appeared in their effect on the physiological function of nitrogen fixation in *Azotobacter*. It is concluded, therefore, that an explanation based on the relative physical properties of hydrogen and nitrogen is unlikely.

Nitrous oxide was found to be a specific inhibitor for nitrogen fixation by *Azotobacter*. Its inhibition may have important implications for the mechanism of the reaction.

MADISON 6, WISCONSIN RECEIVED DECEMBER 9, 1947

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

Kinetics of the Reactions between Iodine and Certain Substituted Phenols

BY CHOH HAO LI

In previous studies[1] it was shown that the reaction of tyrosine with iodine follows a bimolecular rate law and that the most reactive iodinating agent is hypoiodous acid. It was also demonstrated[2] that the formation of diiodotyrosine is catalyzed by phosphate and other basic ions. The present investigation extends such studies with other para substituted phenols.

Reactions were carried out at 25° in acetate buffers of *p*H 5.23 and 5.65 containing different iodide ion concentrations. *p*-Chlorophenol and *p*-hydroxyphenylethylamine (tyramine), C. P. crystalline preparations, were employed without further purification; glycyl-tyrosine was kindly supplied by Dr. J. S. Fruton and the late Dr. Max Bergmann. The reaction rates were followed in the manner previously[1] described. The rate law was found to be identical with that for the formation of diiodotyrosine and may be expressed by the equation

$$-\,\mathrm{d(Phenol)}/\mathrm{d}t = k_2(\mathrm{phenol})(\mathrm{I_3^-}) \qquad (1)$$

where k_2 is the specific rate constant for the reaction Phenol + $2I_2 \rightarrow$ diiodophenol + $2H^+$ + $2I^-$

Table I summarizes the specific rate constants for the formation of diiodophenols[3]; the values for the reaction between iodine and tyrosine were estimated from previous studies.[1] The concentrations are in moles per liter, and time in minutes. It may be noted that the product of $k_2(I^-)^2$ in each buffer appears to be rather constant at the range of iodide concentration studied. In concentrations of iodide beyond the range studied, $k_2(I^-)^2$ was not found to be constant.

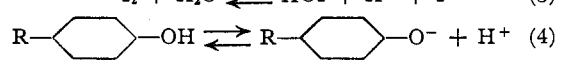
In the study of diiodotyrosine formation,[1] it was found that the reaction involves four paths: namely, iodine and phenol, iodine and phenolate, hypoiodous acid and phenol, and hypoiodous acid and phenolate. It was further noted that the most reactive pair is hypoiodous acid and phenolate, whereas the reaction between iodine and phenol is the least reactive. For first approximations, the reaction between iodine and phenol may be represented by equation (1a).

$$-\mathrm{d(phenol)}/\mathrm{d}t = k'(\mathrm{phenol})(\mathrm{HOI}) + k''(\mathrm{phenolate})(\mathrm{HOI}) \qquad (1a)$$

From the equilibria

$$\mathrm{I^-_3} \rightleftarrows \mathrm{I_2} + \mathrm{I^-} \qquad (2)$$

$$\mathrm{I_2} + \mathrm{H_2O} \rightleftarrows \mathrm{HOI} + \mathrm{H^+} + \mathrm{I^-} \qquad (3)$$



(4)

TABLE I

SPECIFIC REACTION RATE, k_2, OF IODINATING *p*-CHLOROPHENOL, GLYCYLTYROSINE, TYRAMINE AND TYROSINE[a] IN ACETATE BUFFERS OF *p*H 5.23 AND *p*H 5.65 CONTAINING DIFFERENT IODIDE CONCENTRATION AT 25°

k_2 in gram moles per liter per minute

(I^-) $m \times 10^2$	*p*-Chlorophenol		Tyramine		Glycyl-tyrosine		Tyrosine	
	*p*H 5.23	*p*H 5.65	*p*H 5.23	*p*H 5.65	*p*H 5.23	*p*H 5.65	*p*H 5.23	*p*H 5.65
3.34	0.135(0.128)[b]	0.40(0.34)	0.70(0.72)	2.03(2.29)	0.91(0.94)	3.15(3.21)	0.52(0.53)	1.82(1.94)
4.08	.081(.085)	.26(.26)	.45(.45)	1.63(1.49)	.63(.63)	2.16(2.14)	.38(.36)	1.20(1.30)
4.84	.060(.061)	.18(.18)	.35(.38)	1.09(1.07)	.45(.49)	1.55(1.55)	.28(.26)	0.90(0.93)
6.34	.033(.035)	.11(.11)	.20(.22)	0.63(0.62)			.16(.15)	0.53(0.54)

[a] Reaction rates for tyrosine are from a previous paper (see ref. 1). [b] The figures in parentheses are computed values from Equations 7, 8, 9 and 10.

(1) Li, THIS JOURNAL, **64**, 1147 (1942).

(2) Li, *ibid.*, **66**, 228 (1944).

(3) The preparation of N-glycyl-3,5-diiodotyrosine has been reported by Abderhalden and Guggenheim [*Ber.*, **41**, 1241 (1908)]. Diiodotyramine has also been prepared by Abderhalden and his coworkers [*Arch. ges. Physiol.*, **195**, 167 (1922)]. As far as we are aware there is no report concerning the preparation of *p*-chloro-3,5-diiodophenol. In the present experiments we have observed that *p*-chloro-3,5-diiodophenol is very insoluble in acetate buffers and it is gradually crystallized out in fine needles as the reaction proceeds. The colorless crystals have a melting point at 108.5°. *Anal.* Calcd. for $HOC_6H_2I_2Cl$: I, 66.74. Found: I, 66.82.

