the polymerization product was precipitated by adding methanol. Conversion was calculated from the weight of the polymer obtained in the dry state. Viscosity measurements were made in benzene solution with an Ostwald viscometer at 30°. The number-average degrees of polymerization were calculated from the Tobolsky equation[20] for MMA and from that of Mayo[10,21] for styrene: MMA, $\log \bar{P}_n = 3.420 + 1.13 \log [\eta]$; styrene, $\log \bar{P}_n = 3.204 + 1.37 \log [\eta]$.

Counting Procedure.—Radioactive polymers were purified by triple precipitation (toluene–methanol).[22] Radiocarbon analyses were performed with a Packard Tri-Carb liquid scintillation spectrometer, Model 314-AX, using 20-ml. potassium-free, No-Vit glass, liquid scintillation spectrometer vials with tinfoil-lined, toluene-resistant caps. Packard Instrument Co. scintillation grade 2,5-diphenyloxazole (PPO) and *p*-bis[2-(5-phenyloxazole)]-benzene (POPOP) were used as primary and secondary scintillators, respectively. The scintillation solution consisted of 3.50 g. of PPO and 0.100 g. of POPOP dissolved in 1 l. of Wako reagent grade toluene. A sample was prepared for counting by pipetting 20.0 ml. of scintillation solution into a vial containing a known amount of active polymer.

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[20] B. Baysal and A. V. Tobolsky, *J. Polymer Sci.*, **9**, 171 (1952).

[21] D. C. Pepper [*ibid.*, **7**, 347 (1951)] has verified this relation for low molecular weight polymers.

[22] Difficulties encountered in the purification procedure in such experiments are that fractionation of polymer accompanies the purification. J. Bevington's work [*Proc. Roy. Soc* (London), **A221**, 437, 453 (1954); *J. Polymer. Sci.*, **12**, 449 (1952); **14**, 463 (1954)] and E. M. Arnett's work [*J. Am. Chem. Soc.*, **74**, 2031 (1952)] have given assurance that double precipitations can remove almost all the uncombined initiator.

Kinetics of Iodination. II. General Base Catalysis in the Iodination of N-Acetyl-L-tyrosine and N-Acetyl-3-iodo-L-tyrosine

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The effect of buffers over the pH range 5.40 to 9.80 upon the rates of iodination of N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine has been studied. Evidence is presented for general base catalysis in both iodination reactions. The effectiveness of the bases as catalysts follows the order $OH^- \gg CO_3^{-2} > HPO_4^{-2} > C_8H_{11}N_2O_3^- > CH_3COO^- > HCO_3^- > H_2PO_4^- \gg H_2O$. The data fit the Brønsted relationship, $k_b = G_b(1/K)^\beta$. The presence of general base catalysis supports the concept of iodination of phenoxide ion by molecular iodine through a quinoid intermediate but does not rule out H_2OI^+ as the iodinating species.

Halogenation reactions were among the reactions associated with the development of the classic theory of general acid–base catalysis.[2] An effect of the buffer constituents has been noted consistently in the iodination of phenol and derivatives of phenol. Painter and Soper[3] felt that their results in the iodination of phenol were compatible with acid catalysis, while Berliner[4] presented kinetic results for the iodination of aniline and phenol in phosphate buffers which were indicative of base catalysis. The iodination of 2,4-dichlorophenol was thought also to be catalyzed by base.[5]

Li[6] has studied the effect of acetate and phosphate buffers upon the rate of iodination of tyrosine. In phosphate buffer it was reported that a plot of the observed rate constant *vs.* dibasic phosphate ion concentration was linear according to the relationship in eq. 1 where k_{OH}, k_{HA}, and k_{H_2A} are catalytic constants reflecting the efficiency of hydroxyl, dibasic phosphate, and monobasic phosphate ions, respectively.

$$k_{obsd} = k_{OH}K_W\frac{1}{[H^+]} + [HPO_4^{-2}]\left(k_{HA} + \frac{k_{H_2A}}{K_{H_2A}}[H^+]\right) \quad (1)$$

K_W is the ion product of water and K_{H_2A} is the equilibrium dissociation constant for eq. 2. Interpretation of Li's data is complicated in that the kinetic results probably reflect both monoiodination and diiodination.

$$H_2PO_4^- \rightleftharpoons HPO_4^{-2} + H^+ \quad (2)$$

The effect of phosphate buffer components upon the iodination of N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine has previously been shown to follow the form of eq. 3,[7] where k_0 represents the "uncatalyzed" or water reaction.

$$k_{obsd} = k_0 + k_{HA}[HPO_4^{-2}] + k_{H_2A}[H_2PO_4^-] \quad (3)$$

Thus, the evidence presented has been suggestive of general base catalysis in phenolic iodination, but conclusive evidence for such has not been formulated. To study this possibility, the effects of buffers upon the kinetics of iodination has been investigated over a wider range of hydrogen ion concentration and with several different buffers. The studies have been facilitated by a high-speed digital computer program, and evidence for general base catalysis is presented.

Experimental

Materials.—The N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine[8] were the same preparations as recently reported.[9] The buffer reagents were reagent grade chemicals. Glass-distilled water was used.

Buffer solutions were prepared at a given pH in the highest concentration reported, and volumetric dilutions of this concentration were made to achieve varying concentrations of buffer constituents. Total ionic strength was maintained at 0.64 by the addition of sodium chloride.

