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Kinetics of Iodination. I. A Comparison of the Kinetics of Iodination of N-Acetyl-L-Tyrosine and N-Acetyl-3-iodo-L-Tyrosine

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The kinetics of iodination of N-acetyl-L-tyrosine and N-acetyl-3-iodo-L-tyrosine has been studied. The bimolecular second-order rate equation $dx/dt = [A][B]$, where A and B are the stoichiometric concentrations of tyrosyl derivative and iodine, fits both reactions when iodide and hydrogen ion concentrations are held constant. Values for the rate constants have been calculated with the aid of a high speed digital computer. The data for both reactions are compatible with the concept of phenoxide ion iodination by either molecular iodine or hypoiodous acidinium ion. In keeping with the Hammett *meta* σ-constant of +0.352, the rates of iodination of acetyltyrosine exceed those of acetylmonoiodotyrosine by a factor of 30 over the pH range 5.40 to 9.80.

The chemical kinetics of the iodination of tyrosine was studied by Li[2] in 1942. The data were interpreted to show that monoiodination was the rate-limiting step in diiodination and further that once the phenolic ring had been monoiodinated, the second iodine atom entered the ring instantaneously. Biologic and chemical evidence[3] later raised questions regarding the tenability of those conclusions. Recent preliminary studies have shown that N-acetyl-L-tyrosine iodinates more rapidly than N-acetyl-3-iodo-L-tyrosine over the pH range 5.90 to 10.57.[4] Comparisons of the kinetics of monoiodination and diiodination have been made to gain some clue as to the mechanistic detail of phenolic iodination. Such comparisons may have significance in their biologic analogies to *in vivo* thyroxine formation.

A computer program has been employed in the present studies. In addition to increasing speed and facility of rate constant calculations, the statistical fit of the data to the rate equation has been greatly improved by increasing the number of observations per kinetic run. A comparable analysis of the observed data would be precluded by the usual mathematical methods.

Experimental

Equilibria.—The iodination reactions were performed in aqueous solution and were designed with consideration for the equilibria involving the reactants as:

$$I_3^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} I_2 + I^-, \quad K_1 = 1.3 \times 10^{-3} \text{ at } 25° \qquad (1)[5]$$

$$I_2Cl^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} I_2 + Cl^-, \quad K_2 = 0.60 \text{ at } 25° \qquad (2)[6]$$

$$H_2O + I_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} H_2OI^+ + I^-, \quad K_3 = 10^{-11} \text{ at } 25° \qquad (3)[7]$$

$$H_2OI^+ + H_2O \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} HOI + H_3O^+, \quad K_4 = 3 \times 10^{-2} \qquad (4)[8]$$

$$3I_2 + 3H_2O \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} IO_3^- + 5I^- + 6H^+, \quad K_5 = 4 \times 10^{-15} \text{ at } 25° \qquad (5)[9]$$

$$R\text{-}C_6H_4OH \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} R\text{-}C_6H_3O^- + H^+, \quad K_6 \qquad (6)$$

A large ratio (66 to 1 as a minimum) of iodide to iodine has been maintained. Ionic strength has been maintained constant by the addition of sodium chloride; therefore, equilibrium K_2 has significance in the system. This complicates the system somewhat; however, this salt was chosen because of its lack of absorption in the wave length range of the present studies. In addition, as will be subsequently shown, the correction factors necessary because of equilibrium K_2 are not major. Hydrogen ion concentration has been kept constant by the presence of buffer. Iodate formation may result in loss of iodine if iodide and hydrogen ion concentrations are low. This possibility has been studied for each set of experimental conditions. Equilibrium K_6 has been determined for each phenolic compound used. Measurement of the rate constant of iodination requires that all of the equilibria involved be rapid with respect to the actual iodination step.

Materials.—Recrystallized N-acetyl-L-tyrosine (N-acTY), N-acetyl-3-iodo-L-tyrosine (M-acMIT), and N-acetyl-3,5-diiodo-L-tyrosine (N-acDIT) were the phenolic derivatives employed.[10] Paper chromatography with a 1-butanol–1,4-dioxane–2 *N* ammonium hydroxide (30:20:saturated) solvent system and 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.[11]

The acetyltyrosine had a melting point on a Köfler stage of 149.5–151.0° (uncor.), while the literature value is given as 152–154° (cor.).[12]

Anal. Calcd. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27. Found[13]: C, 59.35; H, 5.82, N, 6.31.

The acetylmonoiodotyrosine had a m.p. of 159–160.5° (uncor.), but no literature value was found for comparison.

Anal. Calcd. for $C_{11}H_{12}INO_4$: C, 37.84; H, 3.46; I, 36.35; N, 4.01. Found[13]: C, 38.02; H, 3.47; I, 36.52; N, 4.18.

