of phosphate at 25°. It may be recalled that the iodination of tyrosine[1] follows a bimolecular reaction rate

$$\mathrm{d}x/\mathrm{d}t = k_2\,(a - x)(b - 2x) \quad (3)$$

TABLE I

THE EFFECT OF $\mathrm{HPO_4^=}$ ON THE RATE AT 25°. $(\mathrm{I^-}) = 6.3 \times 10^{-2}$

pH	$(\mathrm{HPO_4^=})\ 10^2$	k_2	k_2 calcd.
6.80	2.23	12.2	11.5
6.80	4.47	15.4	16.9
6.80	8.95	22.0	21.1
6.60	4.02	10.8	10.7
6.60	6.03	13.6	14.0
6.60	8.06	16.0	17.4
6.50	1.79	6.2	6.4
6.50	3.58	8.2	9.5
6.50	7.16	12.31	15.6
6.40	1.50	4.2	5.1
6.40	3.00	5.4	7.8
6.40	5.40	7.2	11.8

where a and b are the initial concentrations of tyrosine and iodine, respectively, and x is that of diiodo-tyrosine. The specific rate, k_2, was calculated by the integrated form of equation (3). The concentrations are in moles per liter, and time in minutes. The rate measurements were the same as previously[1] described. The reaction mixtures contained 1.0 m sodium chloride so that the ionic strength was approximately constant. The pH was measured with a glass electrode.

As shown in Fig. 1, a plot of $(\mathrm{HPO_4^=})$ against the specific rate reveals a linear function

$$k_2 = A + B(\mathrm{HPO_4^=}) \quad (4)$$

The slopes, B, and intercepts, A, were tabulated in Table II. By comparison with equation (2) we find

$$A = k_{\mathrm{OH}}\,K_{\mathrm{W}}\frac{1}{(\mathrm{H^+})} \quad (5)$$

$$B = k_{\mathrm{HA}} + \frac{k_{\mathrm{H_2A}}}{K_{\mathrm{H_2A}}}(\mathrm{H^+}) \quad (6)$$

TABLE II

THE VALUES OF A AND B IN PHOSPHATE BUFFER

pH	A	B
6.80	8.8	130
6.60	5.8	110
6.50	4.3	105
6.40	3.0	85

In Fig. 2 we have drawn the relation[2] between A and $1/(\mathrm{H^+})$ from the slope of this curve and $K_{\mathrm{W}} = 1.2 \times 10^{-14}$ we have computed the catalytic coefficient of hydroxide ion, k_{OH}, to be 1.0×10^8.

Similarly Fig. 3 presents the plot of B *versus* $(\mathrm{H^+})$; it appears that equation (6) holds very well. From the intercept of the curve in Fig. 3

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[1] C. H. Li, THIS JOURNAL, 64, 1147 (1942).

[2] We have assumed that $a_{\mathrm{H}} = (\mathrm{H^+})$ throughout in this paper.

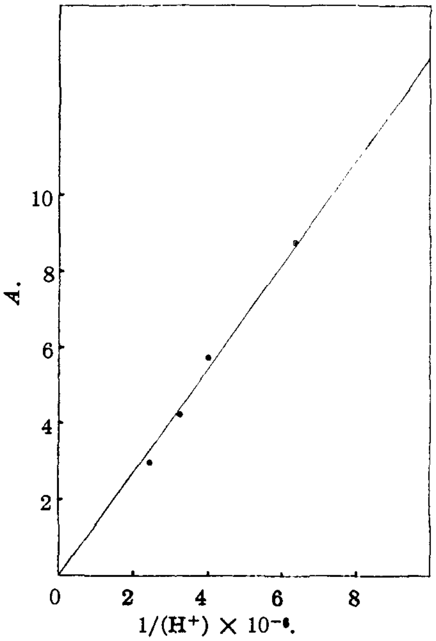

Fig. 2.—The change of intercepts, *A*, with $1/(H^+)$.

we obtain $k_{HA} = 150$, whereas the slope $= k_{H_2A}/K_{H_2A} = 1.6 \times 10^8$. Since the dissociation constant[3] of $H_2PO_4^-$, $K_{H_2A} = 7.5 \times 10^{-8}$ at 25°, k_{H_2A} is calculated to be 12. Thus, the specific rate of diiodo-tyrosine formation in phosphate buffers has the following expression

$$k_2 = 1.0 \times 10^8\ (OH^-) + 150\ (HPO_4^=) + 12\ (H_2PO_4^-) \qquad (7)$$

Hence it may be said that the catalytic effect of dibasic phosphate ion is about 10 times more active than the monobasic phosphate ion while the hydroxide ion is most potent in its catalytic activity due to its highly basic character. This is to be expected if the reaction follows the patterns of generalized base catalysis in phosphate buffer. In the last column of Table I we have calculated the specific rate using equation 7; there is a closer agreement between the observed and calculated values in higher *p*H than at lower *p*H.