The concentration of buffer species was calculated from the appropriate equilibrium dissociation constants at 25° and at infinite dilution for the sodium bicarbonate–sodium carbonate,[10]

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[1] Aided in part by a grant for a Postdoctoral Fellowship from the American Cancer Society. To whom communications should be sent: Mayo Clinic, Rochester, Minn.

[2] See R. P. Bell, "The Proton in Chemistry," Methuen and Co., Ltd., London, 1959, p. 223 ff, for extensive review and reference.

[3] B. S. Painter and F. G. Soper, *J. Chem. Soc.*, 342 (1947).

[4] E. Berliner, *J. Am. Chem. Soc.*, **72**, 4003 (1950); **73**, 4307 (1951).

[5] W. C. Buss and J. E. Taylor, *ibid.*, **82**, 5991 (1960).

[6] C. H. Li, *ibid.*, **66**, 228 (1944).

[7] W. E. Mayberry and J. E. Rall, *Exposes Ann. Biochim. Med.*, **25**, 21 (1964).

[8] Abbreviations, N-acTY and N-acMIT, will be used for N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine, respectively. We are grateful to Dr. Rosalind Pitt-Rivers for generously supplying these compounds.

[9] W. E. Mayberry, J. E. Rall, and D. Bertoli, *J. Am. Chem. Soc.*, **86**, 5302 (1964).

[10] H. S. Harned and S. R. Scholes, Jr., *ibid.*, **63**, 1706 (1941).

5,5′-diethylbarbituric acid–sodium 5,5′-diethylbarbiturate,[11] sodium monobasic phosphate–sodium dibasic phosphate,[12] and acetic acid–sodium acetate[13] buffer systems. Apparent dissociation constants or activity coefficients for these ions under the conditions of the present experiments are not known.[14] The hydroxyl ion concentration was calculated for K_W, 13.996,[15] at 25°.

Kinetic Runs.—Unless indicated otherwise, all experiments were performed with 2×10^{-4} *M* tyrosyl derivative and 5×10^{-5} *M* iodine. Iodide concentrations were varied in order to maintain the velocity of reaction at a conveniently measurable rate. Each run was performed at least in duplicate, and the mean value and standard deviation for the rate constants are reported. The concentration of triiodide ion was followed spectrophotometrically at 350 mμ in a constant-temperature chamber at 25°. The stability of the triiodide ion concentration at each pH and in each buffer system was established by following the optical density of the ion for 15 to 30 min. pH measurements were made with glass electrodes.[16] The pH was determined at the completion of each kinetic run to be sure of buffering stability.

Rate Constant Determinations.—Rate constants for each run were determined from 20 to 40 optical density readings according to the integrated second-order equation (4) derived[9] for computer solution.[17] The observed rate constants from eq. 4 have

$$\text{O.D.} = \alpha\left\{b - \frac{(e^{k_{\text{obsd}}(a-b)t} - 1)}{(e^{k_{\text{obsd}}(a-b)t} - b/a)}b\right\} \tag{4}$$

been corrected for varying iodide, phenoxide, and chloride concentrations according to eq. 5, where K_1 and K_2 are equilibria

$$k = \frac{k_{\text{obsd}}(K_1 + [\text{I}^-] + K_1/K_2[\text{Cl}^-])(K_3 + [\text{H}^+])[\text{I}^-]}{K_1K_3} \tag{5}$$

dissociation constants for eq. 6 and 7. K_3 is the equilibrium dissociation constant for the hydroxyl group of N-acTY and N-acMIT.[9] The γ correction noted in the tables is therefore equal to

$$\text{I}_3^- \rightleftharpoons \text{I}_2 + \text{I}^- \quad K_1{}^{18} = 0.0013 \text{ at } 25° \tag{6}$$

$$\text{I}_2\text{Cl}^- \rightleftharpoons \text{I}_2 + \text{Cl}^- \quad K_2{}^{19} = 0.60 \text{ at } 25° \tag{7}$$

$$\frac{(K_1 + [\text{I}^-] + K_1/K_2[\text{Cl}^-])(K_3 + [\text{H}^+])[\text{I}^-]}{K_1K_3}$$

Electron Spin Resonance in Iodination.—Iodination reactions were studied also with L-tyrosine in a flow cell. The tyrosine and iodine solutions were mixed in the flow cell as e.s.r.[20] scans were performed. The rate of flow of each solution was varied in different runs from 30 to 100 ml./min.

Results

Rate Dependence upon Buffer Concentration.—The linear dependency of the rate of iodination of N-acTY and N-acMIT upon concentration of buffer constituents can be seen in Table I for borate buffer, Table II for carbonate buffer, Table III for barbiturate buffer, Table IV for phosphate buffer, and Table V for acetate buffer. Analysis of the values k_{obsd} or $k_{\text{obsd}}\gamma$ reveals that the rates of iodination at a constant pH of both N-acTY and N-acMIT increase linearly as the concentration of buffer constituents increase. In addition, the data in Tables II, IV, and V at various pH values indicate that the catalysis is due to bases and not acids, since the increase is inversely related to the concentration of the acidic species. The significance of k_{calcd} is discussed below.