The m.p. of the acetyldiiodotyrosine·0.5H_2O was 125–126° (uncor.) confirming the value previously reported for this compound.[14]

Anal. Calcd. for $C_{11}H_{11}I_2NO_4 \cdot 0.5H_2O$: C, 27.30; H, 2.50;

(1) Aided by a grant for a Postdoctoral Fellowship from the American Cancer Society. Address correspondance to the Mayo Clinic, Rochester, Minn.

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(8) K_4 was estimated by Bell and Gelles (ref. 7) by dividing the hydrolysis constant of W. C. Bray and E. L. Connolly, *J. Am. Chem. Soc.*, **33**, 1485 (1911), by K_3.

(9) W. O. Lundberg, C. S. Vestling, and J. E. Ahlberg, *ibid.*, **59**, 264 (1937).

(10) These compounds were synthesized, recrystallized, and supplied generously by Dr. R. Pitt-Rivers to whom we are most grateful.

(11) G. M. Barton, R. S. Evans, and J. A. F. Gardner, *Nature*, **170**, 249 (1952).

(12) V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **98**, 295 (1932).

(13) Microanalysis performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(14) R. Pitt-Rivers, *Biochem. J.*, **43**, 223 (1948).

1, 52.44; N, 2.89. Found[13]: C, 27.25; H, 2.53; I, 53.44; N, 3.23.

The iodine, potassium iodide, sodium chloride, and buffer salts used were the best commercially available reagent grade chemicals. Water redistilled in glass was used.

Kinetic Runs.—The iodination reactions were performed in 1-cm. quartz cuvettes. The level of iodine as a function of time was followed spectrophotometrically in a thermostated chamber at 350 mμ.[15] Each reaction was performed at a constant volume of 3 ml. and at 25 ± 0.02°; 2 ml. of the appropriate concentration of tyrosyl derivative in a 1-cm. quartz cuvette was brought to temperature in the spectrophotometer. One ml. of the appropriate concentration of iodine–iodide solution at temperature was pipetted into the cuvette as rapidly as possible and the cuvette inverted for additional mixing. Time was measured from the moment of initial pipetting. The time required from the beginning of addition of the iodine–iodide solution until the first spectrophotometric reading was generally about 20 sec. Duplicate runs were made for each set of experimental conditions. The total ionic strength, μ, was maintained at 0.64 in all runs by the addition of sodium chloride. pH determinations were made with a Radiometer employing a scale expander and glass electrodes.

pK_a Determinations.—The apparent pK value for the hydroxyl group of each acetyl derivative of tyrosine was determined by spectrophotometric titration.[16] Determinations were carried out for N-acTY and N-acMIT in two buffer systems, borate and carbonate, at 0.6 ionic strength. The duplicate values agreed in each instance within ±0.03 pH unit, and the average value is reported. The value for N-acDIT was determined only in borate buffer.

Rate Constant Calculations.—The second-order rate constant for each kinetic run was calculated from the relationship

$$dx/dt = k(a - x)(b - x) \qquad (7)$$

where a and b represent the initial stoichiometric concentrations (at time = 0) of the tyrosine derivative and iodine, respectively, and x represents the concentration of product at time, t. Upon integration, eq. 7 becomes

$$kt(a - b) = \ln \frac{b}{a} \frac{(a - x)}{(b - x)} \qquad (8)$$

which may be represented exponentially by

$$a/b\, e^{k(a - b)t} = \frac{a - x}{b - x} \qquad (9)$$

Upon rearranging terms and solving for x, eq. 9 gives

$$x = \frac{e^{k(a - b)t} - 1}{e^{k(a - b)t} - b/a} \times b \qquad (10)$$

Since optical density at 350 mμ is followed as a function of time, the concentration of reaction product at any time may be expressed by

$$x = b - (\text{O.D.}/\alpha) \qquad (11)$$

where α is a proportionality constant which converts optical density readings into the stoichiometric concentration of iodine at time t. The proportionality constant approaches the molar extinction coefficient of I_3^- at high iodide concentrations but is significantly less at lower concentrations of iodide and higher concentrations of Cl^-. Upon substituting $b - (\text{O.D.}/\alpha)$ for x and rearranging terms, eq. 11 gives

$$\text{O.D.} = \alpha \left\{ b - \frac{(e^{k(a - b)t} - 1)}{(e^{k(a - b)t} - b/a)} \times b \right\} \qquad (12)$$

If we set y equal to

$$b - \frac{(e^{k(a - b)t} - 1)}{(e^{k(a - b)t} - b/a)} \times b$$

then eq. 12 may be expressed by

$$\text{O.D.} = \alpha y \qquad (13)$$

The kinetic data have been fitted to eq. 12 by a least-squares method using an existing digital computer program.[17] Twenty to forty data points were entered for each kinetic run. Values for k and for α and their uncertainties were calculated by the computer.[18]

Product Identification.—In order to ascertain the extent of diiodination in the iodination reactions of N-acTY, spectral analysis of the reactions were performed following kinetic runs at pH 5.80 and 9.20. Modifications of previously described methods[19] were employed. The reactions were followed spectrophotometrically as before but were terminated by the addition of a 50-fold excess of sodium thiosulfate. The pH 5.80 reactions were titrated to pH 10.22 with sodium hydroxide for spectral analysis, while the pH 9.20 reactions were analyzed at that pH. Optical density readings were taken at 315, 325, 330, and 340 mμ. Since the values at 325 through 340 mμ were small relative to that at 315 mμ, the former group of values were added. The resulting composite value and the value at 315 mμ were used to solve for the concentrations of N-acMIT and N-acDIT by solution of simultaneous equations.

