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Kinetics of Iodination. IV. A Comparison of the Kinetics of Iodination of L-Tyrosine and Some Derivatives*

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ABSTRACT: The kinetics of iodination of L-tyrosine and some derivatives has been studied spectrophotometrically in an aqueous buffered system. The second-order rate constant k for the iodination of L-tyrosine was 5.73×10^3 l. mole^{-1} sec^{-1}. On the basis of the rate constant for tyrosine being 100, the relative rates of iodination for the derivatives were glycyl-L-tyrosine, 162; glycyltyrosylglycine, 134; L-tyrosyl-L-tyrosine, 110; L-tyrosine methyl ester, 77; *N*-acetyl-L-tyrosine, 74; *N*-acetyl-L-tyrosine methyl ester, 61; *N*-acetyl-L-tyrosine ethyl ester, 55; 3-iodo-L-tyrosine, 6; and *N*-acetyl-3-iodo-L-tyrosine, 3. *O*-Methyl-L-tyrosine did not iodinate under the conditions studied. The Arrhenius activation energy E_a in the iodination of the iodinated derivatives was 21 kcal mole^{-1}, while all the other compounds had values of 16–17 kcal mole^{-1}. The kinetics of iodination of all the compounds was compatible with the concept of iodination occurring by way of phenolate anion and molecular iodine through a quinoid intermediate.

The kinetics of iodination of *N*-acetyl-L-tyrosine and *N*-acetyl-3-iodo-L-tyrosine has been studied previously. The rate for iodination of *N*-acetyl-L-tyrosine exceeded that of the iodinated derivative by a factor of 20–30 over the pH range 5.40–9.80 (Mayberry *et al.*, 1964). The kinetic data were interpreted to indicate that the reactive species in phenolic iodination were molecular iodine and phenolate anion and, further, that iodination proceeded *via* a quinoid intermediate. The reactions are subject to general base catalysis, and the function of the base is viewed as proton removal in the "rate-limiting" step from the quinoid intermediate (Mayberry and Bertoli, 1965).

The present study was initiated to determine the effect of the peptide linkage and of the ionizable groups of L-tyrosine upon the rates and activation energies of iodination. Such comparisons may have relevance to *in vivo* iodination of tyrosine within the thyroglobulin molecule.

Experimental

Materials. Recrystallized *N*-acetyl-L-tyrosine, *N*-acetyl-3-iodo-L-tyrosine, and *O*-methyl-L-tyrosine were gifts of Dr. R. Pitt-Rivers. Recrystallized glycyl-L-tyrosine, glycyl-L-tyrosylglycine, L-tyrosine methyl ester, *N*-acetyl-L-tyrosine methyl ester, *N*-acetyl-L-tyrosine ethyl ester, 3-iodo-L-tyrosine, and L-tyrosine were given by Dr. H. Cahnmann. L-Tyrosyl-L-tyrosine was purchased from Cyclo Chemical Corp., Los Angeles, Calif. High voltage electrophoresis in Barbital buffer at pH 8.68 revealed each compound to run as one spot as detected by the ferric chloride–potassium ferricyanide reagent for phenols (Barton *et al.*, 1952). The *O*-methyltyrosine does not stain with the latter, but this serves as a means for detecting trace impurities of phenolic substances in the compound. In addition, the purity of the *O*-methyltyrosine was further determined by ultraviolet spectra in acid and base, a method particularly advantageous for determining impurity in *O*-methyltyrosine (Wetlaufer *et al.*, 1958).

The iodine, potassium iodide, sodium chloride, sodium carbonate, and sodium bicarbonate were the best commercially available reagent grade chemicals. Water redistilled in glass was used in all experiments.

Kinetic Runs. All reactions were performed in a carbonate buffer system containing 0.120 M sodium bicarbonate and 0.075 M sodium carbonate at pH 9.60. Ionic strength was maintained at 0.64 by the addition of sodium chloride which was 0.097 M in the system. The stoichiometric concentration of tyrosine or derivative, iodine, and iodide was constant in each run at 2×10^{-4} M, 5×10^{-5} M, and 0.193 M, respectively. Each run was

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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 as before (Mayberry *et al.*, 1964).

Rate Constant Determinations. Rate constants for each run were determined from 20 to 40 optical density readings according to the integrated second-order equation (1) derived for computer (IBM 7094) solution (Berman *et al.*, 1962).

$$OD = \alpha\left\{b - \frac{e^{k_{obsd}(a-b)t} - 1}{e^{k_{obsd}(a-b)t} - b/a} \times b\right\} \quad (1)$$

where t = time in seconds, a and b are the stoichiometric concentrations of tyrosine derivative and iodine (at time = 0), and α is a proportionality constant which converts OD into concentration of iodine at time t.

