Such a volume increase perhaps results from release of water molecules in the course of charge neutralization.

Acknowledgment

Dr. Richard Podolsky generously loaned us the pressure bomb for an extended period, thereby making possible these studies. As usual we are indebted to several colleagues. Miss Linda Stowring helped with several experiments and the interpretation of this work was greatly aided and stimulated by the suggestions of

Dr. Jean Lecocq, Dr. Giuseppe Inesi, and Visiting Professor Yuji Tonomura.

References

Beardell, A. J. (1954), Ph.D. Dissertation, Washington, D. C., The Catholic University of America Press.
Ebashi, S. (1961), J. Biochem. (Tokyo) 50, 236.
Fiske, C. H., and Subbarow, Y. (1925), J. Biol. Chem. 66, 375.
Noguchi, H., Kasarda, D. D., and Rainford, P. (1964), J. Am. Chem. Soc. 86, 2077.

Kinetics of Iodination. III. Iodination of N-Acetyl-L-tyrosine and N-Acetyl-3-iodo-L-tyrosine Studied in a pH-Stat System*

W. E. Mayberry,† J. E. Rall, M. Berman, and D. Bertoli

ABSTRACT: The kinetics of the iodination of N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine has been studied in a pH-Stat system. A reaction model was proposed to represent the steady-state kinetics of the reactions. The model was based upon the concept of molecular iodine and the phenolate anion of each tyrosine derivative proceeding to a quinoid intermediate plus iodide. The rate-limiting step is then proton removal from the quinoid intermediate to form the iodinated phenolate anion. Using this model and a computer program, the rate k_6 representing the sum of the two steps (see equation 6) has been solved by a high-speed digital computer. The k_6 for each reaction is approximately inversely proportional to iodide concentration.

The values $k_6[I^-]$ are 1400–1600 sec^{-1} and 43–68 sec^{-1}, respectively, in the iodination of N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine. The reactions have previously been shown to be general base catalyzed, and the present values confirm the rate constants for the water-catalyzed reactions. The fit of the data to the model solution and of theoretical considerations to the results adds credence to the proposed mechanism of iodination.

The kinetics of iodination of N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine has been studied previously by following triiodide concentration spectrophotometrically (Mayberry et al., 1964). Hydrogen ion concentration was maintained constant by the presence of buffers. In a subsequent report, evidence of general base catalysis for the two reactions was presented (Mayberry and Bertoli, 1965). In the present study, we wished to study the iodination reactions in the absence of buffer systems and to corroborate our previously determined catalytic constants for water as a base. Hydrogen ion concentration has been maintained by means of a pH-Stat system which has allowed monitor-

ing of the iodination reaction by measuring the release of protons from the aromatic ring. The system is complicated by proton terms other than that of the release of the proton from the ring with iodination. A reaction model was designed, and a solution that fit the experimental observations was derived with the aid of a digital computer program.

Experimental

Materials. N-Acetyl-L-tyrosine, N-acetyl-3-iodo-L-tyrosine, and N-acetyl-3,5-diiodo-L-tyrosine[1] were the same preparations as those previously reported (Mayberry et al., 1964). Water redistilled in glass was used in all experiments. Resublimed iodine and reagent grade potassium iodide were used. Sodium hydroxide solu-

[1] The abbreviations N-acTY, N-acMIT, and N-acDIT will be used, respectively, for N-acetyl-L-tyrosine, N-acetyl-3-iodo-L-tyrosine and N-acetyl-3,5-diiodo-L-tyrosine.
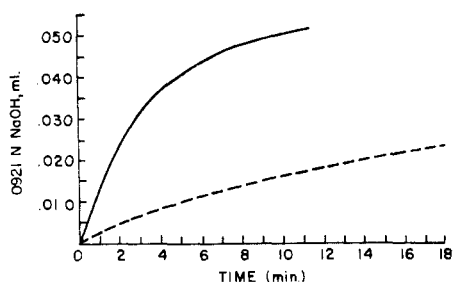
1965

FIGURE 1: pH-Stat titration curves for iodination of N-acTY (———) and of N-acMIT (---------); pH 9.80; T, 20°; tyrosyl derivative, 1.2×10^{-3} M; iodine, 3.0×10^{-4} M; potassium iodide, 0.25 M.

tions were prepared with freshly boiled water which had been redistilled in glass. The concentration of the sodium hydroxide solutions was estimated by titration with potassium acid phthalate as standard (pH Standard 185b, National Bureau of Standards), while the concentration of solutions of the other reactants was verified spectrophotometrically.

Kinetic Runs. The Radiometer TTT1 pH meter was employed as a pH-Stat in conjunction with a magnetic relay, an Ole Dich recorder, and an Agla micrometer syringe outfit. A Radiometer glass electrode with a ground-glass joint fitted to a reaction vessel was used. Temperature was maintained by circulating water around the reaction vessel, and a temperature compensator electrode from the pH meter was placed in the circulating water circuit.