Results

Routine Computer Solutions.—Except for considerable statistical data, information obtained on a typical run for iodination of N-acTY is listed in Table I.

TABLE I

COMPUTER "PRINT-OUT" FOR IODINATION OF N-ACTY[a]

Time, sec.	$y \times 10^4$	Optical density—Calcd.	Optical density—Obsd.	O.D. calcd./O.D. obsd.
28	0.4429	1.006	1.000	1.006
35	.4303	0.977	0.980	0.997
39	.4234	.961	.960	1.001
44	.4148	.942	.940	1.002
49	.4065	.923	.920	1.003
55	.3968	.901	.900	1.001
61	.3874	.880	.880	1.000
67	.3782	.859	.860	0.999
74	.3678	.835	.840	.994
79	.3606	.819	.820	.999
85	.3522	.800	.800	1.000
92	.3427	.778	.780	0.998
99	.3335	.757	.760	.996
106	.3245	.737	.740	.996
113	.3159	.717	.720	.996
120	.3075	.698	.700	.997
127	.2994	.680	.680	1.000
135	.2904	.659	.660	0.999
142	.2828	.642	.640	1.003
151	.2733	.621	.620	1.001
160	.2643	.600	.600	1.000
165	.2594	.589	.590	0.998
169	.2555	.580	.580	1.000
178	.2471	.561	.560	1.002
188	.2382	.541	.540	1.002
197	.2304	.523	.520	1.006
207	.2221	.504	.500	1.009

[a] α = 22707 ± 323; k = 21.183 ± 0.081.

The reaction was performed in pH 7.20 phosphate buffer (HPO_4^{-2}, 0.096 M; $H_2PO_4^-$, 0.024 M) at 0.64 total ionic strength with 0.015 M iodide and with 5×10^{-5} and 2×10^{-4} M concentrations of iodine and

(15) The wave length of maximum absorption for I_3^- is reported as 352 mμ by T. L. Allen and R. M. Keefer, *J. Am. Chem. Soc.*, **77**, 2957 (1955), and 353 mμ by A. D. Awtrey and R. E. Connick, *ibid.*, **73**, 1842 (1951). Both list the ϵ as 26.4×10^4. In the present experiments, 350 mμ was used for convenience and reproducibility of the instrument setting.

(16) J. L. Crammer and A. Neuberger, *Biochem. J.*, **37**, 302 (1943); C. Tanford and G. L. Roberts, *J. Am. Chem. Soc.*, **74**, 2509 (1952).

(17) 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) IBM 7094.

(19) H. Edelhoch, *J. Biol. Chem.*, **237**, 2778 (1962).

tyrosine derivatives. The fit of data for iodination of N-acMIT is comparable and equally good.

Apparent pK Values.—The acetylated tyrosine derivatives were found to have ultraviolet absorption maxima, Table II, at the same wave length as the unacetylated derivatives.[19] The molar extinction coefficients also closely paralleled those of tyrosine, monoiodotyrosine, and diiodotyrosine but were slightly less in each instance. In addition, the isosbestic points, 289 mμ, are the same for N-acMIT and N-acDIT as for monoiodotyrosine and diiodotyrosine. The wave length chosen for spectrophotometric titration was that of the maximum absorption of each in alkali. The pK_a for N-acDIT, 6.83, is near that of 6.95 found for the same compound by Pitt-Rivers[14] at 23° using a potentiometric titration method. Values for N-acTY and N-acMIT could not be found in the literature, but the value for each acetylated derivative again closely parallels that for tyrosine, monoiodotyrosine, and diiodotyrosine.

TABLE II

MOLAR EXTINCTION COEFFICIENTS, ABSORPTION MAXIMA, AND APPARENT HYDROXYL IONIZATION CONSTANTS OF ACETYLATED TYROSINE DERIVATIVES AT 25°

	Acid		Alkali		
	$\epsilon \times 10^{-3}$	λ	$\epsilon \times 10^{-3}$	λ	pK'
N-acTY	1.35	275	2.30	293	10.04
N-acMIT	2.54	283	4.00	305	8.58
N-acDIT	2.50	287	5.52	311	6.83

Reaction Order.—As is seen in Table I, the data for both iodination reactions fit the bimolecular second-order rate equation. Table III lends support to this fact for, with a fourfold variation in either tyrosine derivative, the value of k for each reaction remains relatively constant.