The observed rate constants from eq 1 have been corrected for varying iodide, phenoxide, and chloride concentrations according to eq 2

$$k = \frac{k_{obsd}(K_1 + [I^-] + K_1[Cl^-]/K_2)(K_3 + [H^+])[I^-]}{K_1K_3} \quad (2)$$

where K_1, K_2, and K_3 are equilibria dissociation constants for eq 3–5.

$$I_3^- \rightleftharpoons I_2 + I^- \qquad K_1 = 0.0013 \text{ at } 25° \quad (3)$$

(Davies and Gwynne, 1951; Katzin and Gebert, 1955)

$$I_2Cl^- \rightleftharpoons I_2 + Cl^- \qquad K_2 = 0.60 \text{ at } 25° \text{ (Cason and Neumann, 1961)} \quad (4)$$

$$ArOH \rightleftharpoons ArO^- + H^+ \qquad K_3 \quad (5)$$

pK_a Determinations. The apparent pK value and, thus, apparent value for K_3, eq 5, was determined for the hydroxyl group of tyrosine and each derivative except *O*-methyltyrosine by spectrophotometric titration (Crammer and Neuberger, 1943; Lauford and Roberts, 1952). These titrations were carried out in carbonate buffer at an initial ionic strength of 0.40.

Results

Fit of Data To Second-Order Equation. An example of the fit of the observed data to the second-order rate equation, eq 1, may be seen in Table I for the iodination of tyrosyltyrosine. The fit of the data for the other iodination reactions was equally good. In addition to the information listed for a typical kinetic run in Table I, considerable statistical data is obtained along with a graphic plot of observed and calculated data. In the example shown, the observed velocity constant is 91 l. mole^{-1} sec^{-1}. It may be noted that α in this situation (26.22×10^3) approaches the extinction for triiodide on at the iodide concentration of 0.193 M and stoichiometric iodine concentration at 5×10^{-5} M. The extinction, ϵ, for triiodide is reported as 26.4×10^3 at 352 mμ (Allen and Keefer, 1955) and at 353 mμ (Awtrey and Connick, 1951).

TABLE I: Computer Print-Out for Iodination of L-Tyrosyl-L-tyrosine at 25°.

Time (sec)	$y \times 10^4$ [a]	Optical Density: Calcd	Obsd	Calcd/Obsd
19	0.3587	0.941	0.940	1.001
23	0.3355	0.880	0.880	1.000
27	0.3140	0.823	0.820	1.004
32	0.2893	0.759	0.760	0.998
35	0.2756	0.723	0.720	1.004
39	0.2585	0.678	0.680	0.997
42	0.2465	0.646	0.650	0.994
46	0.2314	0.607	0.610	0.995
49	0.2208	0.579	0.580	0.998
52	0.2107	0.553	0.550	1.005
56	0.1981	0.519	0.520	0.999
60	0.1863	0.489	0.490	0.997
64	0.1753	0.460	0.460	0.999
67	0.1675	0.439	0.440	0.998
70	0.1601	0.420	0.420	0.999
73	0.1530	0.401	0.400	1.003
77	0.1441	0.378	0.375	1.008
82	0.1338	0.351	0.350	1.002
87	0.1243	0.326	0.325	1.003
91	0.1172	0.307	0.310	0.991
95	0.1105	0.290	0.290	0.999
99	0.1042	0.273	0.270	1.012
105	0.0955	0.251	0.250	1.002
111	0.0876	0.230	0.230	0.999
114	0.0839	0.220	0.220	1.000
117	0.0804	0.211	0.210	1.004
121	0.0759	0.199	0.200	0.995

$\alpha = 26223 \pm 132$; $k_{obsd} = 90.71 \pm 0.24$

[a] y is equal to the bracketed term in eq. 1.

Apparent pK for Tyrosine and Derivatives. The values for the apparent pK of tyrosine and the derivatives are listed in Table II. The value for tyrosine is 10.10. It is well known that *ortho* halogen substitution onto the ring of phenolic substances results in a decrease of the pK of the hydroxyl group. This fact is evident in the pK values of this study found for *N*-acetylmonoiodotyrosine and monoiodotyrosine. Substitutions onto the amino or carboxyl groups of tyrosine have much smaller effects. In general, blocking of the carboxyl group (methyl or ethyl ester) decreases the pK of the hydroxyl group of tyrosine, and blocking of the amino group has either little effect or increases the pK.

