

small amount of hydrogen chloride, which was detected in the exhaust gas. This fact explains the elemental analyses for C, H and Cl as compared to the theoretical values for pure 1,1,1-trichloro-2-methyl-2-octene or its allylic isomer, 1,1,3-trichloro-2-methyl-1-octene: C, 47.21; H, 6.54; Cl, 46.37. On a representative number of fractions of the trichlorononenes, infrared spectra indicated the presence of 1,1,3-trichloro-2-methyl-1-octene as there were strong bands at 6.18 and 11.09  $\mu$  characteristic of  $-\text{C}(\text{CH}_3)=\text{CCl}_2$  since  $\text{CH}_2\text{ClC}(\text{CH}_3)=\text{CCl}_2$  has strong bands at 6.19 and 11.09  $\mu$ .

**Reaction of 1-Trichloromethyl-1-cyclohexanol with 95% Sulfuric Acid.**—Seventy-five grams of 1-trichloromethyl-1-cyclohexanol, prepared as above, m.p. 58–59°, was added slowly with stirring and external cooling to 32 ml. of concentrated sulfuric acid, keeping the reaction temperature at 20–40°. When hydrogen chloride substantially ceased to be evolved, the black viscous mixture was poured over ice and water, stirred, the organic layer extracted with carbon tetrachloride, washed with water many times, dried, and fractionated. Only 7 g. (less than 10% yield) of organic acids, b.p. 115–120° (0.1 mm.),  $n_D^{20}$  1.4751, was distillable and obtained. This small yield of acids, as a single fraction, was indicated by its infrared spectrum to be 52% 1-chlorocyclohexanecarboxylic acid and 48% 1-chlorocyclohexene-carboxylic acid, consistent with the 11.4% chlorine and 58.2% carbon contents found for this mixture. These indications from the infrared were based on a broad diffuse band typical of acids (2.8–4.0  $\mu$ ), the carbonyl bands for the carboxylic acid group at 5.86, 7.85 and 7.95  $\mu$ , and one component present to the extent of about 50% contained C=C

because of a very strong band at 6.09  $\mu$ . Bands for C–H at 3.4 and 3.5  $\mu$  were present and absorption at 13.5  $\mu$  is probably due to the carbon-chlorine stretch of the cyclic tertiary chlorine atom in 1-chlorocyclohexanecarboxylic acid. Practically all of the remainder of the products was a gummy polymer which was discarded; 9 g. of tan solid polymers of wide indefinite melting range was also obtained and discarded.

**Reaction of Other Trichloromethylcarbinols with Sulfuric Acid.**—(A) Ten grams of pure 1,1,1-trichloro-3-nitro-2-propanol was mixed with 10 ml. of 100% sulfuric acid and stirred continuously at 50–70° for 0.5 hour, then at 70–80° for 0.5 hour, then at 80–85° for 0.5 hour. No reaction occurred since no HCl was evolved. The reaction mixture was poured over crushed ice and worked up. No indications of formation of an acid chloride or of an organic acid appeared; the original 1,1,1-trichloro-3-nitro-2-propanol was recovered. (B) Five grams of pure 2,2,2-trichloroethanol was mixed with 5 ml. of 100% sulfuric acid and stirred at temperatures up to 80° for several hours; no HCl evolution occurred. The 2,2,2-trichloroethanol was recovered. (C) Twenty grams of 1,1,1-trichloro-2-phenyl-2-ethanol, b.p. 148–150° (16–17 mm.),  $n_D^{20}$  1.5673, was added to stirred 100% sulfuric acid and the temperature gradually increased up to 90° in order to obtain substantial HCl evolution. No organic acid or acid chloride was isolable. Instead, a reddish gum, which was not further investigated, was obtained.

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## Mechanism of the Peroxidatic Activity of Iron(III)-EDTA in the Catalyzed Reaction of Hydrogen Peroxide with *p*-Amino-*N,N*-diethylaniline

BY PAUL M. MADER

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The rate of disappearance of hydrogen peroxide in its reaction with *p*-amino-*N,N*-diethylaniline, catalyzed by the ferric iron-EDTA complex, has been measured polarographically in the pH range 9.67–10.54. The kinetic data are explained by assuming that the catalyst oxidizes the diamine and is rapidly regenerated by reaction of the reduced catalyst with hydrogen peroxide. The kinetic data show that the purple complex formed between hydrogen peroxide and ferric iron-EDTA is not an active catalyst for the diamine-peroxide reaction. The equilibrium constant for the reversible oxidation of the diamine by the catalyst has been determined at several pH values.

In a study of reactions occurring in an aerated photographic color developer containing EDTA [(ethylenedinitrilo)-tetraacetic acid], it was found that traces of iron salts cause the hydrogen peroxide, formed in the autoxidation of the developing agent, to react with the developing agent rather than with the sodium sulfite present in the developer.<sup>1</sup> This effect was shown to be due to catalysis of the reaction of hydrogen peroxide with the developing agent, *p*-amino-*N,N*-diethylaniline. There is little doubt that the active catalyst is the ferric iron-EDTA complex, since catalysis of comparable degree is not observed in the absence of EDTA. In this reaction, then, the complex acts as a peroxidase. The present paper reports the results of a study of the kinetics and mechanism of this catalyzed reaction.