TABLE I

THE EFFECT OF BORATE BUFFER CONSTITUENTS UPON IODINATION RATE CONSTANTS[a]

	N-AcTY, l. mole^{-1} sec.$^{-1}$		N-acMIT, l. mole^{-1} sec.$^{-1}$	
$(\text{Na}_2\text{B}_4\text{O}_7)^b$	k_{obsd} ± std. dev.	$k_{\text{obsd}}\gamma \times 10^{-2}$	k_{obsd} ± std. dev.	$k_{\text{obsd}}\gamma \times 10^{-2}$
0.030	46.55 ± 0.56	27.56	6.71 ± 0.05	1.54
0.024	40.33 ± 0.22	23.87	5.16 ± 0.07	1.18
0.018	34.55 ± 0.32	20.45	4.87 ± 0.09	1.11
0.012			3.92 ± 0.05	0.90

[a] Tyrosyl derivative, 2×10^{-4} *M*; I_2, 5×10^{-5} *M*; KI, 0.167 *M*; $\mu = 0.64$; pH 9.80; OH^-, 84.15×10^{-6} *M*. [b] Molar concentration of $\text{Na}_2\text{B}_4\text{O}_7$ added to the system.

Kinetic Form of Catalyzed Reactions.—Equation 3 was initially utilized to evaluate the catalytic constants for each buffer system except borate. Because of the uncertainty of the type and concentrations of species present in the borate buffer system,[21] further analysis of this system regarding individual ions is not feasible. The value k_0 for each set of buffer data and for each reaction was sought graphically by plotting $k_{\text{obsd}}\gamma$ against the concentration of the more basic anion. Under these conditions k_0 should represent the water reaction and therefore be pH invariant if all pertinent concentration terms have been included in the form of the rate equation. There was, however, some uncertainty in the evaluation of k_0 due to the long extrapolation required in the more concentrated buffers. In the higher pH range, the values tended to increase suggesting that a hydroxyl term should be included in eq. 3. On these grounds, general base catalysis seemed likely. This may be expressed as in eq. 8,

$$k_{\text{obsd}} = \overset{i}{\Sigma} k_i[\text{B}]_i \tag{8}$$

where k_i represents the catalytic constant for a given base and $[\text{B}]_i$ represents its concentration.

Computer Model for Data Analysis.—An existing computer program including a subroutine for solution of linear combination of spectra[17] was employed to analyze simultaneously all the buffer data for each iodination reaction. For this solution, eq. 8 has the form of eq. 9 where y is an artificial time point allowing

$$k_{\text{obsd}}(y) = \overset{i}{\Sigma} k_i[\text{B}]_i(y) \tag{9}$$

a given observed rate constant to be correlated with the appropriate values of base concentration. The computer model for the determination of the catalytic constants for each base in each iodination reaction is seen in Figure 1. For all the data the concentration of water is constant and k_1, the catalytic constant for water, is the same for each set of data. In addition,

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(11) G. G. Manov, K. E. Schnette, and F. S. Kirk, *J. Res. Natl. Bur. Std.*, **48**, 84 (1952).

(12) R. G. Bates and S. F. Acree, *ibid.*, **30**, 129 (1943).

(13) H. S. Harned and R. W. Ehlers, *J. Am. Chem. Soc.*, **54**, 1350 (1932); D. A. MacInnes and T. Shedlovsky, *ibid.*, **54**, 1429 (1932).

(14) Values obtained by extrapolation of the data in dilute solutions could have been used; however, at the ionic strength used in the present studies, extrapolated values may be more tenuous than the use of well-established values for infinite dilution.

(15) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolyte Solution," Reinhold Publishing Corp., New York, N. Y., 1943, p. 485.

(16) Leeds and Northrup "Miniature" glass electrodes with the reference electrode modified with a larger asbestos membrane. Electrodes were used in conjunction with a radiometer TTTI with a scale expander.

(17) IBM 7094 used in conjunction with Program 9B21, Office of Mathematical Research, National Institute of Arthritis and Metabolic Diseases; M. Berman, E. Shahn, and M. F. Weiss, *Biophys. J.*, **2**, 275 (1962).

(18) M. Davies and E. Gwynne, *J. Am. Chem. Soc.*, **74**, 2748 (1952); L. I. Katzin and E. Gebert, *ibid.*, **77**, 5814 (1955).

(19) D. L. Cason and H. M. Neumann, *ibid.*, **83**, 1822 (1961).

(20) The e.s.r. spectrometer used was the Varian V-4500 with 6-in. magnet and with 100-kc. field modulation. The sensitivity of the spectrometer *per se* was such that the 0.00033% pitch sample (supplied by Varian Associates) gave a signal with signal to noise ratio of *ca.* 20.

(21) N. Ingri, *Acta Chem. Scand.*, **16**, 439 (1962); **17**, 573, 581 (1963).