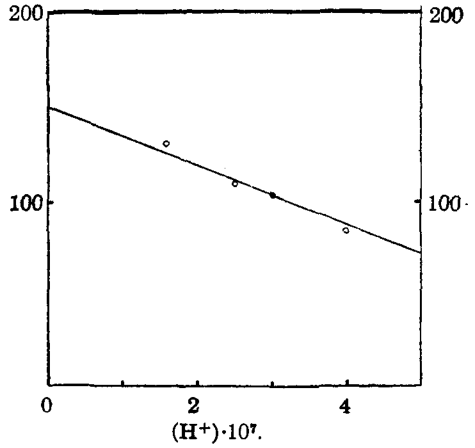

Fig. 3.—The change of slopes, *B*, with (H^+).

Catalytic Activity of Acetate Buffers.—In acetate buffer, the acetate ion itself does not seem to involve the catalytic mechanism. This is surprising because one would expect that the catalytic coefficient of acetate ion should be of the same magnitude as that for monobasic phosphate. Table III summarizes the kinetic data studied in acetate buffers with different concentrations. The relations between the concentration of acetate ion and specific rate do not conform to similar equations given for the phosphate buffer.

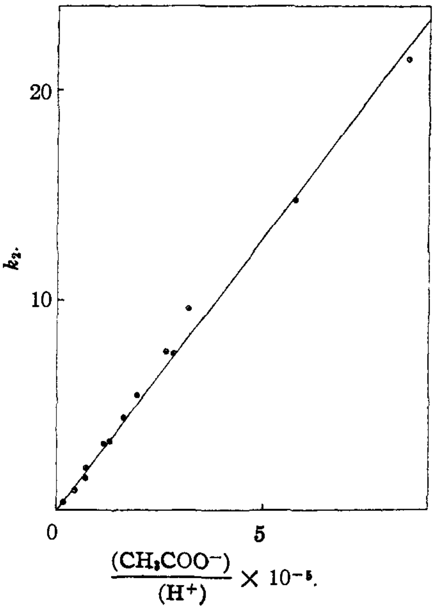

Fig. 4.—Specific rate, k_2, as the function of $(CH_3COO^-)/(H^+)$ at 25°.

As given in Fig. 4 it is possible to show that the specific rate is a function of $(CH_3COO^-)/(H^+)$

$$k = C(CH_3COO^-)/(H^+) \qquad (8)$$

where *C* is a constant. This result may be explained if the acetate buffers are assumed to contain the complex acetate ion $CH_3C(OH)O_2^=$ formed by combination[4] of the simple ion with the hydroxide ion. The relation between the concentrations of these entities is given by $K_c = (CH_3C(OH)O_2^=)/(CH_3COO^-)(OH^-)$, where K_c is the complex constant. The catalytic coefficient for the complex anion being denoted by k_c, equation (8) becomes

$$K_2 = K_w K_c k_c \frac{(CH_3COO^-)}{(H^+)} \qquad (9)$$

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(3) A. Kossiakoff and D. Harker, This Journal, 60, 2047 (1938).

(4) In dilute acetic acid solution we may have some acetic acid hydrate

$$CH_3COOH + H_2O \rightleftarrows CH_3C(OH)_3 \rightleftarrows CH_3C(OH)O_2^= + 2H^+$$

The formation of the complex acetate ion may not be unreasonable.

From Fig. 4 the slope of the curve gives $K_wK_ck_c = 2.5 \times 10^{-5}$. Since the magnitude of the complex anion constant K_c is unknown, it is not possible to assign a value to the catalytic coefficient[5] k_c. However, it may be expected that the complex anion, $CH_3C(OH)O_2^=$, is more basic than the simple hydroxide ion; this in turn suggests that the catalytic coefficient of the complex anion would be greater than that of hydroxide ion. It is of interest to note that Dawson and Spivey[6] have suggested the formation of the complex ion ($CH_3COOH{\cdot}CH_3COO^-$) as an active catalytic species in the reaction of iodine and acetone in acetate buffer.