The tyrosyl derivative was added to the reaction vessel as 9.95 ml of a 1.26×10^{-3} M solution in water. Nitrogen was allowed to flow gradually through the reaction vessel *via* openings in the stopper, while the solution was being brought to temperature. The solution was titrated manually to the desired pH and the nitrogen flow was stopped. The system was then closed to outside air except for a small opening through a glass tube in the stopper. This opening just admitted a small-bore polyethylene tube attached to a syringe containing sodium hydroxide solution. Under these conditions, the pH was stable for 1 hr. The reaction was initiated by quickly adding 0.05 ml of a 0.06 M iodine solution in varying concentration of potassium iodide. This solution was introduced through a 1-cm glass tube within the stopper of the reaction vessel. The glass tube was maintained closed at all other times by a stopper. Stirring was carried out during the reaction by means of a magnetic stirring bar. Reactions were followed to 50–75% completion which required 10–25 min depending upon the pH and iodide concentration. It was necessary to vary the concentration of sodium hydroxide solution and proportional band of the Radiometer instrument with different speeds of reaction in order to maintain optimal regulation of the pH. Typical base addition curves are seen in Figure 1 for both iodination reactions. The volume of base needed to maintain the pH was read directly from each record. Duplicate runs

were made for each set of experimental conditions. Each set of conditions was checked to be sure that spurious loss of iodine was not occurring and that pH changes were due to the reaction under study and not to other factors. These circumstances were investigated by monitoring a simulated kinetic run with iodine and iodide in water at each pH without added tyrosine derivative.

Rate-Constant Determinations. Because of computational complexity, the rate constants for the iodination reactions have been solved using an existing computer program[2] (Berman *et al.*, 1962) on a high speed digital computer.[3] A compartmental model is utilized to represent the nonlinear differential equations proposed to describe the nonsteady-state kinetics of the reaction.

Determination of Apparent pK_a. The pK_a was determined for the carboxyl and hydroxyl groups of N-acTY, N-acMIT, and N-acDIT (Table I) under the

TABLE I: Apparent Dissociation Constants of Tyrosine Derivatives at 25°.

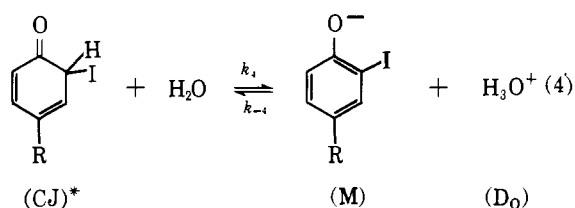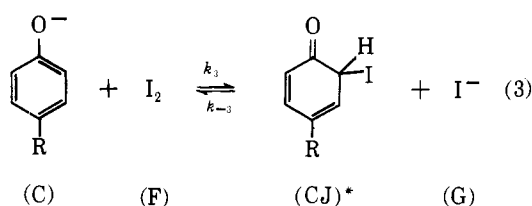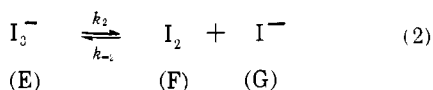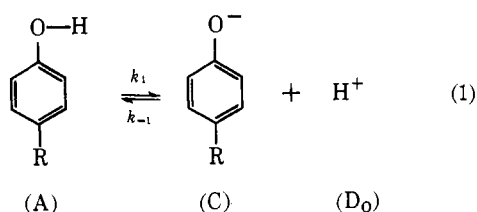|	N-acTY	N-acMIT	N-acDIT
pK_a (OH)	10.22	8.83	7.12
pK_a (COOH)	3.81	3.19	4.13

conditions of these experiments (*i.e.*, low ionic strength). Each compound (25 ml as a 1.2×10^{-3} M solution) was titrated potentiometrically with base in the reaction vessel in an atmosphere of nitrogen at $25 \pm 0.02°$. Duplicate titrations were performed in each instance, one with 0.1739 N potassium hydroxide and the other with 0.2093 N sodium hydroxide. In each instance, the appropriate water titration correction was made. The duplicate values agreed in each instance within 0.01 pH unit.