Equation (1a) becomes

$$-\frac{\mathrm{d(phenol)}}{\mathrm{d}t} = \frac{K_2}{(\mathrm{I^-})^2(\mathrm{H^+})}\left[k'K_3 + \frac{k''K_3K_4}{(\mathrm{H^+})}\right](\mathrm{phenol})(\mathrm{I_3^-}) \qquad (5)$$

where K_2, K_3 and K_4 are the equilibrium constants of equations 2, 3, and 4, respectively. Thus, by comparing the equations (1) and (5), the observed specific rate constant, k_2, is a function of iodide and

hydrogen ion concentration as shown by the expression

$$k_2 = \frac{K_2}{(I^-)^2(H^+)}\left[k'K_3 + \frac{k''K_3K_4}{(H^+)}\right] \quad (6)$$

It is obvious from equation (6) that when the hydrogen-ion concentration is maintained unchanged, the product of $k_2(I^-)^2$ becomes a constant. Since the equilibrium constants[4] of equations (2) and (3) and the dissociation constants of *p*-chlorophenol,[5] tyramine,[6] glycyltyrosine[7] and tyrosine[8] are known, the specific rate constants k' and k'' can be computed from the data in Table I. The results are summarized in Table II. Therefore, in a solution of known hydrogen-ion and iodide-ion concentrations, the observed specific rates for iodinating *p*-chlorophenol, tyrosine, tyramine and glycyltyrosine may be computed from the equations

TABLE II

THE COMPUTED VALUES OF k' AND k'' FOR *p*-CHLOROPHENOL, TYRAMINE, TYROSINE AND GLYCYLTYROSINE IN ACETATE BUFFER AT 25°

Phenols	Dissociation constant K (10^{11})	$k' \times (10^{-6})$	$k'' \times (10^{-10})$
p-Chlorophenol	66.0 (5)[a]	1.7	0.2
Tyrosine	8.5 (8)	8.1	1.1
Glycyltyrosine	4.0 (7)	12.0	40.0
Tyramine	1.26 (6)	10.0	58.0

[a] Numbers in parentheses refer to the reference in the text.

p-Chlorophenol:

$$k_2 = \frac{1}{(I^-)^2(H^+)}\left[7.2 \times 10^{-10} + \frac{5.5 \times 10^{-16}}{(H^+)}\right] \quad (7)$$

Tyrosine:

$$k_2 = \frac{1}{(I^-)^2(H^+)}\left[3.4 \times 10^{-9} + \frac{4.0 \times 10^{-16}}{(H^+)}\right] \quad (8)$$

Tyramine:

$$k_2 = \frac{1}{(I^-)^2(H^+)}\left[4.2 \times 10^{-9} + \frac{3.06 \times 10^{-15}}{(H^+)}\right] \quad (9)$$

Glycyltyrosine:

$$k_2 = \frac{1}{(I^-)^2(H^+)}\left[5.0 \times 10^{-9} + \frac{6.7 \times 10^{-15}}{(H^+)}\right] \quad (10)$$

The computed specific rates are listed in Table I; it may be noted that the agreements between the calculated and observed values are satisfactory.

From Table II, it is evident that, in each phenol studied, the value of k' is always much smaller than that of k''. This indicates that the phenolate ion is far more reactive than the undissociated phenol. It is also clear that the smaller is the dissociation constant of a phenol, the faster is the reaction rate. For instance, the dissociation constant of *p*-chlorophenol is fifty times higher than that of tyramine and yet the latter reacts with hypoiodous acid much faster than *p*-chlorophenol.

It is of interest to note that the specific rate for glycyltyrosine is greater than the value for tyrosine. It appears to indicate that the presence of a peptide linkage enhances the rate of iodination. This inference may also be arrived at by an experiment using carbobenzoxyglutamyltyrosine. In acetate buffer of *p*H 5.65 containing an iodide concentration of 3.34×10^{-2} *m* at 25°, the biomolecular specific rate, k_2, of iodinating carbobenzoxyglutamyltyrosine was found to be 4.24 gram-mols per liter per minute which is more than twice the specific rate for tyrosine. Whether the conclusion can be generalized for other peptides requires further investigation.

The absence of the carboxyl group in tyrosine causes an increase in iodination rate. Thus, k'' for tyramine is about 50 times larger than that for tyrosine. On the other hand, a replacement of $-CH_2CH_2NH_2$ group in tyramine by —Cl radical greatly diminishes the specific rates of iodination. No satisfactory explanation can be offered at present to correlate the structure of substituents on the iodination rate of phenols.

Summary

The rate of the reactions between iodine and *p*-chlorophenol, tyramine and glycyltyrosine have been determined at 25° in acetate buffers. In the range of iodide concentrations studied, the rate can be represented by: rate = k' (phenol)(HOI) + k'' (phenolate)(HOI). The specific rate of the reaction between phenolate and hypoiodous acid decreases in the order: tyramine > glycyltyrosine > tyrosine > *p*-chlorophenol.

BERKELEY, CALIFORNIA RECEIVED MAY 29, 1947

(4) (a) Bray and MacKay, THIS JOURNAL, **32**, 914 (1910); (b) Bray, *ibid.*, **32**, 932 (1910).

(5) Murray and Gordon, *ibid.*, **57**, 110 (1935).

(6) Ogston, *J. Chem. Soc.*, 1713 (1936).

(7) Greenstein, *J. Biol. Chem.*, **95**, 485 (1932).

(8) Hitchcock, *J. Gen. Physiol.*, **6**, 747 (1925).