The meaning of the listed apparent pK for tyrosyltyrosine is uncertain, though it probably represents an apparent average pK for two ionizing groups. This

TABLE II: Apparent Hydroxyl pK of Tyrosine and Derivatives at 25°.[a,b]

L-Tyrosine	10.10
Glycyl-L-tyrosine	10.14
N-Acetyl-L-tyrosine	10.04
L-Tyrosine methyl ester	9.98
N-Acetyl-L-tyrosine methyl ester	9.96
Glycyl-L-tyrosylglycine	9.95
N-Acetyl-L-tyrosine ethyl ester	9.88
L-Tyrosyl-L-tyrosine	9.74
N-Acetyl-3-iodo-L-tyrosine	8.54
3-Iodo-L-tyrosine	8.24

[a] Determined by spectrophotometric titration using 1 N NaOH and 1 N HCl. [b] Tyrosine or derivative, 2.4 × 10^{-4} M; $NaHCO_3$, 0.120 M; Na_2CO_3, 0.075 M.

value has been used subsequently, as well as the values of 9.80 and 10.26 as given by Greenstein (1932) for the two hydroxyl groups of tyrosyltyrosine, to correct for phenoxide concentration. These factors will be discussed subsequently.

TABLE III: Rate Constants for Iodination of L-Tyrosine and Derivatives in Carbonate Buffer, pH 9.60, at 25°.[a]

	k_{obsd} ± SD	$k_{obsd}\gamma$[b]	Relative Rates
L-Tyrosine	47.68 ± 0.22	5729	100
Glycyl-L-tyrosine	71.79 ± 0.40	9262	162
Glycyltyrosylglycine	81.95 ± 0.43	7666	134
L-Tyrosyl-L-tyrosine	90.71 ± 0.24	6321[c]	110
		6771[d]	118
		14594[e]	255
L-Tyrosine methyl ester	45.17 ± 0.22	4433	77
N-Acetyl-L-tyrosine	38.90 ± 0.17	4217	74
N-Acetyl-L-tyrosine methyl ester	36.57 ± 0.11	3476	61
N-Acetyl-L-tyrosine ethyl ester	37.56 ± 0.14	3151	55
3-Iodo-L-tyrosine	10.87 ± 0.12	328	6
N-Acetyl-3-iodo-L-tyrosine	5.19 ± 0.03	163	3
O-Methyl-L-tyrosine	0	0	0

[a] Rate constants in l. mole^{-1} sec.$^{-1}$ Tyrosine or derivative, 2 × 10^{-4} M; I_2, 5 × 10^{-5} M; Cl^-, 0.097 M; I^-, 0.193 M; $NaHCO_3$, 0.120 M; Na_2CO_3, 0.075 M. [b] $\gamma = (K_1 + [I^-] + K_1[Cl^-]/K_2)[I^-](K_3 + [H^+])/K_1K_3$. [c] Based upon p$K_a$ = 9.74. [d] Based upon pK_a = 9.80. [e] Based upon pK_a = 10.26.

Rate Constants for Iodination. The rate constants for iodination of tyrosine and the derivatives are listed in Table III. Under the conditions of these experiments and at pH 7.20 in phosphate buffer, there was no detectable iodination of *O*-methyltyrosine. Since phenolate anion is the species undergoing iodination, comparison of the rates of iodination on the basis of the observed rate constants is only possible when the rate constants are corrected for the equilibria dissociation of each tyrosine derivative. The corrected rate constant for iodination of tyrosine is 5729 l. mole^{-1} sec^{-1}. Relative to this value, the corrected rates for the iodination of the other derivatives are given in column 3 of Table III. It is significant that the rate for iodination of tyrosine is about 17 *times* greater than that for monoiodotyrosine, and similarly the rate for *N*-acetyltyrosine exceeds that for *N*-acetylmonoiodotyrosine by a factor of 25.

Glycyltyrosine and glycyltyrosylglycine iodinate more rapidly than tyrosine. All of the acetylated tyrosine derivatives have slower iodination rate constants than tyrosine. Further, the esters of both tyrosine and *N*-acetyltyrosine have rate constants smaller than the parent nonesterified compounds. The relative rates of 61 and 55, respectively, for the methyl and ethyl esters of *N*-acetyltyrosine suggest that the ethyl group reduces the rate more than the methyl group. This same situation exists in the pK effects (Table II).