Results

Proposed Reaction Mechanism. Based upon evidence for an isotope effect in phenolic iodination (Grovenstein *et al.*, 1956, 1957, 1962) and on previous kinetic data (Mayberry *et al.*, 1964; Mayberry and Bertoli, 1965) for iodination of N-acTY and N-acMIT, iodination of phenolic compounds seems likely to occur by way of phenoxide ion and molecular iodine through a quinoid intermediate. The proposed mechanism for the reaction is given in chemical equations 1–7, where

(A)　　　　(C)　　　　(D₀)

$$I_3^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} I_2 + I^- \qquad (2)$$

(E)　　　　(F)　　(G)

(C)　　(F)　　　(CJ)*　　(G)

(CJ)*　　　(M)　　(D₀)

(M)　　(D₀)　　　(P)
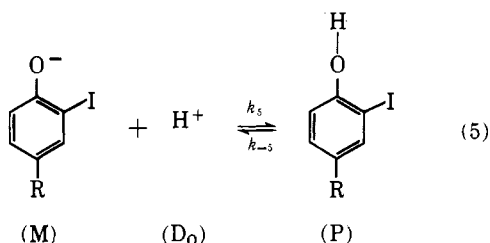
R represents the *N*-acetylalanyl side chain, and the letters beneath the chemical formulas are abbreviations for that compound. Since the quinoid intermediate is in a rapid but unknown equilibrium (k_3/k_{-3}) with the reactants and $k_{-3} \gg k_4$ (Hine, 1962; Mayberry *et al.*, 1964), equations 3 and 4 have been combined for purposes of this kinetic study into equat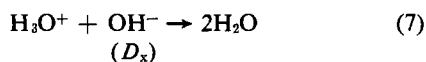ion 6. This is valid since I^- is large initially and essentially unchanged during the reaction. The neutralization of proton buildup

(C)　　(F)　　(M)　　　(D₀)　　(G)
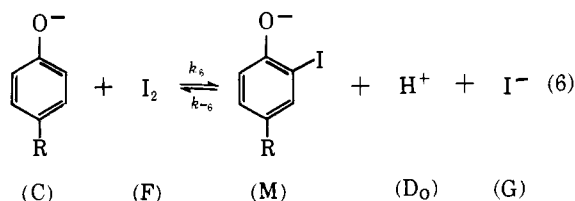
in the system is represented in chemical equation 7.

$$H_3O^+ + OH^- \rightarrow 2H_2O \qquad (7)$$
$$(D_x)$$

Therefore, the system is maintained at the constant hydrogen ion concentration (D_0), since the potential buildup of hydrogen ions in the system is neutralized by an equivalent amount of base (D_x).

The differential equations describing the kinetics of the reactions 1, 2, 5, and 6 are represented for the present system by equations 8-15 where abbreviations represent the molar concentrations of the involved chemicals. Equations 12 and 14 have been set equal to

$$dA/dt = -k_1A + k_{-1}CD_o \qquad (8)$$

$$dC/dt = k_1A - k_{-1}CD_o - k_6CF + k_{-6}MD_oG \qquad (9)$$

$$dE/dt = -k_2E + k_{-2}FG \qquad (10)$$

$$dF/dt = k_2E - k_{-2}FG - k_6CF + k_{-6}MD_oG \qquad (11)$$

$$dG/dt = k_2E - k_{-2}FG + k_6CF - k_{-6}MD_oG = 0 \qquad (12)$$

$$dM/dt = k_6FC - k_{-6}MD_oG - k_5MD_o + k_{-5}P \qquad (13)$$

$$dD_o/dt = k_1A - k_{-1}CD_o + k_6FC - k_{-6}MD_oG - k_5MD_o + k_{-5}P - dD_x/dt = 0 \qquad (14)$$

$$dP/dt = k_5MD_0 - k_{-5}P \qquad (15)$$

$$dD_x/dt = k_1A - k_{-1}CD_0 + k_6FC - k_{-6}MD_oG - k_5MD_o + k_{-5}P \qquad (16)$$

zero, since the initial concentration or iodide is large compared to that formed during the reaction and hydrogen ion concentration has been maintained constant by the addition of sodium hydroxide.

Compartmental Model. When the rate of equilibrium of some reactions is very rapid compared to the rate of the over-all reactions, it is possible to introduce certain simplifying assumptions. For example, equations 1 and 8 depict the equilibrium between the un-ionized and ionized tyrosine derivative. Since k_1 and k_{-1} represent rate constants and hydrogen ion concentration is maintained constant in our system, the ratio of un-ionized to ionized tyrosine derivative also should be constant. This is based on the assumption that the equilibrium represented in equation 1 is very rapid and that the rate k_6 of reaction of ionized tyrosine derivative is slow as compared to its rate of formation k_1. This concept may be expressed as in equation 17.

$$A/C = k_{-1}D_o/k_1 \qquad (17)$$

Defining $A' = A + C$ and $K_1 = k_1/k_{-1}$, we get

$$A'/C = (A + C)/C = 1 + D_o/K_1 \qquad (18)$$

where

$$C = A'/(1 + D_o/K_1) \qquad (19)$$

and

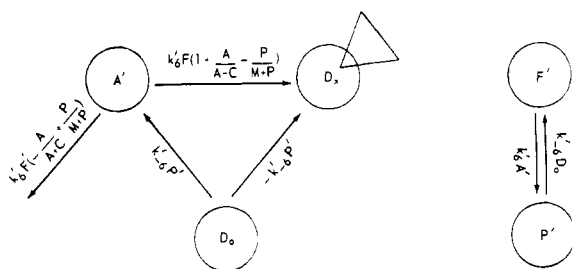$$A = A'/(1 + K_1/D_o) \qquad (20)$$

1967

FIGURE 2: Compartmental model based on nonsteady-state kinetic treatment of proposed mechanism for phenolic iodination.