TABLE III

EFFECT OF VARYING INITIAL CONCENTRATION OF TYROSINE DERIVATIVE UPON REACTION RATE CONSTANT

Na_2HPO_4, 0.160 M; NaH_2PO_4, 0.040 M; μ 0.64; I_2, 5×10^{-5} M; KI, 0.015 M; pH 7.20

10^5·[N-acTY], moles/l.	$k_{N\text{-acTY}}$ ± S.D. l./mole/sec.	10^5·[N-acMIT] moles/l.	$k_{N\text{-acMIT}}$ ± S.D. l./mole/sec.
5.0	32.50 ± 0.46	5.0	26.14 ± 0.21
	32.94 ± .41		25.50 ± .17
10.0	29.31 ± .27	10.0	21.98 ± .48
	30.40 ± .21		22.64 ± .49
20.0	31.38 ± .31	20.0	25.93 ± .43
	30.13 ± .39		25.15 ± .39

Similarly, when the tyrosine derivative and iodide concentrations are kept constant, a threefold variation in initial iodine concentration again results in fairly constant values for k, Table IV.

TABLE IV

EFFECT OF VARYING INITIAL CONCENTRATIONS OF IODINE UPON REACTION RATE CONSTANT

Na_2HPO_4, 0.160 M; NaH_2PO_4, 0.040 M; μ 0.64; KI, 0.015 M; tyrosine derivative, 10^{-4} M; pH 7.20

10^5·[I_2], moles/l.	$k_{N\text{-acTY}}$ ± S.D., l./mole/sec.	$k_{N\text{-acMIT}}$ ± S.D., l./mole/sec.
2.5	30.71 ± 0.27	25.68 ± 0.40
	30.13 ± .29	25.64 ± .39
4.0	29.31 ± .27	21.98 ± .39
	30.40 ± .21	22.64 ± .43
7.5	32.75 ± .42	25.54 ± .12
	33.45 ± .44	24.43 ± .10

Iodide Effect.—The effect of varying iodide concentrations at constant pH and buffer concentration is shown in Table V for iodination of N-acTY and N-acMIT.

TABLE V

EFFECT OF VARYING CONCENTRATION OF IODIDE UPON REACTION RATE CONSTANT OF IODINATION

Na_2HPO_4, 0.160 M; NaH_2PO_4, 0.04 M; μ 0.64; pH 7.20

10^5·[I_2], mole/l.	10^2·[KI], mole/l.	k, ± S.D. l./mole/sec.	$k_{obsd}\beta^a$ l./mole/sec.
		N-acTY, 10^{-4} M	
2.50	0.75	114.18 ± 1.35	5.94
		111.42 ± 1.20	5.79
5.00	1.50	29.31 ± 0.27	5.57
		30.40 ± .21	5.78
7.50	2.25	12.88 ± .10	5.35
		12.88 ± .06	5.35
		N-acMIT, 10^{-4} M	
2.50	0.75	79.97 ± 0.60	4.16
		82.93 ± .58	4.31
5.00	1.50	21.98 ± .48	4.18
		22.64 ± .49	4.30
7.50	2.25	9.70 ± .04	4.03
		9.73 ± .03	4.04

[a] $\beta = \dfrac{(K_1 + [I^-] + K_1/K_2[Cl^-])\,[I^-]}{K_1}$.

Varying concentrations of iodide have a profound effect upon both iodination reactions. The effect upon the rate constant roughly is proportional to the inverse square of iodide concentration. Certainly the iodide term must be considered in equilibrium K_1, but this pre-equilibrium could explain the rate's dependence only upon the first power of iodide concentration. Obviously, the iodide concentration term is a factor in another of the equilibria concerned with the iodination reaction. Taking equilibria K_1 and K_2 into consideration and iodide concentration in a second reaction without implying which one, the observed rate constant according to steady-state approximation may be expressed by

$$k_{obsd} = \frac{kK_1}{(K_1 + [I^-] + K_1/K_2[Cl^-])[I^-]} \quad (14)$$

Although this relationship can be only an approximation, it should produce an invariant k with varying iodide concentration so long as iodide concentration is considerably larger than the second or unknown iodide equilibrium. That such is likely the case may be seen in the constancy of the values, $k_{obsd}\beta$, in the fourth column of Table V.

pH Effect.—With consideration for effects of buffer concentration, the effect of pH on the two reactions was compared. These results are listed in Table VI. Each value represents the mean of duplicate experiments.