If the apparent pK found in the present study is used to correct for the observed rate constant in the iodination of tyrosyltyrosine, the rate constant is approximately the same as for tyrosine. If the lower pK of Greenstein is used, the relative rate of tyrosyltyrosine is 118. However, if the larger pK is used, the relative rate is 255. On the basis of the other data, it seems reasonable that the apparent pK of this study may more nearly reflect the actual situation.

Effect of Heat on Reaction Rates. The rate of each iodination reaction was determined at 20, 27, and 30° in addition to that at 25°. In calculating k at 20, 25, and 30°, the appropriate values for K_1 are given by Katzin and Gebert (1955). K_1 at 27° was estimated by interpolation of that data. The value at 25° was used for all temperatures for K_2. This is a relatively insignificant correction under the conditions of these studies. Since apparent values for K_3 are known only at 25°, these values were necessarily used at all the temperatures.

The Arrhenius activation energies for all the reactions are given in Table IV. These values are actually composite terms which include the ΔH for ionization of tyrosine or derivative and for the buffer constituents. The value for iodination of tyrosine is 17.4 ± 0.8 kcal mole^{-1}. There is little if any significant difference in the values for all the other derivatives except those for the iodinated derivatives. The activation energies for iodination of monoiodotyrosine and *N*-acetylmonoiodotyrosine are approximately 4 kcal greater than the values for the other compounds.

The most striking feature about the values for entropy of activation for these reactions, as noted in Table IV, is that all the values are positive (Bunnett, 1961). In

TABLE IV: Energy and Entropy of Activation for Iodination of Tyrosine and Derivatives at pH 9.60.

	E_a[a] (kcal mole^{-1})	ΔS^*[b] (eu (cal deg^{-1} mole^{-1}))
L-Tyrosine	17.4 ± 0.8	+15.0
Glycyl-L-tyrosine	16.6 ± 0.4	+13.2
Glycyltyrosylglycine	16.7 ± 0.8	+13.1
L-Tyrosyl-L-tyrosine	17.4 ± 0.5	+15.2
L-Tyrosine methyl ester	16.3 ± 0.8	+10.7
N-Acetyl-L-tyrosine	17.7 ± 0.8	+15.6
N-Acetyl-L-tyrosine methyl ester	16.4 ± 0.5	+10.5
N-Acetyl-L-tyrosine ethyl ester	16.2 ± 0.6	+ 9.7
3-Iodo-L-tyrosine	21.2 ± 1.6	+22.2
N-Acetyl-3-iodo-L-tyrosine	21.5 ± 0.5	+21.9

[a] ±SD (standard deviation) as determined by method of least squares. [b] Calculated at 25°.

addition, there seems to be significance in the similarity of the values for comparable substituents. ΔS^* is about 15 eu for tyrosine, tyrosyltyrosine, and *N*-acetyltyrosine; 13 eu for glycyltyrosine and glycyltyrosylglycine; 10 eu for the niethyl ester of tyrosine and for the methyl and ethyl esters of *N*-acetyltyrosine; and 22 eu for the monoiodinated derivatives of tyrosine and *N*-acetyltyrosine.

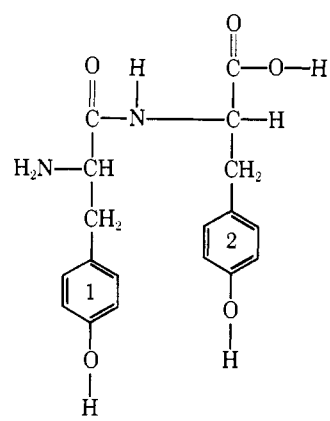
Discussion

When the concentrations of iodide, buffer constituents, and hydrogen ion are maintained constant, the iodination of tyrosine and tyrosine derivatives fit the bimolecular second-order rate equation quite well. The single exception has been that *O*-methyltyrosine does not iodinate under the conditions of these experiments. This finding fits well with the concept of the phenolate anion being the species iodinated (Berliner, 1951; Mayberry *et al.*, 1964).

In comparing the reaction rates for iodination of tyrosine and the derivatives in the present study, there is only one critical variable. This is the phenolic dissociation for each as expressed in eq 5. Although correction has been made for the equilibria expressed in eq 3 and 4, these were not necessary for comparison of relative rates since the concentrations of iodine, iodide, and chloride were constant in the runs.