In the kinetic experiments, the concentration of hydrogen peroxide was measured continuously using the polarograph. The reactions were carried out in the presence of 4-chloro-1-naphthol, which reacts rapidly with the oxidation product of *p*-amino-*N,N*-diethylaniline to give an insoluble indoaniline dye.<sup>2</sup> It was helpful to remove

the oxidation product, since it gives a polarographic current which interferes with the analysis for hydrogen peroxide, and since it apparently reacts further with the hydrogen peroxide, thus complicating interpretation of the rate data.

Carbonate buffers containing  $5.4 \times 10^{-3}$  *M* EDTA were used in all the experiments. The temperature was 25°, and the ionic strength was 0.38.

### Experimental

**Materials.**—All inorganic chemicals were reagent-grade (except  $\text{NaHSO}_3$  and  $\text{Na}_2\text{S}_2\text{O}_4$ ) and were obtained from the General Chemical Division of Allied Chemical and Dye Corp. The (ethylenedinitrilo)-tetraacetic acid tetrasodium salt and the reagent grade disodium salt were obtained from the Geigy Chemical Corp. Photographic grade *p*-amino-*N,N*-diethylaniline hydrochloride was recrystallized from ethanol. The 4-chloro-1-naphthol,<sup>3</sup> crystallized from ligroin, melted at 124–126°. Double-distilled water was used.

**Preparation of the Indoaniline Dye.**—A solution of 5 g. of  $\text{K}_3\text{Fe}(\text{CN})_6$  in 100 ml. of water was added rapidly to a solution consisting of 1.0 g. of *p*-amino-*N,N*-diethylaniline hydrochloride, 0.5 g. of 1-naphthol (1-naphthol gives the same dye as 4-chloro-1-naphthol<sup>3</sup>), 5 g. of  $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$ , 12 ml. of

(1) P. M. Mader, *Phot. Sci. and Eng.*, **3**, 49 (1959).

(2) L. K. J. Tong and M. Carol Giesmann, *THIS JOURNAL*, **79**, 583 (1957).

(3) H. Kast, *Ber.*, **44**, 1337 (1911).

methanol (the naphthol was first dissolved in the methanol) and 250 ml. of water. After 3 min., the crude dye was extracted into 200 ml. of benzene. The benzene phase, after being washed three times with 2%  $\text{NaH}_2\text{PO}_4$  solution and dried over  $\text{Na}_2\text{SO}_4$ , was run into a 4- $\times$  26-cm. chromatographic column of Florisil (Floridin Co.). The absorbed dye was washed on the column with 100 ml. of benzene and then eluted with benzene containing 2% acetone. Evaporation of the eluate gave 0.9 g. of crystalline dye ( $\epsilon_{585} = 1.59 \times 10^4$  in benzene). The dye was chromatographed a second time and finally crystallized from water-ethanol (1:4). There was no significant change in the extinction coefficient.

*Anal.* Calcd. for  $\text{C}_{20}\text{H}_{20}\text{O}_2\text{N}_2$ : C, 78.92; H, 6.62; N, 9.21. Found: C, 79.0; H, 6.7; N, 9.4.

**Preparation of Ferric Sodium (Ethylenedinitrilo)-tetraacetate.**—This salt was obtained by dissolving 0.046 mole of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and 0.049 mole of tetrasodium EDTA in 60 ml. of water at 80°. The product, which crystallized from the cooled solution, was washed with acetone, dried, and recrystallized from 60 ml. of water containing 1 g. of  $\text{NaNO}_3$ , 1 ml. of 0.3 *N*  $\text{HNO}_3$  and 0.1 g. of disodium EDTA. The crystals were washed with acetone, dried at 105°, and allowed to stand in moist air until they reached constant weight. The gain in weight indicated absorption of two moles of water per mole of salt.

*Anal.* Calcd. for  $\text{C}_{10}\text{H}_{12}\text{O}_8\text{N}_2\text{NaFe} \cdot x\text{H}_2\text{O}$ : Fe, 13.87 for  $x = 2$ ; 13.26 for  $x = 3$ . Found: Fe, 13.3.

**Stock Solutions.**—The carbonate buffer stock solutions were prepared at twice the concentration at which the buffers were used in the reaction mixtures. Disodium EDTA was included in these stock buffers at a level of 4 g./l.

A stock solution of Fe(III)-EDTA was prepared by dissolving 0.80 g. of tetrasodium EDTA and 0.48 g. of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 1 liter of water. *Anal.*: Fe, 0.100<sub>5</sub> g./l. This solution was used as the source of catalyst in all the *p*-amino-N,N-diethylaniline- $\text{H}_2\text{O}_2$  reaction-rate experiments.