Similarly, for rapid equilibria in equations 10 and 15, equations 21-24 are obtained when $F' = E + F$, and $P' = M + P$.

$$F = F'/(1 + G/K_2) \tag{21}$$

$$E = F'/(1 + K_2/G) \tag{22}$$

$$M = P'/(1 + D_o/K_5) \tag{23}$$

$$P = P'/(1 + K_5/D_o) \tag{24}$$

where $K_2 \equiv k_2/k_{-2}$ and $K_5 \equiv k_{-5}/k_5$.

Substitution of equations 8 and 19-24 into equation 9 yields equation 25.

$$\frac{1}{1 + D_o/K_1} \frac{\mathrm{d}A'}{\mathrm{d}t} = -\frac{1}{1 + K_1/D_o} \frac{\mathrm{d}A'}{\mathrm{d}t}$$
$$- k_6 \frac{A'}{1 + D_o/K_1} \frac{F'}{1 + G/K_2}$$
$$+ k_{-6} \frac{P'}{1 + D_o/K_5} D_o G \tag{25}$$

Solving for $\mathrm{d}A'/\mathrm{d}t$, we get

$$\frac{\mathrm{d}A'}{\mathrm{d}t} = -k_6 \frac{1}{1 + D_o/K_1} \frac{F'A'}{1 + G/K_2}$$
$$+ k_{-6} \frac{D_o G P'}{1 + D_o/K_5} \tag{26}$$

Setting

$$k_6' = \frac{1}{1 + D_o/K_1} \frac{k_6}{1 + G/K_2} \tag{27}$$

and

$$k_{-6}' = \frac{G k_{-6}}{1 + D_o/K_5} \tag{28}$$

we get

$$\frac{\mathrm{d}A'}{\mathrm{d}t} = -k_6'F'A' + k_{-6}'D_oP' \tag{29}$$

Similar substitution for equations 11-14 yields

$$\mathrm{d}F'/\mathrm{d}t = \mathrm{d}A'/\mathrm{d}t = -k_6'F'A' + k_{-6}'D_oP' \tag{30}$$

$$\mathrm{d}G/\mathrm{d}t = 0 \tag{31}$$

$$\mathrm{d}P'/\mathrm{d}t = -\mathrm{d}F'/\mathrm{d}t \tag{32}$$

$$\mathrm{d}D_o/\mathrm{d}t = 0 \tag{33}$$

$$\mathrm{d}D_x/\mathrm{d}t = \left(\frac{1}{1 + K_1/D_o} \right.$$
$$\left. + \frac{1}{1 + K_5/D_o} + 1 \right)\mathrm{d}A'/\mathrm{d}t \tag{34}$$

The above set of equations is shown schematically in Figure 2. This representation[4] lends itself directly to the computational techniques of the computer program employed.

As a first approximation, it may be assumed that equation 4 is virtually irreversible. Then the relationship of k_6 to equations 3 and 4 may be expressed according to the steady-state assumption as in equation 35.

$$k_6 = k_3k_4/(k_4 + k_{-3}G) \tag{35}$$

Upon solving for G, iodide, equation 35 gives equation 36.

$$G = \frac{k_3k_4}{k_{-3}} \times \frac{1}{k_6} - \frac{k_4}{k_{-3}} \tag{36}$$

Therefore, it can be seen that with varying iodide concentrations a plot of G vs. $1/k_6$ will yield a straight line with an intercept of $-k_4/k_{-3}$ and a slope of k_3k_4/k_{-3}.

The concentration of ionized and un-ionized tyrosyl derivative at each pH studied was calculated from the hydroxyl pK of each noted in Table I. These values are somewhat less in each instance than was found by spectrophotometric titration (Mayberry et al., 1964). This is not unexpected, since the spectrophotometric titrations were performed in buffered solutions at much higher ionic strength. The carboxyl pK_a values for these compounds have not been determined for these compounds previously as far as we could determine. The values for N-acTY and N-acMIT are compatible with values for related compounds (Greenstein and Winitz, 1961), while the value for N-acDIT is higher than ex-

---

[4] This is a compartmental representation of the simplified set of differential equations (30-34). This schematic representation is used in connection with computer solution of nonlinear differential equations and the fitting of data and is discussed elsewhere (Berman, 1965).