Because of the importance of K_3 for each compound, we include some comparison of the observed values in this study with those in the literature. The apparent pK for the hydroxyl group of tyrosine has been reported by Martin *et al.* (1958) to be 10.13. This value is similar to that in the present study. Martin and associates have also reported a complete ionization scheme for tyrosine. The pK for the phenolic hydroxyl group of tyrosine with the other two ionizing groups in the COO^- and NH_2 form should correspond to the situation for *N*-acetyltyrosine in this study. In fact, their value and ours are identical at 10.04. The pK for tyrosine ethyl ester has been given as 9.80 (Martin *et al.*, 1958) and 9.98 (Beaven and Holiday, 1952). On the basis of these values and the other values in the present study, the apparent pK values for the esters of tyrosine seem consistent. Values for the pK of glycyltyrosine are given as 9.96 (Beaven and Holiday, 1952) and 10.40 (Greenstein, 1932). The pK for glycyltyrosine in the present study is 10.14, which fits well. The pK value, 8.24, for monoiodotyrosine in the present study is also that reported previously (Herriott, 1947) for this compound.

As pointed out from previous data (Mayberry *et al.*, 1964; Mayberry and Bertoli, 1965), the rate of iodination of *N*-acetyltyrosine exceeds that of *N*-acetylmonoiodotyrosine by a factor of 25 in the present study. This has been ascribed (Mayberry *et al.*, 1964) to the electrophilicity of the iodinium ion which has a *meta* σ constant of +0.352 (Hammett, 1940). On this basis, a similar ratio would be anticipated for the rates of iodination of tyrosine and monoiodotyrosine; in agreement, a ratio of 17 is found. Glycyltyrosine iodinated more rapidly than any of the other derivatives by a factor of 1.6 over that for tyrosine. Li (1948) found a value of 1.5 for this ratio at pH 5.65 in acetate buffer and suggested that the presence of a peptide linkage enhanced the rate of iodination. However, *N*-acetyltyrosine with a comparable linkage has a rate of iodination of only about 0.7 of that for tyrosine. It seems that, when the NH_2 group of tyrosine is involved in a peptide bond with a compound containing a free NH_2, the rate of iodination is enhanced. When, in addition, the COOH group of tyrosine is involved in peptide linkage, even with a group that contains a free COOH, the rate of iodination is decreased. This assumption is based on comparison of the rates of glycyltyrosine and glycyltyrosylglycine. It is also noted that, when the COOH group of tyrosine is involved in an ester linkage, the rate of iodination is decreased. All of these factors may be indicative of inductive effects. The effect of the peptide linkage in tyrosyltyrosine is more complicated. Consider the formula for tyrosyltyrosine (I)



I

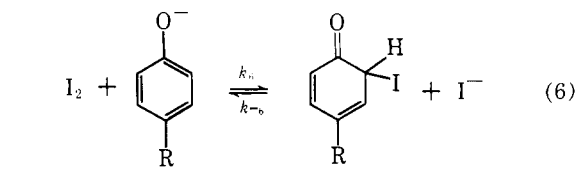
in which the ring of the tyrosine moiety whose carboxyl

group is involved in peptide linkage is labeled "1" and the ring of the tyrosine moiety whose amino group is involved in peptide linkage is labeled "2." Greenstein (1932) has attributed the pK of 9.80 to the hydroxyl group of ring 1 and pK of 10.26 to ring 2. This assignment is also reasonable on the basis of the data in Table II.

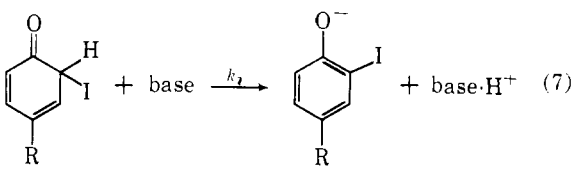
As seen in Table III, when the apparent pK of this study is used to correct the initial stoichiometric concentration of tyrosyltyrosine, the value of $k_{obsd}\gamma$ is near that for iodination of tyrosine. When the pK value of 10.26 is used, the rate constant for tyrosyltyrosine is about 2.6 times greater than that for tyrosine. Obviously, neither situation reflects the actual events. On the basis of the other data in Table III, ring 2 of tyrosyltyrosine should iodinate more rapidly than ring 1; in contrast, there should be a greater number of moles of tyrosyltyrosine with the OH group of ring 1 ionized than of tyrosyltyrosine with the OH group of ring 2 ionized. Since our value for the pK of tyrosyltyrosine should reflect the approximate mean of the hydroxyl groups of the two rings, the true pK of ring 1 should be somewhat lower than 9.74. If such a lower value were used to correct the observed rate constant, then a $k_{obsd}\gamma$ significantly lower than that for tyrosine would result. If a higher pK were used, as for ring 2, a $k_{obsd}\gamma$ significantly greater than that for tyrosine would be found. However, since ring 1 is in greater concentration in the ionized form and since ring 2 iodinates more readily, it seems inherently likely that both rings 1 and 2 are undergoing iodination. Since the rate constant for iodination of monoiodotyrosine is so much slower than for tyrosine and since the total combined stoichiometric concentrations of rings 1 and 2 are 8 times greater than the stoichiometric concentrations of iodine, there seems little likelihood of either ring 1 or 2 being diiodinated to any appreciable extent (Mayberry *et al.*, 1964). In view of these considerations, the over-all rate for iodination of tyrosyltyrosine may approximate that for tyrosine.