Table III includes the specific rate calculated by equation (9). It will be noted that the calculated and observed binolecular rate constants are in good agreement.

TABLE III

EFFECT OF ACETATE BUFFER ON THE RATE AT 25°. $(I^-) = 3.15 \times 10^{-2}$

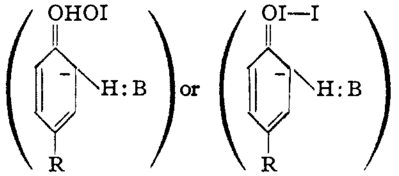
*p*H	(CH_3COO^-)	k_2	k_2 calcd.
6.0	0.280	7.4	7.0
6.0	.570	14.8	14.3
6.0	.850	21.5	21.2
5.6	.268	3.2	2.7
5.6	.398	4.5	4.0
5.6	.645	7.5	6.5
5.6	.805	9.7	8.1
5.4	.250	2.0	1.6
5.4	.760	5.4	4.8
5.0	.209	0.6	0.5
5.0	.418	1.0	1.1
5.0	.626	1.5	1.6

Mechanism.—Base catalysis has thus been clearly demonstrated in both acetate and phosphate buffers. The possibility that the catalysis may belong to the generalized base type needs further investigations. In a recent study[7] of the iodination of histidine the base catalysis is also observed. It may be recalled that the iodination of aliphatic compounds, *e. g.*, ketones,[8] however, involves both acid and base catalysis.

Previous studies[1] have found that the most reactive species in the iodination of phenols are hypoiodous acid and phenolate ion. It is possible that the base present in the solution participates in the equilibria

$$C_6H_5OH \rightleftharpoons C_6H_5O^- + H^+$$
$$I_2 + H_2O \rightleftharpoons HOI + H^+ + I^-$$

and hence increases the concentration of either HOI or $C_6H_5O^-$. Since the solution was kept at constant *p*H, such action of the base is very unlikely. On the other hand, the base probably formed a complex with the reactants in the transition state of the reaction



where :B denotes the base. Thus the removal of hydrogen atom from the ring is assisted by :B and in turn enhances the rate of the iodine atom entering into the ortho position. It is obvious that the catalytic coefficients of :B depend on its basicity. In the present investigation we have obtained an order of the catalytic effect: $CH_3C(OH)O_2^= > OH^- > HPO_4^= > H_2PO_4^-$, which approximately follows the order of their basicity.

Summary

1. The catalytic effect of phosphate and acetate buffers in the reactions between iodine and tyrosine has been investigated at 25°. In phosphate buffer, the specific rate is $k_2 = 1.0 \times 10^8 (OH^-) + 150 (HPO_4^=) + 12 (H_2PO_4^-)$.

2. In acetate buffer, the specific rate is found proportional to the ratio $(CH_3COO^-)/(H^+)$. The existence of a complex anion $CH_3C(OH)O_2^=$ as the catalytic agent is postulated.

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[5] We may assume that K_c is very small and has a magnitude of 10^{-10}. This is not unreasonable if we compare the following equilibria

$$OP(OH)_2O^- \rightleftharpoons OP(OH)O_2^= + H^+$$
$$OC(OH)_2 \rightleftharpoons OC(OH)O_2^- + H^+$$
$$H_3CC(OH)_2O^- \rightleftharpoons H_3CC(OH)O_2^= + H^+$$

Since we know the dissociation constants of monobasic phosphate and carbonic acid of about 10^{-6}, the assumption of taking 10^{-10} as K_c for the complex anion $CH_3C(OH)_2O^-$ may be justified. Thus, if $K_c = 10^{-10}$, k_c is computed to be 2.5×10^{19}: it is far greater than the catalytic coefficient for hydroxide ion as obtained in phosphate buffer.

[6] H. M. Dawson and E. Spivey, *J. Chem. Soc.*, 2180 (1930).

[7] C. H. Li, THIS JOURNAL, 66, 225 (1944).

[8] H. M. Dawson and co-workers, *J. Chem. Soc.*, 2282 (1926): 2844 (1928); 1884 (1929).