W. E. MAYBERRY, J. E. RALL, M. BERMAN, AND D. BERTOLI

TABLE II: Typical Computer "Print-Out" for Iodination of N-acMIT.[a]

| Time (sec) | $10^3 D_x$ (M) | | D_x(calcd)/ D_x(obsd) |
	Calcd	Obsd	
30	0.033	0.026	1.283
60	0.064	0.055	1.147
90	0.092	0.087	1.059
120	0.118	0.114	1.032
150	0.142	0.138	1.025
180	0.164	0.162	1.013
240	0.204	0.205	0.993
300	0.238	0.241	0.989
360	0.268	0.271	0.989
420	0.294	0.296	0.994
480	0.317	0.319	0.993
540	0.337	0.340	0.993
600	0.355	0.356	0.998
660	0.371	0.373	0.996
720	0.385	0.386	0.997
780	0.397	0.400	0.994
840	0.408	0.409	0.999
900	0.418	0.418	1.000
960	0.427	0.425	1.005
1020	0.435	0.433	1.005
1080	0.442	0.439	1.006
1140	0.448	0.447	1.003
1200	0.454	0.454	1.000

[a] $\alpha = 1.005 \pm 0.006$; $k_6' = 1.903 \pm 0.028$ l. mole^{-1} sec^{-1}; $k_{-6}' = 9.857 \pm 38.18$ l. mole^{-1} sec^{-1}.

pected and cannot be explained.[5] However, the value for the hydroxyl group of N-acDIT seems compatible with other comparative data (Mayberry et al., 1964; and Greenstein and Winitz, 1961).

The concentration of free iodine was found from the equilibrium in equation 2. The triiodide equilibrium is given by Katzin and Gebert (1955) at several temperatures appropriate to this study. If the initial stoichiometric concentration of iodine in solution is set equal to x, and y is the iodide concentration, then equation 2 may be expressed as

$$K_2[\text{I}_3^-] = (x - [\text{I}_3^-])(y - [\text{I}_3^-]) \qquad (37)$$

Since K_2, x, and y are known, concentration of I_3^- may be solved from the quadratic equation. The concentration of free iodine is then found by subtracting the concentration of I_3^- from x.

Fit of Data to Kinetic Model. The solution of the simplified set of differential equations (18–24) and the

---

[5] Since the value is lower for N-acMIT than for N-acTY, it might be expected that the pK_a for the carboxyl group of N-acDIT would be lower than for N-acMIT.

adjustment of parameter values to yield a least-squares fit of the data were performed on the digital computer using the existing general purpose program.[2] An example of the fit of the data to the compartmental model is shown in Table II for the iodination of N-acMIT at pH 8.40 and 25° with initial stoichiometric concentrations of N-acMIT, 1.2×10^{-3} M; I_2, 3×10^{-4} M; and I^-, 0.105 M. There was no significnt difference in the fit for either iodination reaction. The data entered into the computer run were the volumes of sodium hydroxide required to maintain a given pH and time. The volume data were converted into molar concentration of sodium hydroxide, D_x. This calculation was based upon the value for sodium hydroxide concentration determined titrimetrically. This latter term was subsequently corrected by a proportionality constant, α. This value allowed the computer to correct the initial estimate of base concentration for the best fit of the data. As further noted in Table II, the observed and calculated concentration of compartment D_x based upon the kinetic model are listed along with their ratio. The values for k_6' and k_{-6}' including their uncertainties are given also. The values of k_6 and k_{-6} may then be found from the relationships in equations 27 and 28. The standard deviation in the rate k_{-6}' is quite large. This is due to equilibrium K_6 being markedly in favor of k_6. This situation was observed in all of the experiments. Thus, the values of the k_{-6} are actually meaningless and the conditions for k_{-6} are unknown. Since the reaction proceeds essentially in one direction, application of steady-state kinetics (equation 35) seems reasonable. The ratio of D_x(calcd) to D_x(obsd) for the initial 60 sec may be seen to be larger than at subsequent times. This is explained by the fact that the 0.05 ml. of iodine–iodide solution was not at the pH of the run when added to the system.