**Analyses.**—The  $\text{H}_2\text{O}_2$  solution was analyzed iodometrically. Analyses for iron in the presence of EDTA were carried out as follows: Two ml. of 0.25% aqueous *o*-phenanthroline was introduced into a 25-ml. volumetric flask. There was added a measured quantity of sample (containing ca.  $10^{-4}$  g. of iron). The walls of the flask were washed down with water after each of these additions. After addition of a mixture of 0.01 g. of  $\text{Na}_2\text{S}_2\text{O}_4$ , 0.05 g. of  $\text{Na}_2\text{SO}_3$  and 0.05 g. of  $\text{NaHSO}_3$ , the solution was diluted to 25.0 ml. with water. (The  $\text{Na}_2\text{S}_2\text{O}_4$  quickly reduces the iron to the ferrous state, while the  $\text{Na}_2\text{SO}_3$  plus  $\text{NaHSO}_3$  prevents precipitation of sulfur and maintains a desirable pH.) The absorbance of the *o*-phenanthroline-Fe(II) complex was measured at 510  $m\mu$ . A correction of 0.003, necessitated by traces of impurity iron, was subtracted, and the iron concentration in the sample was calculated from the corrected absorbance. The analytical procedure was calibrated using reagent grade ferrous ammonium sulfate.

**Kinetic Runs.**—A mixture of stock buffer solution and water was swept with Linde pure nitrogen to remove oxygen. The 4-chloro-1-naphthol and then the *p*-amino-N,N-diethylaniline hydrochloride were dissolved, and, last, the nitrogen-swept Fe(III)-EDTA solution was added. Ten ml. of this mixture was transferred by pipet to the nitrogen-swept, thermostated ( $25 \pm 0.1^\circ$ ) polarographic cell. Reaction was started by adding a drop of aqueous  $\text{H}_2\text{O}_2$ , while the solution was stirred with a rapid stream of nitrogen. The flow of nitrogen was stopped, and polarographic current was recorded at  $-1.50$  v. vs. S.C.E., using the Brown Elektronik Strip chart recorder. The initial  $\text{H}_2\text{O}_2$  concentration was calculated from the current extrapolated to reaction time zero. It was shown that the concentration determined in this way was essentially the same as that expected from the amount of  $\text{H}_2\text{O}_2$  used. The relation between polarographic current and  $\text{H}_2\text{O}_2$  concentration was determined experimentally.

**Dye Yield.**—Twenty ml. of an air-free solution consisting of 25 ml. of stock buffer (giving pH 10.33), 20.7 ml. of water, 0.015 g. of 4-chloro-1-naphthol, 0.05 g. of *p*-amino-N,N-diethylaniline hydrochloride and 2.0 ml. of the Fe(III)-EDTA stock solution was placed in a nitrogen-swept, 70-ml. separatory funnel. Nitrogen flow was continued while 0.99 ml. of an air-free solution consisting of 67.0 mg. of 26.0%  $\text{H}_2\text{O}_2$  diluted to 25.0 ml. was added. The nitrogen flow was stopped and the funnel stoppered. After 16 min. at 25°, the solu-

tion was shaken with 20.0 ml. of air-free benzene. The dye-containing benzene layer was washed twice with 2%  $\text{NaH}_2\text{PO}_4$  solution and dried over  $\text{Na}_2\text{SO}_4$ . Five ml. of the solution was diluted to 50.0 ml. with benzene and the absorbance determined. In two experiments, absorbance at 585  $m\mu$  was 1.567 and 1.583. The spectrophotometric curve shape was identical to that for the dye prepared using ferricyanide as oxidant. Next a blank experiment was performed using water in place of the  $\text{H}_2\text{O}_2$  solution. In this case, the absorbance was 0.030. These two absorbances were corrected by subtracting 0.030, giving values corresponding to yields of dye of  $1.92 \times 10^{-3}$  and  $1.94 \times 10^{-3}$  mole, or an average of 95% of the dye expected from the amount of  $\text{H}_2\text{O}_2$  used. Dye formation in the blank experiment was due to oxidation of the diamine by the Fe(III)-EDTA and traces of oxygen.

**Reaction of Fe(III)-EDTA with *p*-Amino-N,N-diethylaniline in the Presence of 4-Chloro-1-naphthol.**—The amount of dye formed in this reaction was very small. Therefore, it was important to exclude oxygen. The apparatus consisted of a 70-ml., cylindrical separatory funnel, serving as a reaction vessel, at the top of which was connected a triple nozzle assembly having a nitrogen exit tube. Each of the three nozzles was connected by a short length of rubber tubing to the upper end of one of three solution chambers, A, B and C. Each solution chamber had a nitrogen inlet tube extending to near the bottom of the chamber and a stoppered tube for introduction of solutions. The apparatus was not placed in a water-bath, but the temperature of the laboratory was adjusted to  $25 \pm 0.2^\circ$ .