The observed rate constants have been corrected for iodide concentration as noted previously. The most obvious fact about the values, irrespective of individual buffer concentrations, is that the rates increase rapidly with increasing pH over the range 5.40 to 9.80 which represents a 2.5×10^{-4} decrease in hydrogen ion concentration. This is also the approximate increase noted in the N-acTY iodination rates, but the increase in N-acMIT iodination rates is smaller by a factor of 10. This fact is reflected in the last column of Table VI in that the ratio of the rate constants increases

TABLE VI

COMPARISON OF THE EFFECTS OF pH ON IODINATION RATE CONSTANTS

N-acTY or N-acMIT, 2×10^{-4} *M*; I_2, 5×10^{-5} *M*; μ 0.64

Buffer	pH	[KI]	N-acTY $k_{obsd}\beta$,[a] l./mole/sec.	N-acMIT $k_{obsd}\gamma$,[a] l./mole/sec.	$k_{N\text{-acTY}}/k_{N\text{-acMIT}}$
Acetate	5.40[b]	0.0033	.096 ± 0.0007	0.105 ± 0.0005	0.91
Phosphate	6.80	.0150	2.098 ± .008	1.766 ± .008	1.19
Phosphate	7.80	.0200	22.79 ± .15	14.97 ± .07	1.52
Barbital	8.80	.1000	250.9 ± 1.1	119.4 ± 1.3	2.10
Carbonate	9.20	.1670	408.7 ± 1.1	92.36 ± 0.43	4.42
Carbonate	9.80	.1670	1869. ± 5.6	193.6 ± 3.2	9.65

[a] $\beta = \dfrac{(K_1 + [I^-] + K_1/K_2\,[Cl^-])\,[I^-]}{K_1}$. [b] Tyrosyl derivatives were 4×10^{-4} *M* in these runs.

rather markedly with decreasing hydrogen ion concentration. Since there is formation of iodate at pH 10 and above during the usual time limits for a kinetic run, comparison of the two reactions above this pH are not reported, although the ratio of the two reactions continues to increase. The observed rate constants have been based on the initial concentrations of the sum of the phenoxide and nonionized phenolic fractions. Since iodination of phenolic compounds most probably occurs by way of the phenolate ion,[20] a comparison of the pH effects on this basis may explain the increasing ratio of N-acTY to N-acMIT rate constants with increasing pH. Including the iodide ion correction of eq. 14, the phenolate ion term in the observed rate constant should lead to

$$k_{obsd} = \frac{kK_1K_6}{(K_1 + [I^-] + K_1/K_2[Cl^-])(K_6 + [H^+])[I^-]} \quad (15)$$

As can be seen in eq. 15, the observed rate constant for N-acTY might be expected to correlate well with an inverse hydrogen ion term, since K_6, 9.12×10^{-11}, is small compared to the hydrogen ion concentrations. However, K_6 for N-acMIT, 2.63×10^{-9}, becomes a significant factor in the correlation of the observed rate constants of N-acMIT.

The data in Table VI have been recalculated on the basis of eq. 15 and are listed in Table VII. The ratio $k_{N\text{-acTY}}/k_{N\text{-acMIT}}$ following correction of the rate constants for phenoxide ion in Table VII remains reasonably constant. The ratio at pH 7.80 was rechecked several times with similar results. Reasons for this variation along with that at pH 6.80 will be discussed subsequently.

TABLE VII

COMPARISON OF EFFECTS OF pH ON CORRECTED RATE CONSTANTS

Buffer	pH	$k_{obsd}\gamma$[a] N-acTY	$k_{obsd}\gamma$[a] N-acMIT	$k_{N\text{-acTY}}/k_{N\text{-acMIT}}$
Acetate	5.40	4186 ± 33	158.2 ± 0.75	26.46
Phosphate	6.80	3650 ± 13	107.9 ± .47	33.83
Phosphate	7.80	3979 ± 27	105.0 ± .51	37.89
Barbital	8.80	4616 ± 20	190.9 ± 2.0	24.18
Carbonate	9.20	3240 ± 9	114 5 ± 0.54	28.30
Carbonate	9.80	5121 ± 15	204.8 ± 3.4	25.00

[a] $\gamma = \dfrac{(K_1 + [I^-] + K_1/K_2[Cl^-])(K_6 + [H^+])[I^-]}{K_1K_6}$

Reaction Products.—In the kinetic runs performed at pH 5.80 and subsequently titrated to pH 10.22, the optical densities at 315 mμ have been corrected for the N-acTY present. The ϵ for N-acTY in 0.1 *M* sodium hydroxide at 315 mμ is only 13, but in these runs the concentration of N-acTY was relatively large. The correction was made on the assumption that the initial concentration of N-acTY was reduced by the amount of iodine lost within the time lapse of the kinetic run and on the basis of N-acTY being 60% ionized at pH 10.22. Table VIII shows the results of runs at the two pH's. It can be seen that small amounts of N-acDIT are formed but only when the reaction is run virtually to completion does this exceed a few per cent of the N-acMIT formed. In general, reactions were run only to 50% completion so that it is unlikely that N-acDIT formation is significant.