Varying Iodide Concentration in Iodination of N-acTY. The values k_6' and k_6 for the iodination of N-acTY are listed in Table III. The velocity constant k_6 decreases markedly as iodide concentration increases. The last column in the table shows that k_6 approximately is inversely proportional to iodide concentration in that the values $k_6[\text{I}^-]$ are reasonably constant. Since phenolic iodination is general base catalyzed (Mayberry and Bertoli, 1965), $k_6[\text{I}^-]$ values reflect catalysis by both water and hydroxyl ions. The water plus hydroxyl ions over the pH range 5.80–9.80 have been found previously to contribute 1187–1396 l. mole^{-1} sec^{-1} to observed rate constants in buffer systems. These latter values should be comparable to the present $k_6[\text{I}^-]$ values, and they in fact are remarkably close.
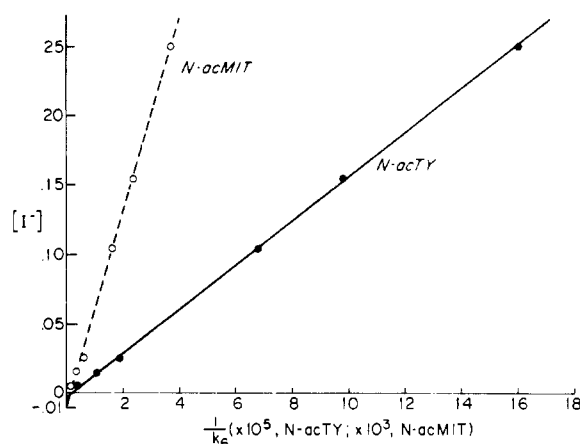
Varying Iodide Concentration in Iodination of N-acMIT. For the iodination of N-acMIT, the dependence of k_6 upon the inverse first power of iodide concentration may be seen in Table IV. The values $k_6[\text{I}^-]$ are reasonably constant as was the case for N-acTY iodination. However, there is more variation in these values than was the case for N-acTY. This variation occurs near pH 8.40. Since the hydroxyl ion catalysis contributes less to N-acMIT iodination than to N-acTY (Mayberry and Bertoli, 1965), this is not likely the

1969

TABLE III: Effect of Varying Iodide Concentration upon k_6 for Iodination of N-acTY at 25°.

pH	[KI] (M)	$k_6' \pm$ S.D. (l. mole^{-1} sec^{-1})	$10^{-3}k_6$ (l. mole^{-1} sec^{-1})	$k_6[I^-]$ (sec^{-1})
5.80	0.005	2.29 ± 0.03	281.	1407
6.80	0.015	3.04 ± 0.02	98.9	1483
7.80	0.025	10.37 ± 0.09	54.8	1369
8.40	0.105	2.59 ± 0.04	14.9	1561
8.80	0.155	3.19 ± 0.04	10.5	1203
9.80	0.250	8.63 ± 0.18	6.27	1566

TABLE IV: Effect of Varying Iodide Concentration upon k_6 for Iodination of N-acMIT at 25° and Correlation with N-acTY.

pH	[KI] (M)	$k_6' \pm$ S.D. (l. mole^{-1} sec^{-1})	$10^{-3}k_6$ (l. mole^{-1} sec^{-1})	$k_6[I^-]$ (sec^{-1})	$\dfrac{k_6(N\text{-acTY})}{k_6(N\text{-acMIT})}$
5.80	0.005	1.90 ± 0.15	9.75	48.7	28.9
6.80	0.015	2.36 ± 0.01	3.19	47.9	31.0
7.80	0.025	7.18 ± 0.32	1.72	43.0	31.9
8.40	0.105	1.90 ± 0.03	0.61	64.3	24.3
8.80	0.155	1.66 ± 0.004	0.42	64.9	25.0
9.80	0.250	1.22 ± 0.01	0.27	67.8	23.1

FIGURE 3: Correlation of k_6 (l. mole^{-1} sec^{-1}) with iodide concentration (M) according to equation 36 in the iodination of N-acTY and N-acMIT. Lines drawn between data points represent the least-squares fit.

explanation. The variation probably is due to the correction for phenolate ion concentration. The pH range is near that of the pK_a of N-acMIT and any discrepancy in the actual pK for the compound and that of the pK used in the correction factor would result in a maximal error in the calculation of phenolate ion concentration.

Nevertheless, the values of $k_6[I^-]$ again are quite close to the comparable value, 45 (l. mole^{-1} sec^{-1}), found in the buffer studies.

The ratio of k_6 for N-acTY to that for N-acMIT is given in the last column of Table IV. Again, this ratio remains fairly constant and is just that ratio previously found for these reactions by different methods (Mayberry et al., 1964).