Twenty mg. of *p*-amino-N,N-diethylaniline hydrochloride was placed in the reaction vessel and the system swept with nitrogen, which was purified by passage through two wash bottles of vanadium(II) solution<sup>4</sup> and one of water. In solution chamber A was placed 5 ml. of the EDTA-containing stock buffer and a weighed quantity of ferric sodium (ethylenedinitrilo)-tetraacetate. This solution was swept free of air with nitrogen and then 5.0 ml. of an air-free ferrous ammonium sulfate solution was added. The amounts of ferric and ferrous iron used were varied. In chamber B was placed 10.0 ml. of an air-free solution made by dissolving 30 mg. of 4-chloro-1-naphthol in 50.0 ml. of nitrogen-swept 1:1-diluted stock buffer. Twenty ml. of benzene was placed in chamber C. The purified nitrogen was passed through the solution chambers and reaction vessel for about 20 min. (The nitrogen passed through chamber C was first passed through a wash bottle of benzene to minimize evaporation of benzene in C.) Chamber A was tilted so the solution was forced by nitrogen into the reaction vessel, which was swirled until the diamine had dissolved. Then the solution in B was quickly transferred to the reaction vessel in like manner and the solutions rapidly mixed by shaking the vessel. Reaction was allowed to proceed until about  $3 \times 10^{-8}$  mole of dye had formed. Then the benzene was introduced into the reaction vessel. The nozzle assembly was removed and rapidly replaced with a glass stopper. The vessel was shaken vigorously for 45 sec. The dye-containing benzene phase was washed twice with 2%  $\text{NaH}_2\text{PO}_4$ , and the suspended aqueous phase was removed by centrifuging. The amount of dye formed was calculated from the absorbance of the benzene solution at 585  $m\mu$ . Dividing this quantity by the reaction time gave the rate of dye formation plotted in Fig. 4 or 5. The averages of the initial and final Fe(II) and Fe(III) concentrations were used for Figs. 4 and 5. The extent of reaction was so small that these average values differ little from the initial and final concentrations themselves.

**Formation Constant of the  $\text{H}_2\text{O}_2$ -Fe(III)-EDTA Complex.**—Basically the method was like that used by Ringbom, *et al.*,<sup>5</sup> in that the concentration of the complex was measured spectrophotometrically. Five-ml. portions of carbonate-buffered solutions of Fe(III)-EDTA and of  $\text{H}_2\text{O}_2$  were brought to 25° and mixed rapidly at the recorded time, *t*, giving solutions containing  $5.6 \times 10^{-3}$  *M* total EDTA and having an ionic strength of 0.38. The absorbance at 520  $m\mu$  was measured at several recorded times after mixing, using the Beckman DU instrument with a water-jacketed cell compartment held at 25°. The absorbance diminished slowly, owing to decomposition of the  $\text{H}_2\text{O}_2$ . The data

(4) L. Meites and T. Meites, *Anal. Chem.*, **20**, 984 (1948).

(5) A. Ringbom, S. Siitonen and B. Saxén, *Anal. Chim. Acta*, **16**, 541 (1957).

were extrapolated to time,  $t$ , to find the absorbance at the time of mixing. This value was corrected by subtracting the absorbance due to the free Fe(III)-EDTA.

At  $pH$  9.68 and 10.32, series of runs were made in which total iron concentration was always the same ( $2.13 \times 10^{-4} M$ ) and  $H_2O_2$  concentration was varied. For each run, a value of  $K$ , defined by equation 4, was calculated, using 547 and 7 as the molar extinction coefficients of the  $H_2O_2$ -Fe(III)-EDTA complex and of free Fe(III)-EDTA, respectively, and using  $4 \times 10^{-12}$  as the dissociation constant<sup>6</sup> of  $H_2O_2$ . The values obtained are given in Table I.

TABLE I

FORMATION CONSTANT FOR  $H_2O_2$ -Fe(III)-EDTA COMPLEX<sup>a</sup>

Total $H_2O_2$ ( $M \times 10^2$ )	$pH$ 9.68		$pH$ 10.32	
	$K \times 10^{-3}$		$K \times 10^{-3}$	
0.278	$6.65 \pm 0.14$	0.364	$6.95 \pm 0.23$	
.510	$7.23 \pm .13$	0.697	$7.21 \pm .16$	
.879	$6.33 \pm .11$	1.275	$7.62 \pm .14$	
1.54	$6.49 \pm .13$	2.20	$7.53 \pm .14$	
3.08	$7.82 \pm .25$	3.85	$7.50 \pm .16$	
		7.71	$7.21 \pm .22$	
		30.8	$8.2 \pm .9$	

<sup>a</sup> The errors are those corresponding to an error of 0.001 in the transmittance of the solutions. When the  $K$ -values are weighted in inverse proportion to the errors, the average value obtained is  $7.1 \times 10^3$ .

**Minimum Rate of Oxidation of Fe(II)-EDTA by  $H_2O_2$ .**—An attempt was made to follow this reaction polarographically at  $pH$  10.33, using  $2 \times 10^{-4} M$  Fe(II)-EDTA and  $8 \times 10^{-5} M$   $H_2O_2$ . The reaction was too fast to follow, but it appeared to be at least 80% complete in 10 sec., indicating a rate constant greater than  $ca. 1.5 \times 10^3 M^{-1} sec^{-1}$ .

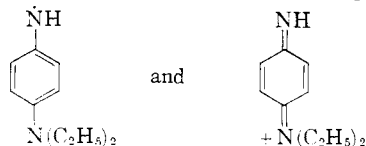
$pH$  measurements were made at  $25^\circ$  using the Beckman model GS instrument equipped with 1170 calomel and 1190-80 glass electrodes calibrated against borate buffer.