TABLE VIII

Reaction[a]	Time, sec.	Calcd.[b] $\times 10^5$ N-acMIT	Obsd. $\times 10^5$ N-acMIT	Obsd. $\times 10^5$ N-acDIT
I	228	3.24	2.98	0.03
I	701	5.12	4.76	.19
II	263	5.46	5.23	.07

[a] Reaction I: pH 5.80; phosphate buffer; I_2, 5.78×10^{-5} *M*; KI, 3.33×10^{-3} *M*; N-acTY, 4×10^{-4} *M*. Reaction II: pH 9.20; carbonate buffer; I_2, 5.78×10^{-5} *M*; KI, 0.1033*M*; N-acTY, 2×10^{-4} *M*. [b] Calculated on basis that all I_2 consumed resulted uniquely in the formation of N-acMIT.

Salt Effect.—The effect of ionic strength, μ, upon the reaction rate constants for each iodination reaction was investigated at three hydrogen ion concentrations. The results are listed in Table IX. At each pH the concentration of the buffer pair was kept constant, and the total ionic strength of the medium was varied by addition of sodium chloride. With the higher concentrations of added salt, it was necessary to add sodium hydroxide to maintain the desired pH.

Statistical information does not accompany the rate constants, since these determinations were made by a graphical method of calculation before inception of the computer program. At pH 7.11, no significant change in the rate constant for either reaction occurred. However, at pH 7.90 and 7.45, acceleration in the velocity of both reactions occurred. When log *k* was plotted against $\mu^{1/2}$ for the latter two pH series, straight line plots were realized for both reactions at pH 7.90 and 9.45. The slopes of the plots for each reaction are approximately the same, *i.e.*, +1 at pH 9.45 and +0.5 at pH 7.90.

TABLE IX

INFLUENCE OF IONIC STRENGTH UPON REACTION RATE CONSTANTS

Buffer	pH	μ	[KI]	N-acTY $k_{obsd}\beta$,[a] l./mole/sec.	N-acMIT $k_{obsd}\beta$,[a] l./mole/sec.
Phosphate	7.11	0.4	0.017	1.96	1.58
		.4	.017	1.89	1.52
		.5	.017	1.91	1.58
	7.90	.3	.017	20.28	11.70
		.4	.017	23.27	12.69
		.5	.017	25.05	14.00
Bicarbonate	9.45	.4	.250	337.3	66.70
		.5	.250	425.6	80.76
		.6	.250	479.2	92.93

[a] $\beta = \dfrac{(K_1 + [I^-] + K_1/K_2[Cl^-])}{K_1}$.

(20) E. Berliner, *J. Am. Chem. Soc.*, **73**, 4307 (1951); B. S. Painter and F. G. Soper, *J. Chem. Soc.*, 342 (1947).

Discussion

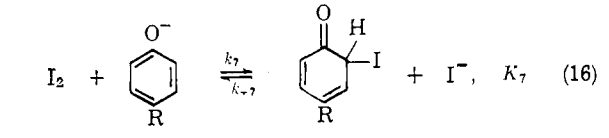
The kinetic data for the iodination of acetyltyrosine and acetylmonoiodotyrosine do not support the concept that the formation of monoiodotyrosine is the rate-limiting step in diiodination. Quite clearly, iodination of N-acTY is faster than iodination of N-acMIT at all hydrogen ion concentrations above pH 5.40. The diminished reactivity of the monoiodinated compound toward further iodination is supported in principle by the success of preparative methods of monoiodinated compounds.[21] Moreover, the electrophilic character of the substituted iodine atom with a Hammett *meta* σ-constant of $+0.352$[22] is such that iodination on the second position *ortho* to the hydroxyl group and *meta* to the first iodine atom should be more difficult. This concept is analogous to the assumption[23] that the inductive effect is responsible for the lowering of the pK of the hydroxyl group of phenolic compounds by approximately two units for each iodine atom added to the ring (Table II).

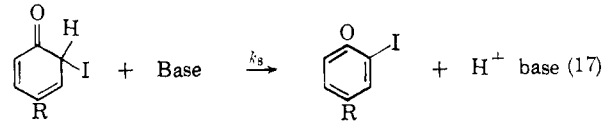
In the present studies, the identification of products by spectral analysis confirms the fact that little diiodination occurs under the conditions of an excess of tyrosine. In Li's studies, the iodine concentration exceeded that of tyrosine by a factor of two. Under those conditions, there undoubtedly would be more diiodination than in the present studies. The possibility that the differences in absolute iodine concentrations in our studies and in Li's[2] or that differences in mechanisms of iodination of tyrosine and N-acTY would explain the divergent views seems unlikely. Irrespective of these aspects, the concept that monoiodination is the rate-determining step in iodinating phenols cannot be reconciled with existing evidence[3] or the present data.