Table II

The Effect of Carbonate Buffer Constituents upon Iodination Rate Constants[a]

				N-acTY			N-acMIT		
				l. mole^{-1} sec.$^{-1}$			l. mole^{-1} sec.$^{-1}$		
					10^{-2}			10^{-2}	
pH	$10^6[OH^-]$	$[CO_3^{-2}]$	$[HCO_3^-]$	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}
9.80	84.15	0.0441	0.1489	86.33 ± 0.26	51.16	50.39	8.94 ± 0.15	2.05	1.91
9.80	84.15	0.0352	0.1189	66.06 ± 0.37	39.20	43.02	6.83 ± 0.04	1.57	1.61
9.80	84.15	0.0265	0.0895	59.14 ± 0.65	35.11	35.85	5.95 ± 0.04	1.37	1.33
9.80	84.15	0.0176	0.0594	43.94 ± 0.32	26.12	28.49	4.32 ± 0.01	0.99	1.03
9.60	74.88	0.0297	0.1593	56.16 ± 0.59	45.66	42.87	6.36 ± 0.15	1.51	1.55
9.60	74.88	0.0238	0.1272	47.65 ± 0.33	38.79	37.01	5.44 ± 0.03	1.29	1.33
9.60	74.88	0.0178	0.0952	41.95 ± 0.17	34.17	31.15	4.72 ± 0.02	1.12	1.11
9.60	74.88	0.0120	0.0640	32.74 ± 0.22	26.69	25.45	3.66 ± 0.05	0.87	0.89
9.40	60.19	0.0203	0.1727	35.50 ± 0.10	41.18	38.24	5.29 ± 0.16	1.32	1.33
9.40	60.19	0.0162	0.1378	31.03 ± 0.10	36.10	33.21	4.72 ± 0.09	1.18	1.15
9.40	60.19	0.0122	0.1038	24.13 ± 0.08	28.11	28.31	3.95 ± 0.02	0.99	0.98
9.40	60.19	0.0081	0.0689	21.24 ± 0.08	24.77	23.29	3.33 ± 0.02	0.83	0.80
9.20	36.90	0.0132	0.1787	18.85 ± 0.05	32.37	34.15	4.26 ± 0.02	1.15	1.16
9.20	36.90	0.0107	0.1434	16.65 ± 0.05	28.60	29.92	3.55 ± 0.02	0.96	1.02
9.20	36.90	0.0080	0.1071	14.85 ± 0.06	25.53	25.58	3.47 ± 0.02	0.93	0.87
9.20	36.90	0.0053	0.0717	11.18 ± 0.03	19.23	21.35	2.81 ± 0.01	0.76	0.73

[a] Tyrosyl derivative, 2×10^{-4} *M;* I_2, 5×10^{-5} *M;* KI, 0.167 *M;* $\mu = 0.64$.

Table III

The Effect of Barbital Buffer Constituents upon Iodination Rate Constants[a]

	N-acTY			N-acMIT		
	l. mole^{-1} sec.$^{-1}$			l. mole^{-1} sec.$^{-1}$		
		10^{-2}			10^{-2}	
$[C_8H_{11}N_2O_3^-]$	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}
0.2588	32.04 ± 0.14	46.11	44.97	15.25 ± 0.16	1.91	1.98
0.2067	26.05 ± 0.07	37.54	38.35	13.46 ± 0.18	1.69	1.67
0.1555	23.63 ± 0.09	34.10	31.71	10.97 ± 0.18	1.38	1.36
0.1034	16.21 ± 0.04	23.42	25.21	8.63 ± 0.08	1.09	1.06

[a] Tyrosyl derivative, 2×10^{-4} *M;* I_2, 5×10^{-5} *M;* KI, 0.100 *M;* $\mu = 0.64$; OH^-, 84.17×10^{-7} *M;* pH 8.80.

Table IV

The Effect of Phosphate Buffer Constituents upon Rate Constants[a]

				N-acTY			N-acMIT		
				l. mole^{-1} sec.$^{-1}$			l. mole^{-1} sec.$^{-1}$		
					10^{-2}			10^{-2}	
pH	$[OH^-] \times 10^8$	$[HPO_4^{-2}]$	$[H_2PO_4^-]$	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}
7.80	84.15	0.1512	0.0378	68.85 ± 0.46	39.81	43.73	45.22 ± 0.22	1.05	1.20
7.80	84.15	0.1208	0.0302	57.26 ± 0.54	33.41	37.33	42.47 ± 0.27	1.00	1.05
7.80	84.15	0.0904	0.0226	47.98 ± 0.64	28.26	30.93	33.65 ± 0.48	0.80	0.90
7.80	84.15	0.0605	0.0151	39.74 ± 0.56	23.72	24.63	27.58 ± 0.36	0.66	0.75
7.20	36.90	0.1003	0.0998	30.76 ± 0.35	40.57	36.73	25.54 ± 0.41	1.21	1.04
7.20	36.90	0.0802	0.0798	23.87 ± 0.41	32.06	31.76	20.90 ± 0.34	1.01	0.92
7.20	36.90	0.0602	0.0599	21.54 ± 0.09	29.27	26.79	18.37 ± 0.05	0.90	0.80
7.20	36.90	0.0401	0.0399	15.59 ± 0.07	21.44	21.82	13.88 ± 0.04	0.69	0.69
6.80	8.415	0.0571	0.1429	10.87 ± 0.04	36.43	30.34	9.15 ± 0.04	1.08	0.89
6.80	8.415	0.0457	0.1143	9.69 ± 0.03	32.86	26.64	8.34 ± 0.03	1.00	0.81
6.80	8.415	0.0343	0.0857	7.75 ± 0.04	26.44	22.95	7.07 ± 0.03	0.85	0.71
6.80	8.415	0.0229	0.0572	7.03 ± 0.03	24.26	19.25	6.29 ± 0.04	0.76	0.63
5.80[b]	0.8415	0.0077	0.1923	8.76 ± 0.03	20.87	23.01	7.76 ± 0.03	0.64	0.72
5.80[b]	0.8415	0.0062	0.1539	7.82 ± 0.03	19.01	20.78	7.14 ± 0.03	0.60	0.66
5.80[b]	0.8415	0.0046	0.1154	7.15 ± 0.02	17.73	18.55	6.57 ± 0.02	0.57	0.61
5.80[b]	0.8415	0.0031	0.0769	6.07 ± 0.04	15.34	16.32	5.90 ± 0.02	0.52	0.56