Estimation of Values of k_3 and k_4/k_{-3}. A plot of $1/k_6$ for each iodination reaction against iodide concentration (Figure 3) yields a linear relationship as predicted by equation 36. k_6 is not completely invariant with pH, but its variation over the pH range under study is quite minor (Mayberry and Bertoli, 1965). For our purposes in the present study, k_6 may be assumed to be invariant with pH and as a first approximation to reflect only the water reaction. The intercepts, $-k_4/k_{-3}$, of the lines drawn according to the least-squares fit in Figure 3 are -0.0012 ± 0.0009 and -0.0080 ± 0.0018 M for N-acTY and N-acMIT, respectively. Whether these values are significantly different from zero is conjectural. Nevertheless, it is of interest to examine these values further with respect to the slopes of the lines drawn in Figure 3. The slopes, k_3k_4/k_{-3}, were 1588 ± 13 sec^{-1} for N-acTY and 69.2 ± 1.0 sec^{-1} for N-acMIT. Dividing the slope by the intercept gives a value for k_3 of 1.3 × 10^6 l. mole^{-1} sec^{-1} in the iodination of N-acTY and 8.7 × 10^3 l. mole^{-1} sec^{-1} in the iodination of N-acMIT. Therefore, the rate k_3 of the formation of the quinoid intermediate in the iodination of N-acTY is about 150

W. E. MAYBERRY, J. E. RALL, M. BERMAN, AND D. BERTOLI

times that in the iodination of N-acMIT. However, the ratio of k_4/k_{-3} for N-acTY to that for N-acMIT is only 0.15. The ratio of the value k_3k_4/k_{-3} for the two reactions, 22.93, is approximately that previously found for the ratio of k_6 for the two reactions (Table IV).

Salt Effect. A comparison of the rate constants of both iodination reactions in the presence of 0.3 M sodium chloride (Table V) with the corresponding rate

TABLE V: Effect of Sodium Chloride upon Rate Constants for Iodination of N-acTY and N-acMIT.

pH	[NaCl] (M)	$10^{-4}k_6$ (l. mole^{-1} sec^{-1}) N-acTY	N-acMIT
5.80	0	28	0.98
5.80	0.30	26	1.10
9.80	0	0.63	0.03
9.80	0.30	0.67	0.03

constants in the absence of salt reveals little or no apparent salt effect.

Activation Energies. The rate of both iodination reactions was determined at 20, 25, and 30° at pH 9.80. The plots of log k_6 vs. $1/T$ were linear for both reactions. The activation energies were 24.49 ± 0.99 kcal mole^{-1} for N-acTY and 24.22 ± 2.54 kcal mole^{-1} for N-acMIT.

Discussion

The fit of the data to the compartmental model solution adds credence to the concept of phenolate ion iodination by molecular iodine by way of a quinoid intermediate, but, of course, does not exclude other possible mechanisms. The present study supports the data previously reported for iodination of N-acTY and N-acMIT in buffer systems. The ratio of k_6 for N-acTY to k_6 for N-acMIT is that previously found in buffer systems. In addition, the value $k_6[I^-]$ for each iodination reaction correlates well with the sum of the water and hydroxyl terms determined by analysis of a great number of buffer studies. This fact seems quite remarkable in view of the divergent methods and analysis used in obtaining the two sets of data.

The activation energies, 24.49 and 24.22, found for N-acTY and N-acMIT, are very close to those previously found for these compounds in borate buffer at pH 9.08 (24.1 and 24.3 kcal mole^{-1} (Mayberry and Rall, 1964)). Both sets of values reflect composite ΔH values for all equilibria involved except that the ΔH for equation 2 has been removed from the activation energy values of the present study, since I_2 concentration has been calculated using the appropriate equilibrium value at each temperature. Though there is no

statistical difference in the activation energies for the two iodination reactions under study, a possible difference is not ruled out due to the size of the standard deviations.

Small and variable salt effects have been noted previously in the iodination rate constants for N-acTY and N-acMIT in buffer systems (Mayberry et al., 1964). Since the relative effect was the same for both reactions, the assumption has been made that the salt effect was secondary and probably upon the buffer equilibria. In the present studies, salt has not been added to maintain a constant ionic strength. The total ionic strength has varied in the standard procedure and has depended upon the concentration of iodide in the system. To study salt effects, sodium chloride was added. This complexes a small amount of molecular iodine as I_2Cl^- (Cason and Neumann, 1961). As previously noted (Mayberry et al., 1964), this effect upon the calculated rate constants is small except at very low iodide and very high chloride concentrations. Because the reactions are base catalyzed and dependent upon OH^- concentration, a significant change in the activity coefficient for these ions occurs. This effect should not be major, however, in the pH range studied. It is also seen that in the present studies the pK values for the tyrosine derivatives are significantly higher than was previously found for the compounds by the spectrophotometric titration at a much higher ionic strength. Unless a primary salt effect is hidden within the complexities indicated, the absence of a significant salt effect in the present study supports the assumption that k_6 results from a reaction between a neutral molecule and an ion (Gould, 1959). Since it seems clear that phenolic iodination proceeds by way of the phenolate ion (Berliner, 1951; Painter and Soper, 1947), the apparent lack of a primary salt effect suggests that the iodinating species is not an ion, i.e., I^+ or H_2OI^+, and adds some evidence in favor of iodination by molecular iodine. However, this interpretation is not conclusive since k_6 is not a true rate constant but rather an apparent constant of composite terms.