### Results and Discussion

**Symbols.**—The following abbreviations and symbols will be used

- R = *p*-amino-*N,N*-diethylaniline
- S = semiquinone of R
- T = quinonediimine of R
- P = hydrogen peroxide
- III = ferric-EDTA complex
- II = ferrous-EDTA complex
- $Y^{-4}$  = EDTA anion
- C = 4-chloro-1-naphtholate ion
- ( ) = concentration

It is considered that, in the  $pH$  range studied, the semiquinone and quinonediimine exist largely as the neutral molecule and cation, respectively

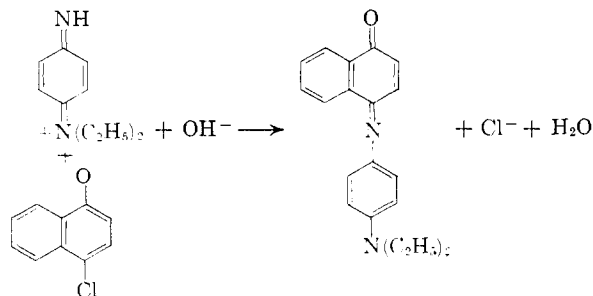


The symbols S and T represent the molecules in these ionization states.

**Reaction Products and Stoichiometry.**—The main product of the III-catalyzed reaction of P with R carried out in the presence of C is the same indaniline dye which is formed when ferricyanide is the oxidant.<sup>2</sup> The formation of this dye is evidence that R is oxidized to T in the III-catalyzed reaction, since it has been shown<sup>2</sup> that T is the intermediate involved in the dye-forming reaction when ferricyanide is the oxidant.

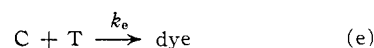
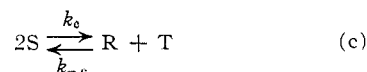
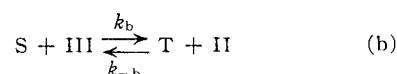
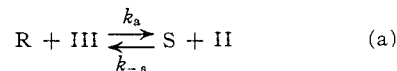
When the reaction was carried out at  $pH$  10.33 using  $10^{-3} M$  P,  $5 \times 10^{-3} M$  R,  $3.6 \times 10^{-5} M$

III and  $1.68 \times 10^{-3} M$  C, the yield of dye was 0.95 mole per mole of P used. Hence, it may be concluded that close to one mole of T was formed for each mole of P which reacted, since a mole of T is used in forming a mole of dye.



**Kinetic Experiments and Mechanism.**—Rate data are given in Figs. 1-3. A separate kinetic experiment was run for each datum. The  $-d(P)/dt$  values were determined from the slopes of the polarographic chart records (current *vs.* reaction time). In most cases, the slope was measured at a point near time zero. The value of (P) corresponding to this point was calculated directly from the polarographic current. The values of (R) and (C) were obtained by subtracting the moles per liter of P reacted from the initial concentrations of R and C.

The kinetic data suggest that III is the oxidant for R, the II formed in the reaction being rapidly oxidized to III by P. The mechanism may be formulated as



Reaction d occurs in at least two steps and is first-order in II and P.<sup>7</sup> Reactions d and e and forward c are assumed to be much faster than forward and reverse a, so concentrations of S, T and II are low. (It was on the basis of this assumption that the values of (R) and (C) of Figs. 1-3 were taken equal to the difference between the initial concentrations of these reactants and the amount of P reacted, a relation which would not be true if concentrations of T and S were not negligible.) It is assumed further that forward reaction c is much faster than forward b and that reverse b is much faster than reverse c. Under these conditions, forward b and reverse a and c may be considered to occur to a negligible extent, and the steady-state (T, S and II) approximation gives

$$-d(P)/dt = -k_t(C)(P) + \frac{\sqrt{[k_t(C)(P)]^2 + k_a k_t(C)(P)(R)(III)}}{k_a} \quad (1)$$

(7) (a) B. Matyska and D. Dušková, *Coll. Czech. Chem. Commun.*, **22**, 1747 (1957); (b) B. Matyska, *ibid.*, **22**, 1758 (1957).

(6) M. G. Evans and N. Uri, *Trans. Faraday Soc.*, **45**, 224 (1949).

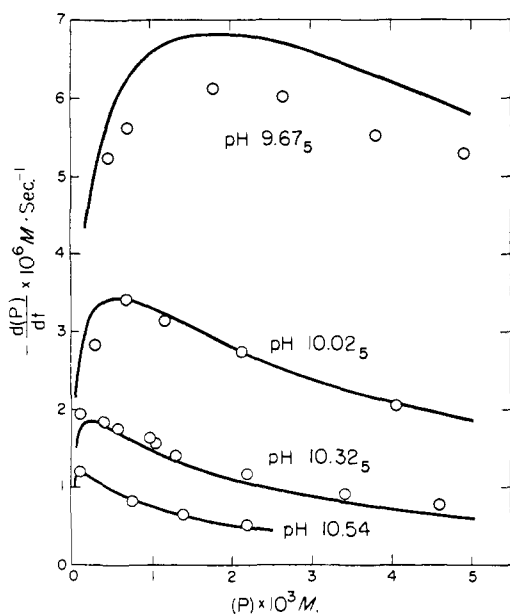


Fig. 1.—Rate of loss of P vs. (P) and pH; total iron concentration =  $3.59 \times 10^{-5} M$ ; (R) =  $4.53 \times 10^{-3} M$ ; 4-chloro-1-naphthol concentration =  $1.23 \times 10^{-3} M$ ; points, data; curves, calcd. using eq. 1.