[a] Tyrosyl derivative, 2×10^{-4} *M;* I_2, 5×10^{-5} *M;* KI, 0.020 *M* at pH 7.80, 0.15 *M* at pH 7.20 and 6.80, 0.0033 *M* at pH 5.80; $\mu = 0.64$. [b] Tyrosyl derivative concentration was 4×10^{-4} *M* in these experiments.

k_2, the catalytic constant for hydroxyl ions, is the same in each set of data, though the actual concentration of ions varies. Equation 9 for each buffer system according to the model may be expressed by eq. 10 for the carbonate system, eq. 11 for the barbital system, eq. 12 for the phosphate system, and eq. 13 for the acetate system. By using this model and simultaneously considering all the data for each iodination reaction, the catalytic constants for water and hydroxyl ions can be more precisely determined than is possible by analyzing individually each set of data at each pH.

$$k_{obsd\,5} = k_1[H_2O] + k_2[OH^-] + k_3[CO_3^{-2}] + k_4[HCO_3^-] \qquad (10)$$

$$k_{obsd\,8} = k_1[H_2O] + k_2[OH^-] + k_7[C_8H_{11}N_2O_3^-] \qquad (11)$$

$$k_{obsd\,12} = k_1[H_2O] + k_2[OH^-] + k_{10}[HPO_4^{-2}] + k_{11}[H_2PO_4^-] \qquad (12)$$

$$k_{obsd\,15} = k_1[H_2O] + k_2[OH^-] + k_{14}[CH_3COO^-] \qquad (13)$$

Catalytic Constants.—On the basis of the previous model, the catalytic constants and their uncertainties for each base species in the iodination of N-acTY and N-acMIT are listed in Table VI. There is a large uncertainty in the catalytic constant for hydroxyl ions in the iodination of N-acTY. Even though the constant is very large, 2.5×10^6, the hydroxyl concentration is quite small. The over-all term, $k_{OH}[OH^-]$, thus contributes little to the observed rate constants until pH 8.80 is reached. In view of these facts, the uncertainty in this term is not surprising, and it follows that the corresponding term in N-acMIT iodination would be even more uncertain. This has been the

TABLE V

EFFECT OF ACETATE BUFFER CONSTITUENTS UPON IODINATION RATE CONSTANTS[a]

			N-acTY			N-acMIT		
			l. mole^{-1} sec.$^{-1}$			l. mole^{-1} sec.$^{-1}$		
				10^{-2}			10^{-2}	
pH	$[OH^-] \times 10^{10}$	$[C_2H_3O_2^-]$	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}	k_{obsd} ± std. dev.	$k_{obsd}\gamma$	k_{calcd}
5.80	84.15	0.4469	23.44 ± 0.43	50.61	50.13	22.70 ± 0.27	1.71	1.75
5.80	84.15	0.3579	17.89 ± 0.12	40.25	42.51	17.89 ± 0.09	1.49	1.40
5.80	84.15	0.2679	15.43 ± 0.17	36.08	34.81	15.63 ± 0.11	1.22	1.27
5.80	84.15	0.1789	11.75 ± 0.13	28.52	27.19	11.69 ± 0.20	0.97	0.99
5.40	60.19	0.3825	7.71 ± 0.06	42.20	44.62	8.43 ± 0.04	1.60	1.56
5.40	60.19	0.3060	6.35 ± 0.02	36.17	38.07	6.75 ± 0.03	1.33	1.34
5.40	60.19	0.2294	5.83 ± 0.02	34.56	31.52	5.76 ± 0.06	1.19	1.12
5.40	60.19	0.1530	4.23 ± 0.02	26.01	24.97	4.18 ± 0.04	0.89	0.90

[a] Tyrosyl derivative, 4×10^{-4} *M;* I_2, 5×10^{-5} *M;* KI, 3.3×10^{-3} *M;* $\mu = 0.64$.

TABLE VI

CATALYTIC CONSTANTS FOR BASE CATALYSIS OF IODINATION REACTIONS

		N-acTY	N-acMIT
Base	pK[a]	10^3k ± std. dev., l. mole^{-1} sec.$^{-1}$	$10^{-3}k$ ± std. dev., l. mole^{-1} sec.$^{-1}$
H_2O	−1.745[b]	0.02139 ± 0.00139	0.000817 ± 0.000039
OH^-	15.747[b]	2488 ± 2117	62.0[c]
CO_3^{-2}	10.329[d]	56.45 ± 6.81	2.637 ± 0.174
HCO_3^-	3.86[e]	7.76 ± 0.86	0.197 ± 0.030
$C_8H_{11}N_2O_3^-$	7.98[f]	12.71 ± 0.64	0.590 ± 0.025
HPO_4^{-2}	7.198[g]	19.81 ± 0.88	0.463 ± 0.025
$H_2PO_4^-$	2.148[g]	5.00 ± 0.62	0.119 ± 0.018
CH_3COO^-	4.756[h]	8.56 ± 0.35	0.290 ± 0.011

[a] −Log K_A of the acid corresponding to the base indicated. [b] R. P. Bell, "Acids and Bases," Methuen and Co., Ltd., London, 1952, pp. 15–17. [c] Estimated value. [d] Ref. 10. [e] Ref. 25. [f] Ref. 11. [g] Ref. 12. [h] Ref. 13.

case and the computer analysis has set this value at zero because of its large uncertainty.