The data obtained by varying the concentration of hydrogen ion suggest for both N-acTY and N-acMIT that phenoxide ion is the species undergoing iodination. The ratio $k_{\text{N-acTY}}$ to $k_{\text{N-acMIT}}$ remains relatively constant at about 30 when the rates are based on initial concentration of phenoxide ion for each reaction. There is significant variation in this ratio at pH 7.80 and 6.80. The fact that these variations occur at hydrogen ion concentrations approaching the pK_a of N-acMIT may be significant. A slight deviation in the pK_a found during spectrophotometric titration and that existing in a different buffer during a kinetic run would result in a sizable discrepancy in the rate constant correction for phenoxide ion. There are factors other than concentration of phenoxide ion concentration that enter into the observed rate constants; for, after both iodide and phenoxide corrections in Table VII, there remain differences in the rate constants for each reaction too great to be experimental error. The concentrations of the buffer salts are important,[20] and the results of an extensive study of these variables will be the subject of a subsequent report. Therefore, a discussion of the catalytic effects of buffers will be postponed.

The rate dependence upon the concentration of phenoxide ion in the present study is the same as that found by Buss and Taylor[24] for iodination of 2,4-dichlorophenol. Berliner[20] found an inverse hydrogen ion dependence for the iodination of phenol, while Zeltman and Kahn[25] found the same dependence for the exchange reaction between molecular iodine and diiodotyrosine. However, Painter and Soper[20] found an inverse hydrogen ion dependence greater than the first power and less than the second power of hydrogen ion concentration for the iodination of phenol. All the latter studies were performed in the acid pH range, and their findings can be reconciled with phenoxide ion as the reactive species.

The dependence of the velocity of iodination upon the inverse square of iodide concentration has been reported for the iodination of several aromatic compounds.[20,24-26] This is essentially the relationship found in the iodination of N-acTY and of N-acMIT, for equilibrium K_1 must be involved in any iodination reaction in the presence of iodine and iodide. However, the iodide dependency term does not preclude iodination by either H_2OI^+ or HOI, since a second iodide term would be involved kinetically with either species. Grovenstein and associates[27] have also pointed out that the available results do not exclude a rapid and reversible attack of molecular iodine upon the phenoxide ion followed by a slow loss of proton to the solvent or buffer base. A fourfold isotope effect (k_H/k_D) in the iodination of deuterated derivatives of phenol and 4-nitrophenol iodination was demonstrated. The conclusion[27] was reached that iodination proceeded through a quinoid intermediate which was in equilibrium with the reactants. Such a mechanism, excluding Cl^- from the system, may be represented by eq. 1, 6, 16, and 17 for iodination of N-acTY.





The kinetic equation representing this mechanism may be expressed as in eq. 18, and the dependence of the observed rate constant upon the inverse square of iodide concentration is evident. However, in so far

$$k_{obsd} = \frac{k_8 K_1 K_6 K_7}{(K_1 + [I^-])(K_6 + [H^+])(K_7 + [I^-])} \quad (18)$$

as the rate dependency upon the iodide concentration term is concerned, iodination by either hypoiodous acidinium ion or by hypoiodous acid would explain the present kinetic results equally well. However, hypoiodous acid is ruled out by the hydrogen ion dependence, unless iodination occurs on the nonionized tyrosyl derivative. This seems unlikely.[20]

The effects of salt upon iodination of phenolic com-

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(23) A. E. Remick, "Electronic Interpretations of Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 14.

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(25) A. H. Zeltman and M. Kahn, *ibid.*, **76**, 1554 (1954).

(26) E. Berliner, *ibid.*, **72**, 4003 (1950); C. Bostic, Q. Fernando, and H. Freisen, *Inorg. Chem.*, **2**, 232 (1963).

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

pounds have been reported with varying results.[2,20] In the iodination of N-acTY and N-acMIT, significant salt effects are not observed at pH 7.11 and are small at pH 7.90 and 9.45. These results and the variance of the previous reports suggest that the salt effects are secondary. The fact that the effects on N-acTY and N-acMIT are of a similar magnitude further suggests that the equilibria displaced by the salts may well be on the buffer constituents rather than the reactants themselves. According to Debye–Hückel theory for dilute soutions, the slope of a plot of log k against $\mu^{1/2}$ depends in part on the product of the ionic charge of the reactants. This seems quite unlikely for more concentrated solutions.