where  $k_t = k_d k_e / k_{-b}$ . The rate data indicate that both  $k_a$  and  $k_t$  are pH-dependent. The equation predicts an approach to zero-order dependence of rate on (P) as the value of (P) is increased. Actually, the rate goes through a maximum and then decreases as (P) is increased (Fig. 1). This decrease is attributed to a decrease in concentration of the catalyst III, resulting from formation of the purple, III-peroxide complex.<sup>8</sup> In this explanation, it is implicit that the purple complex has little or no catalytic activity for the P-R reaction. In the experiments, the P tied up in the III-P complex constituted a small fraction of the total P. Consequently, the concentration of free P has been taken equal to the total (P).

To show the extent of agreement between the theoretical and experimental rates, the curves of Figs. 1-3 were calculated using equation 1, taking  $k_a = 10^{11.78 - pH}$  and  $k_t = 10^{pH - 8.795}$ . The values of (C) were calculated by using

$$(C) = aK_D / (10^{-pH} + K_D) \quad (2)$$

where  $a$  is the total concentration of 4-chloro-1-naphthol and  $K_D$  is its dissociation constant ( $2.14 \times 10^{-9}$ ).<sup>9</sup>

Values of the free catalyst concentration (not tied up in the purple III-P complex) were calculated by using

$$(III) = b(10^{-pH} + K_A) / [10^{-pH} + K_A + KK_A(P)] \quad (3)$$

where  $b$  is the total concentration of III and III-P complex ( $b$  is considered equal to the total iron concentration, since ferrous iron concentration is assumed very low, and most of the ferric iron should be tied up in III and in III-P complex),  $K_A$  is the dissociation constant of P (estimated as  $4 \times 10^{-12}$  from data of Evans and Uri<sup>6</sup>) and  $K$  is the forma-

(8) K. L. Cheng and P. F. Lott, *Anal. Chem.*, **28**, 462 (1956).

(9) L. K. J. Tong and M. Carol Giesmann, *THIS JOURNAL*, **79**, 4305 (1957).

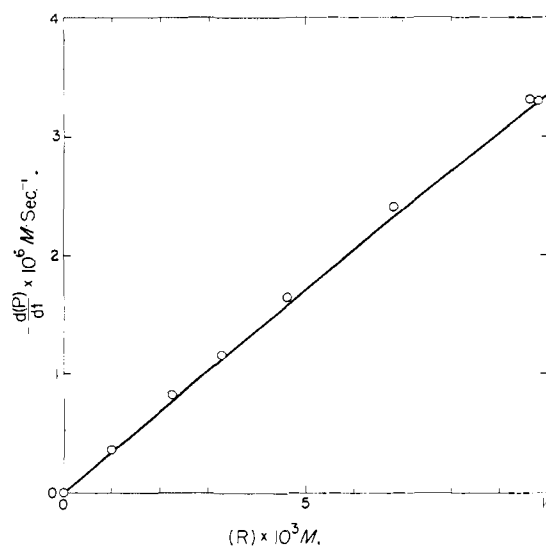


Fig. 2.—Rate of loss of P vs. (R); (P) =  $8.02 \times 10^{-4} M$ ; total iron concentration =  $3.59 \times 10^{-5} M$ ; 4-chloro-1-naphthol concentration =  $1.38 \times 10^{-3} M$ ; pH 10.32; points, data; curves, calcd. using eq. 1.

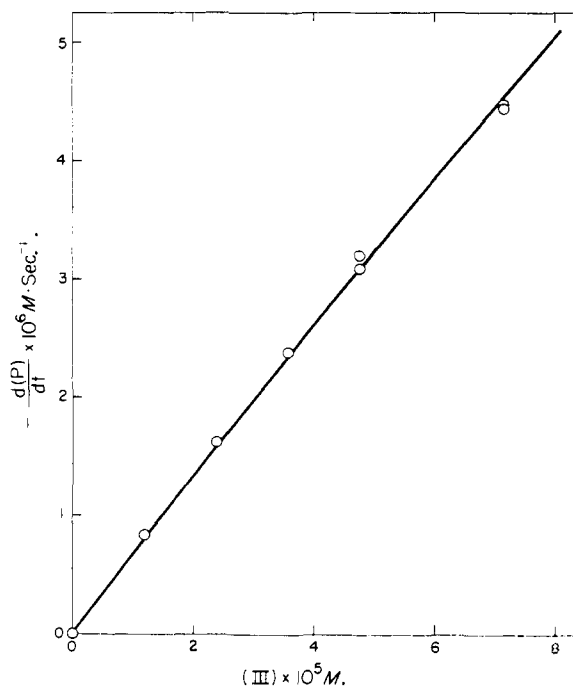


Fig. 3.—Rate of loss of P vs. (III); (R) =  $4.73 \times 10^{-3} M$ ; (P) =  $9.1 \times 10^{-4} M$ ; 4-chloro-1-naphthol concentration =  $1.43 \times 10^{-3} M$ ; pH 10.32; points, data; curves, calcd. using eq. 1.