The rate of iodination of N-acTY is faster than the iodination of N-acMIT, presumably as a result of the electrophilicity of the substituted iodine atom in N-acMIT. This increased difficulty in adding a second iodine atom to the ring might be expected to result in an increased activation energy value for N-acMIT as compared to N-acTY. The fact that such is not the case may be in keeping with the proposed mechanism of iodination by molecular iodine. The activation energies in these studies are based on the velocity constant k_6. It might well be that, if separate energy terms were available for k_3 and k_4, the E_a for k_3 in the iodination of N-acMIT would be significantly larger than the corresponding term for N-acTY. The reverse situation would then be necessary for the composite energy term for k_4/k_{-3} in the two reactions, since the activation energies for k_6 are similar.

The values of k_3 for each reaction do conform to theoretical considerations in support of the proposed mechanism. The substitution of the first iodine atom on N-acTY should make the substitution of a second iodine

atom more difficult and be reflected in a slower rate constant. This fits well with the Hammett *meta* σ constant of +0.352 for the iodine atom (Hammett, 1940). In the present study, the value k_4/k_{-3} for N-acMIT is 6.78 times as large as the corresponding value for N-acTY. This must be a reflection of the fact that k_4 is significantly greater or k_{-3} significantly less for N-acMIT than for N-acTY.

As noted in equation 3, k_{-3} is the rate constant for the nucleophilic attack of iodide upon the quinoid intermediate. The nucleophilicity of iodide is reflected in the Swain and Scott (1953) nucleophilic constant for iodine of 5.04. Hine (1962) has called attention to the susceptibility of organic iodides to nucleophilic attack on halogen ions in support of the quinoid intermediate mechanism in phenolic iodination. There are no directly applicable nucleophilic substrate constants which would allow some estimation of the degree of reactivity of the quinoid intermediate of N-acMIT iodination as compared to that of N-acTY. Nevertheless, if, as seems likely, k_{-3} were larger or as large for N-acMIT as for N-acTY, then the value k_4/k_{-3} for N-acMIT gives evidence for the greater velocity of proton loss for N-acMIT than for N-acTY. This also supports the concept of the rate-limiting step of proton removal, since this reaction, equation 4, is an acid–base reaction. Since the energy curves for proton transfer make no distinction between substrate and catalyst (Bell, 1952), a relationship between the reaction velocity and acid–base strengths of substrates should exist for a given catalyst. In the present instance, the intermediate in the iodination of N-acMIT would be more acid than that for N-acTY (Table I). This should result in a faster rate of k_4 for N-acMIT than for N-acTY. The fit of these theoretical considerations to the present data lends further support to the proposed mechanism of iodination.

References

Bell, R. P. (1952), *Advan. Catalysis 4*, 198.
Berliner, E. (1951), *J. Am. Chem. Soc. 73*, 4307.
Berman, M. (1965), *in* Computers in Biomedical Research, Vol. II, Waxman, B. D., and Stacy, R. W., eds., New York, Academic, Chapter 7 (in press).
Berman, M., Shahn, E., and Weiss, M. F. (1962), *Biophys. J. 2*, 275.
Cason, D. L., and Neumann, H. M. (1961), *J. Am. Chem. Soc. 83*, 1822.
Gould, E. S. (1959), Mechanism and Structure in Organic Chemistry, New York, Holt, pp. 185, 186.
Greenstein, J. P., and Winitz, M. (1961), Chemistry of the Amino Acids, Vol. 1, New York, Wiley, pp. 486–491.
Grovenstein, E., and Aprahamian, N. S. (1962), *J. Am. Chem. Soc. 84*, 212.
Grovenstein, E., Jr., and Henderson, U. V., Jr. (1956), *J. Am. Chem. Soc. 78*, 569.
Grovenstein, E., Jr., and Kildy, D. C. (1957), *J. Am. Chem. Soc. 79*, 2972.
Hammett, L. P. (1940), "Physical Organic Chemistry," New York, McGraw-Hill, p. 188.
Hine, J. (1962), "Physical Organic Chemistry," 2nd ed., New York, McGraw-Hill, pp. 209, 360.
Katzin, L. I., and Gebert, E. (1955), *J. Am. Chem. Soc. 77*, 5814.
Mayberry, W. E., and Bertoli, D. (1965), *J. Org. Chem. 30*, 2029.
Mayberry, W. E., and Rall, J. E. (1964), *Exposes Ann. Biochim. Med. 25*, 21.
Mayberry, W. E., Rall, J. E., and Bertoli, D. (1964), *J. Am. Chem. Soc. 86*, 5302.
Painter, B. S., and Soper, F. G. (1947), *J. Chem. Soc. 342*.
Swain, C. G., and Scott, C. B. (1953), *J. Am. Chem. Soc. 75*, 141.