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The Synthesis and Oxidative Rearrangement of Some 1,4-Thiazepines Related to the Penicillins[1-3]

BY NELSON J. LEONARD AND G. EDWIN WILSON, JR.[4]

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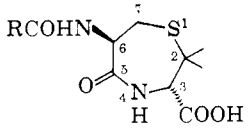
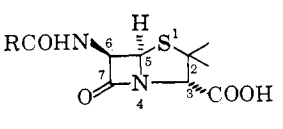
We have studied the synthesis, stereochemistry, and oxidative rearrangement of some 1,4-thiazepines related to the penicillins. 3D-Carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (XIII) was formed by the reaction of α-phenylacetamidoacrylic acid and D-penicillamine methyl ester hydrochloride in acetonitrile in the presence of dicyclohexylcarbodiimide and triethylamine, but none of the 3D-6L-diastereomer was isolated. Proof of the induced configuration 6D was provided by desulfurization of XIII with Raney nickel to give methyl N-(N'-phenylacetyl-D-alanyl)-D-valinate. The stereoisomer synthesized (XIII) is the thermodynamically more stable form. The oxidation of XIII by means of chlorine produced 3D-carbomethoxy-2,2-dimethyl-5-oxo-6-phenylacetamido-2,3,4,5-tetrahydro-1,4-thiazepine (XVI) and two isomeric 3-isothiazolones, methyl α-D-isopropenyl-4-phenylacetamido-3-isothiazolone-2-acetate (XXI) and methyl α-isopropylidene-4-phenylacetamido-3-isothiazolone-2-acetate (XXII). The oxidative formation of 3-isothiazolones was shown to have some generality. Photoreduction of XVI in ethyl mercaptan solution at 55° regenerated 3D-carbomethoxy-2,2-dimethyl-5-oxo-6D-phenylacetamidoperhydro-1,4-thiazepine (XIII) while photoadducts of XVI were formed at 20° with ethanol, 2-propanol, and ethyl mercaptan (XVII–XX).

While an advanced stage has been reached in the elucidation of the intermediates involved in the pathway of penicillin biosynthesis,[5,6] including the isolation of the tripeptide γ-(α-aminoadipyl)cyst(e)inylvaline from the mycelium of *Penicillium chrysogenum*, the place in the sequence where oxidative condensation between the β-position of the cyst(e)ine moiety and the peptide nitrogen atom occurs has not been determined. We considered that the earlier suggestions of the transannular formation of the C–N bond across a substituted 1,4-thiazepine derivative (I → II)[7-9] had not as yet received a definitive test, and we were intrigued with the possibility of effecting a transannular synthesis of the bicyclic system present in penicillin. The requirement that the configurations at C-6 and C-3 of II be fixed before creation of the bicyclic ring system is implicit in the findings of Arnstein and his co-workers,[5,10] so that, at least in the biosynthetic conversion to penicillin, 3D-carboxy-2,2-dimethyl-5-oxo-6L-phenylacetamidoperhydro-1,4-thiazepine (I, R = $C_6H_5CH_2$) would be the desired stereomer to test, with the added provisions that it could serve as a competitive precursor and that the cells would be permeable to the compound.



I



II

The 1,4-thiazepines related to the penicillins have received relatively little attention, and the stereochemistry of these compounds has not been investigated. Previous syntheses leading to 3-carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (III)[11-13] and its closely related derivatives[9,14] have utilized open-chain starting materials and have generally employed a ring-closure step involving either addition of a thiol to an acrylate derivative or the formation of the amide C–N bond. Thus, 3-carbomethoxy-7-chloro-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (IV) and 3-carbomethoxy-2,2-dimethyl-5-oxo-2,3,4,5-tetrahydro-1,4-thiazepine (VIII) were reported as products from condensations of

(1) This work was supported in part by a research grant (USPHS-RG 5829, currently GM-05829-06) from the National Institutes of Health, U. S. Public Health Service.

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(5) For lead references, see (a) H. R. V. Arnstein, M. Artman, D. Morris, and E. J. Toms, *Biochem. J.*, **76**, 353 (1960); (b) H. R. V. Arnstein and D. Morris, *ibid.*, **76**, 357 (1960); (d) H. R. V. Arnstein, D. Morris, and E. J. Toms, *Biochim. Biophys. Acta*, **35**, 561 (1959); (e) H. R. V. Arnstein and D. Morris, *Biochem. J.*, **71**, 8P (1959); (f) F. R. Batchelor, F. P. Doyle, J. H. C. Nayler, and G. N. Rolinson, *Nature*, **183**, 257 (1959).

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(11) T. Wieland, G. Ohnacker, and W. Ziegler, *Chem. Ber.*, **90**, 194 (1957).

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(13) I. L. Knunyants and M. G. Lin'kova, *ibid.*, 62 (1955); *Chem. Abstr.*, **50**, 1592 (1956).

(14) I. L. Knunyants, O. V. Kil'disheva, M. P., Krasuskaya, M. G. Lin'kova, V. V. Shokina, Z. V. Benevolenskaya, and L. P. Rasteikene, *ibid.*, 1777 (1959); *Bull. Acad. Sci. U.S.S.R., Div. Chem. Sci.*, 1702 (1959); *Chem. Abstr.*, **54**, 8843 (1960).