tion constant for the purple III-P complex, abbreviated IIIP, defined by Ringbom, *et al.*,<sup>5</sup> as

$$K = (IIIP) / (III)(HO_2^-) \quad (4)$$

The value ( $7.1 \times 10^3$ ) of  $K$  was determined experimentally at 25° and ionic strength 0.38, using carbonate buffers containing the same concentration of EDTA as that used in the kinetic experiments. (At pH 10.33, C had no noticeable effect on the value of  $K$ .) The abscissa values of Fig. 3 were calculated using equation 3.

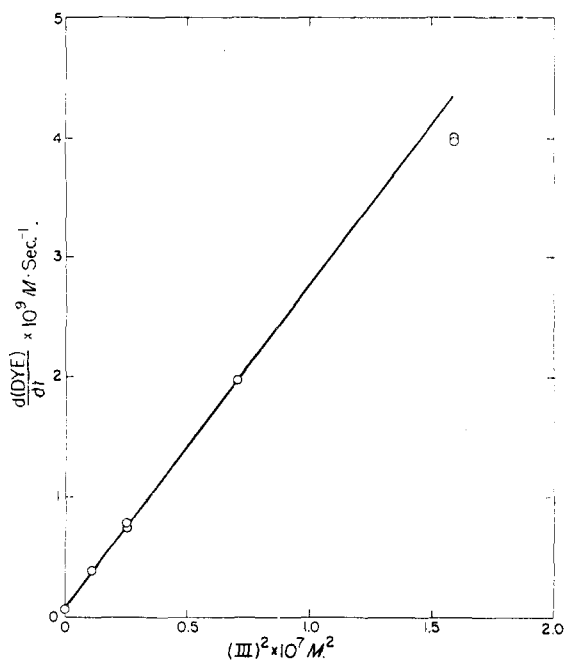


Fig. 4.—Rate of dye formation vs. (III)<sup>2</sup>; (II) =  $1.703 \times 10^{-4} M$ ; (R) =  $5.11 \times 10^{-3} M$ ; 4-chloro-1-naphthol concentration =  $1.72 \times 10^{-3} M$ ; pH 10.32<sub>5</sub>.

The relation between  $-d(P)/dt$  and (C), although studied but briefly, was found to be in accord with equation 1. At higher values of the product, (P)(C), the rate is close to zero-order in (C), while at low (P)(C) values the rate becomes positive-order in (C).

**Discussion of the Mechanism.**—The assumptions made regarding the relative rates of the various steps of the proposed mechanism might be considered sufficiently justified by the agreement between the calculated and experimental rates. Some of these assumptions are supported by additional evidence, however. The rates of d and e and forward c are known to be very fast. The second-order rate constant for d is at least  $10^3 M^{-1} \text{sec}^{-1}$  in the alkaline solutions used (reported to be  $7 \times 10^3 M^{-1} \text{sec}^{-1}$  in acid solution<sup>7</sup>), and the rate constant for e is  $7 \times 10^6 M^{-1} \text{sec}^{-1}$  (ionic strength 0.27).<sup>10</sup> The equilibrium for c lies well to the right in alkaline solution and is established rapidly,<sup>11</sup> implying a fast forward reaction. The fact that the experimental  $-d(P)/dt$  values are not very large indicates that forward a, rather than one of the fast reactions c, d or e, tends to be the slow, rate-determining step.

The curves of Fig. 1 relating  $-d(P)/dt$  and (P) consist of sharply rising segments at low (P) and gradually decreasing segments at higher (P). In the rising segments, reaction d is rate-determining to a significant extent. The position of the maximum is determined in part by the value of  $k_t$ . The higher the value of  $k_t$ , the closer is the maximum to the  $-d(P)/dt$  axis. The rate data indicate that  $k_t$  increases with increasing pH. The precision is not sufficient, however, to allow more than a rough

(10) Determined by Miss M. Carol Giesmann, of these Laboratories, as described previously: L. K. J. Tong and M. Carol Giesmann, *This Journal*, **79**, 4310 (1957).

(11) L. K. J. Tong, *J. Phys. Chem.*, **58**, 1090 (1954).