The activation energies for *N*-acetyltyrosine and *N*-acetylmonoiodotyrosine have been determined previously over a wider temperature range (Mayberry and Rall, 1964) than in the present study and by a different method of monitoring iodination (Mayberry *et al.*, 1965). No difference in E_a was found for the two reactions, but the size of the standard deviations precluded ruling out such a possibility. In the present study, a difference in the E_a for the two iodination reactions seems clearly indicated. This difference is also supported by the fact that the E_a for iodination of monoiodotyrosine is also 4 kcal mole^{-1} greater than for iodination of tyrosine. There is no significant difference among the activation energies for the iodination of the previously noniodinated tyrosine derivatives.

The positive values for the entropies of iodination in the reactions studied (Table IV) can be used to rationalize the fast rates of these reactions. As is well established, the entropies of activation reflect in part a probability factor, p. This factor reflects in part the probability that molecules having the necessary energy will react upon collision, and log p has been found to be nearly equal to $\Delta S^*/2.303R$ (Gould, 1959). On this basis the value of p for tyrosine is 1.9×10^3 while that for monoiodotyrosine is 70.8×10^3 or about 30 times greater. A similar situation exists for the comparable acetylated derivatives. The availability of two sites *ortho* to the hydroxyl group on tyrosine might be expected to result in a greater probability factor in the iodination of tyrosine than the one site available in the iodination of monoiodotyrosine. This possibility is not ruled out by the present evidence if one considers our previously proposed mechanism (Mayberry *et al.*, 1964) for iodination of *N*-acetyltyrosine and *N*-acetylmonoiodotyrosine. This mechanism is represented by eq 6 and 7, and our

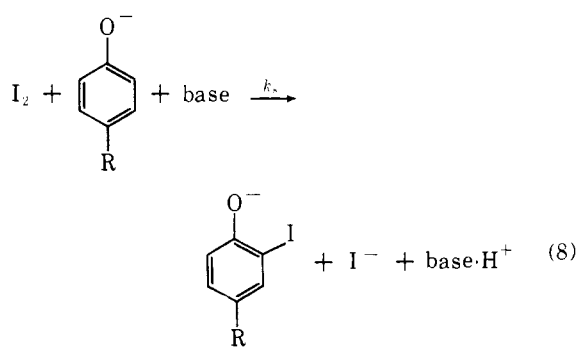


(6)



(7)

rate constant values $k_{obsd}\gamma$ actually only approximate the sum of these reactions as represented by eq 8.



(8)

Since the equilibrium k_6/k_{-6} is not known, the value $k_{obsd}\gamma$ or k_8 is as representative of k_7 as is possible by these methods. The relationship between eq 6 and 7 and eq 8 has been discussed previously (Mayberry and Bertoli, 1965). The pertinent point for the present discussion is that the energy terms in Table IV reflect the sum of two reactions, k_6/k_{-6} and k_7. It seems likely that E_a for k_6 in iodination of monoiodotyrosine may be significantly greater than that for tyrosine than indicated by the 4 kcal difference. By contrast, since eq 7 represents an acid–base reaction and since the quinoid intermediate in iodination of monoiodotyrosine should be more acid than that for tyrosine, the E_a for k_7 in iodination of monoiodotyrosine might be less than that for tyrosine. By the same analysis, the randomness of the system as expressed by ΔS^* may actually be greater in k_7 for monoiodotyrosine than that for tyrosine. It may well be that similar but smaller discrepancies are hidden within

the consistency of the activation energies for the non-iodinated compounds.

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