As may be seen by comparing the ratio of the other constants for N-acTY to the same terms for N-acMIT, the catalytic constants for N-acTY are 20–40 times larger than those for N-acMIT. This ratio is similar to that found for this sum of the buffer terms at varying pH in a previous study.[9] If this ratio were of a similar magnitude for the hydroxyl ion constants, then the constant for N-acMIT would be 62–124 l. mole^{-1} sec.$^{-1}$. The smaller value has been used as an estimate. In the pH range studied, neither the smaller nor larger value would contribute significantly to the over-all observed rate constant except at very high OH^- concentration.

Using the catalytic constants in Table VI except that for OH^- in N-acMIT iodination and using the concentration of bases corresponding to the observed rate constant for each reaction, the computer has calculated a rate constant for each reaction according to eq. 10–13. These calculated rate constants, k_{calcd}, are listed in Tables II–V. As may be seen, the calculated rate constants compare reasonably well with the observed data. This seems remarkably so in view of the magnitude of the correction factors applied for varying iodide and phenoxide ion concentrations and, also, in view of the lack of apparent equilibrium dissociation constants for calculating individual base ion concentrations. The values for the phosphate system seem the least precise. Whether this is due to a large discrepancy in buffer ion concentration in the entire system, but particularly at pH 6.80, is not apparent. Under the circumstances, however, even the data for the phosphate buffers lend support for the model and the presence of general base catalysis.

Correlation of Catalytic Constants.—As noted in Table VI, the catalytic constants for hydroxyl ions are large for both iodination reactions and those for water are small. The other base anions are intermediate between these values and correlate well with the strength of the corresponding acid. According to the Brønsted[22] relationship for general base catalysis, eq. 14, which relates the effectiveness of a catalyst to its acid–base strength, a plot of log k_b against −log K_A of the corresponding acid should form a straight line.

$$k_b = G_b(1/K)^{\beta} \qquad (14)$$

That such is the case for both iodination reactions may be seen in Figure 2. As expected, the values for water and hydroxyl ions deviate widely in both reactions. Excluding these values, the slope, B, for iodination of both N-acTY and N-acMIT is +0.15. The slope of the values for water and hydroxyl ions is +0.30 for both reactions.

Since statistical corrections for the K_A values have limited practical application,[23] none have been made in these plots. The conventional pK for carbonic acid, 6.35,[24] reflecting the pK of dissolved carbon dioxide in water has not been used. Since only a small proportion of carbon dioxide is present as carbonic acid, the true pK of the species is 3.86.[25] Bell[26] has found that it is this last figure that gives a true tendency of the species HCO_3^- to accept a proton.

Pfluger[27] has noted a relationship between the rates of general basic catalysis in different reactions. In the present instance, when the logarithms of the catalytic constants for iodination of N-acTY are plotted against the corresponding values for iodination of N-acMIT, a straight-line plot with a slope of 1 is obtained as shown in Figure 3. In this plot, the values for water and hydroxyl ions fall upon the line. The slope of 1 reflects the ratio of the slopes of the plots in Figure 2 and adds further evidence to the contentions that the slopes for the two reactions in Figure 2 are equal.

Free Radical in Iodination.—The possibility of chemical iodination occurring by way of a free-radical reaction was studied by e.s.r. in a flow cell. Because of the large quantities of reactants required, L-tyrosine was used rather than the N-acetyl derivative. Reactions were performed in pH 9.60 carbonate buffer,

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(22) J. N. Brønsted and K. J. Pedersen, *Z. physik. Chem.* (Leipzig), **108**, 185 (1924).

(23) Reference 2, p. 159.

(24) H. S. Harned and R. Davis, *J. Am. Chem. Soc.*, **65**, 2030 (1943).

(25) D. Berg and A. Patterson, *ibid.*, **75**, 5197 (1953).

(26) See ref. 2, pp. 178, 179.

(27) H. L. Pfluger, *J. Am. Chem. Soc.*, **60**, 1513 (1938).

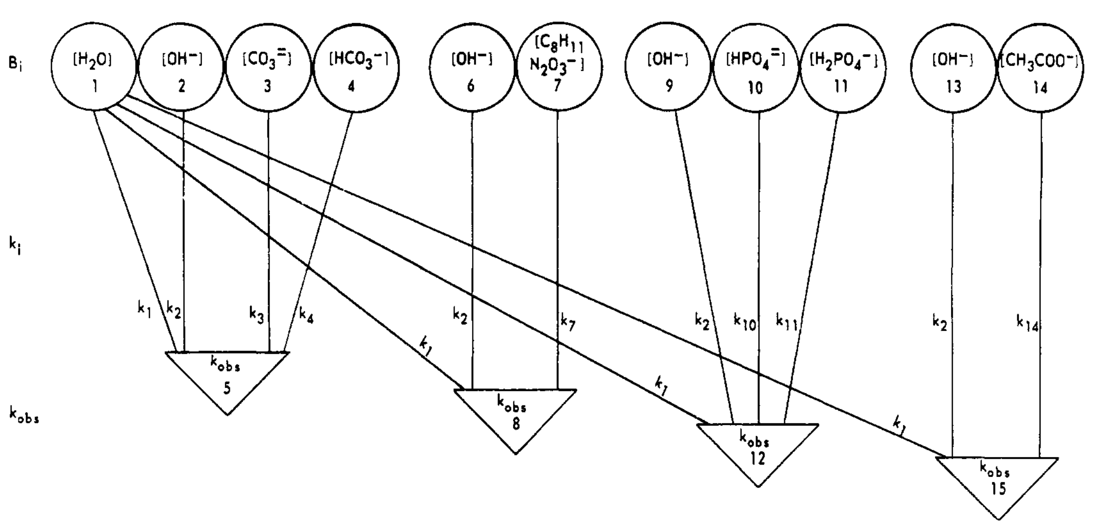

Figure 1.—Computer model for determination of catalytic constants for base catalysis of iodination reactions.

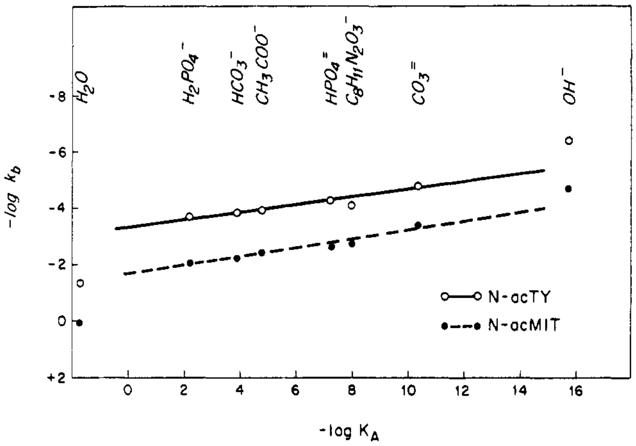

Figure 2.—Brønsted plot of the relation between catalytic constant for a base and the equilibrium dissociation for the corresponding acid in the iodination reactions.

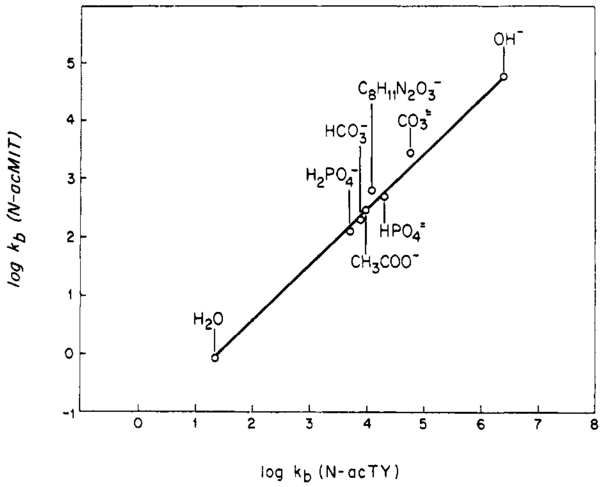

Figure 3.—A plot of the catalytic constant for each base species for iodination of N-acTY against the corresponding constant for the iodination of N-acMIT.

in 0.01 N NH_4OH, and in 0.01 N HCl. The L-tyrosine concentration was 3.38×10^{-3} M in all runs except in the NH_4OH medium in which the concentration was 6.76×10^{-3} M. Iodine concentration in the runs varied from 1.6×10^{-3} to 8.0×10^{-4} M; while 0.1–0.3 M concentrations of iodide were used. There was no detectable free radical in any of the experiments. Under the conditions employed, a free radical with a half-life of 50 msec. or an average concentration of 10^{-5} M over the flat flow cell should have produced a detectable signal.

Discussion

The data lend support to the concept of general base catalysis in the iodination of both N-acTY and N-acMIT. The computer analysis of the data has allowed the determination of the catalytic constant for each base species with a higher degree of certainty than the more conventional mathematical methods would have permitted.

In investigating the kinetics of iodination of phenol and tyrosine, neither Berliner[4] nor Li[6] was able to show a water reaction. However, in each instance only a small pH range was studied in phosphate buffer, and it seems likely that their terms reflecting the hydroxide ion catalysis may have contained both water and hydroxide terms. Even in the present, wider pH-range study, the catalytic constants for hydroxyl ions are quite uncertain. This is not surprising since the hydroxyl ion concentration in the permissible pH range of iodine stability is quite small, while the catalytic constants required are large. The value, k_b, for hydroxyl ions in the iodination of N-acTY is 2.49×10^6 l. mole^{-1} sec.$^{-1}$. This term would not appreciably affect observed rate constants in buffer systems until the pH of the buffer systems approached pH 8. For the iodination of N-acMIT, the estimated k_b for hydroxyl ions is 6.20×10^4, and this factor becomes appreciable only when the pH approaches pH 9 or 10. These situations correlate well with those described for general base catalyzed water reactions.[28]

In the present study, the acetate buffer data fit well with base catalysis by the acetate ion. This is in contrast to Li's interpretation[6] of the kinetics of iodination of tyrosine. In that instance, it was postulated that the acetate ion was not involved in the catalytic mechanism and further that the complex acetate

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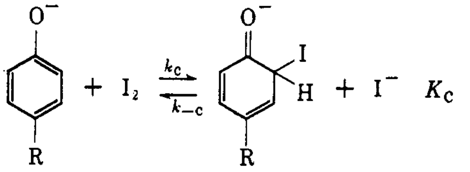
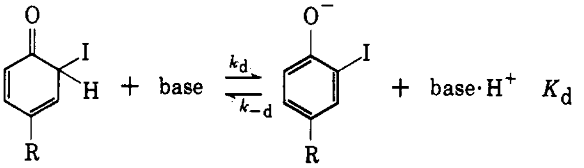
[28] M. Kilpatrick, *Advan. Catalysis*, **6**, 244 (1954).

ion, $CH_3C(OH)O_2^{-2}$, formed by acetate and hydroxide ions was the active species.

The presence of general base catalysis in the present studies lends support to the concept of phenoxide ion iodination by molecular iodine by way of a quinoid intermediate.[29] This mechanism may be represented by eq. 15 and 16. Since I_3^- dissociation to I_2 and tyrosyl ionization to the tyrosinate anion are virtually instantaneous, either eq. 15 or 16 may be rate limiting. If the indicated mechanism is the correct one, then the presence of general base catalysis requires that k_d be the "rate-limiting" or slower step in phenolic iodination. Eq. 16 represents an acid–base reaction where the quinoid intermediate is the acid. Though acid–base reactions are usually considered to be too fast to measure, slow reactions are known, particularly those involving C–H bonds. In addition, Bell[26] has pointed out that some slower acid–base reactions involve a considerable structural change in one of the acid–base pair taking part in the reaction. It has been surmised that the relative slowness of such reactions is connected with this electronic reorganization.[30] In the present instance, the rearrangement of electronic structure in going from the acid quinoid form to the base anion structure is the type of acid–base reaction that might be expected to be relatively slow.[26] However, whether the electronic reorganization is responsible or not has not been established.



(15)



(16)

Bell[31] has pointed out that proton transfer makes no distinction between substrate and catalyst and that a relationship between the reaction velocity and acid–base strength of a series of substrates should exist. In the present reactions, the quinoid intermediate in iodination of N-acTY would be related to the p*K* of N-acMIT, 8.58, while the intermediate for iodination of N-acMIT would be comparable to that for N-acDIT, 6.83. On this basis, the intermediate in the iodination of N-acMIT would be expected to be a stronger acid than that in the iodination of N-acTY. Therefore, with the same base catalyst, k_b for N-acMIT should be faster than that for N-acTY. Table VI shows, however, that the catalytic constants for N-acTY are 20–40 times as fast as those for N-acMIT. The previous assumption may still be valid, since the values, $k_{\text{obsd}}\gamma$, are not actually k_d but are related to k_d as in eq. 17. As may be seen in eq. (17), $k_{\text{obsd}}\gamma$

$$k_{\text{obsd}}\gamma = \frac{k_d}{1/[I^-] + k_{-c}/k_c} \tag{17}$$

could reflect a larger value for N-acTY than for N-acMIT even if the reverse relationship held for the values of k_d. This would require that either k_{-c} be smaller or that k_c be larger for N-acTY than for N-acMIT. It is possible that both situations exist. Certainly, on the basis of the Hammett *meta* σ-constant of +0.352[32] for the substituted iodine atom, k_c for N-acTY should be greater than k_c for N-acMIT.

In the present study, the quantitative values for catalytic constants of each base and for each iodination reaction are not true values but are related to the actual values as $k_{\text{obsd}}\gamma$ is related to k_d in eq. 17. It may be further noted that the values, $k_{\text{obsd}}\gamma$, may be expected to change little with varying iodide concentration only if the value k_{-c}/k_c is quite large relative to $1/[I^-]$. The present data support such a contention. In addition, the postulated mechanism requires just this situation, that the reactants be in rapid equilibrium with the quinoid intermediate and that k_{-c} be much greater than k_d.

Although general base catalysis has been postulated[5] for formation of HOI and H_2OI^+, there seems to be little evidence for it. In addition, iodination by HOI would require an additional H^+ term in the kinetic rate expression due to the equilibria for HOI formation. This would not fit the present data nor those presented by Buss and Taylor.[5] Therefore, only H_2OI^+ might be conceived to support base catalysis as well as the iodide dependency term. However, in view of Grovenstein and associates' convincing arguments[29] regarding the quinoid intermediate and in view of the presence of general base catalysis, iodination of phenolic compounds seems best viewed currently as proceeding through phenoxide ion and molecular iodine by way of a quinoid intermediate.

Acknowledgment.—Drs. J. E. Rall and M. Berman have given continued help and criticism in these studies. The authors are most grateful to them and to Dr. H. Kon who performed and interpreted the e.s.r. studies.

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[29] E. Grovenstein, Jr., and N. V. Henderson, Jr., *J. Am. Chem. Soc.*, **78**, 569 (1956); E. Grovenstein, Jr., and D. C. Kilby, *ibid.*, **79**, 2972 (1957); E. Grovenstein, Jr., and N. S. Aprahamian, *ibid.*, **84**, 212 (1962).

[30] R. P. Bell, *Advan. Catalysis*, **4**, 192 (1952).

[31] R. P. Bell, *ibid.*, **4**, 198 (1952).

[32] L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 188.