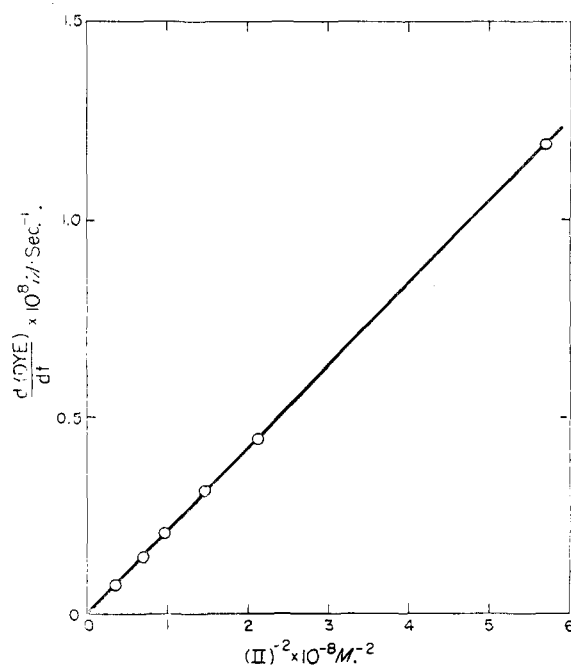


Fig. 5.—Rate of dye formation vs. (II)<sup>-2</sup>; (III) =  $1.597 \times 10^{-4} M$ ; (R) =  $5.11 \times 10^{-3} M$ ; 4-chloro-1-naphthol concentration =  $1.72 \times 10^{-3} M$ ; pH 10.32<sub>5</sub>.

estimate of the functional relation between  $k_t$  and (H<sup>+</sup>). It was arbitrary, therefore, to make  $k_t$  proportional to (H<sup>+</sup>) in calculating the curves. Actually, there is indication that, at the higher pH values,  $k_t$  may be larger than this dependency predicts. In the gradually decreasing segments of the curves, reaction a is rate-determining. As stated above, decrease in rate with increasing (P) is attributed to an increase in the amount of III removed by complexation with P. The agreement between the data and the calculated slopes of these decreasing segments indicates quite strongly that this interpretation is correct. In the case of the enzyme peroxidases, the active oxidizing agents are P-enzyme complexes.<sup>12</sup> Such a mechanism appears to be ruled out for the III-catalyzed R-P reaction.

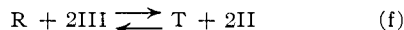
In Fig. 1 the displacement of the entire curve toward higher rates as pH is lowered is due to the increase in the value of  $k_a$ . The data indicate that  $k_a$  is not exactly proportional to (H<sup>+</sup>) as assumed in calculating the curves. The dependence of rate on pH is not due to catalysis by the buffer ions, since changing the buffer concentration while holding pH and ionic strength constant (with sodium sulfate) had no effect on the rate.

The data of Figs. 2 and 3 follow closely the near-first-order dependence of rate on (R) and (III) predicted by the mechanism.

**The III-R Reaction and its pH Dependence.**—The key reaction of the proposed mechanism is the oxidation of R by III. It was of interest, therefore, to study this reaction in the absence of peroxide. The reaction was carried out in the presence of C in order that dye formation might be used as a

(12) W. D. McElroy and B. Glass, editors, "A Symposium on the Mechanism of Enzyme Action," The Johns Hopkins Press, Baltimore, Md., 1954, p. 389.

measure of reaction. The course of dye formation indicates that the equilibrium for the reaction



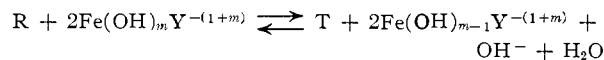
lies far to the left. When even a small amount of II is present, the rate of dye formation is so low that T may be assumed to have close to its equilibrium concentration. Under this condition, if f has been correctly formulated, the rate of dye formation should be given by

$$d(\text{dye})/dt = K_f k_e (C)(R)(III)^2/(II)^2 \quad (5)$$

where  $K_f$  is the equilibrium constant for f. This relation has been tested at several pH values by determining  $d(\text{dye})/dt$  as a function of  $(III)^2$  and of  $(II)^{-2}$ , other variables being held almost constant. Results for pH 10.32<sub>5</sub> are given in Figs. 4 and 5. The data are in good accord with equation 5, showing that reaction f is reversible and that the coefficients of (III) and (II) in f are 2, as formulated.

A value of  $K_f$  can be calculated, using differentiated equation 5, from the slope of the data of either Fig. 4 or Fig. 5. The values obtained are  $1.3 \times 10^{-11}$  and  $1.4 \times 10^{-11}$ , respectively. The negative logarithm of the average of the two  $K_f$  values and the negative logarithms of similar averages for other pH values are plotted vs. pH in Fig. 6. The value of  $K_f$  is rather closely proportional to  $H^+$  concentration.

The proportionality of  $K_f$  to  $(H^+)$  suggests the following more detailed formulation of f



Thus, it would appear that, in the pH range studied, one hydroxide ion is lost from each molecule of complex reduced to the ferrous state. This formulation is not in accord with the equilibrium constants given by Schwarzenbach and Heller<sup>13</sup> for the reactions at 20° of hydroxide ions with  $FeY^-$  and  $FeY^{-2}$ . For pH values above ca. 10, for example, these constants indicate that both the ferric and ferrous complexes exist primarily in the dihydroxylated form. Therefore, the value of  $pK_f$  would be expected to decrease with increasing pH above 10. Expected slope values, calculated using these constants, for the plot of Fig. 6 are: pH 8, +0.9; pH 9, +0.4; pH 10, -0.5; pH 11, -0.9. Disagreement with the experimental data is marked.

(13) G. Schwarzenbach and J. Heller, *Helv. Chim. Acta*, **34**, 576 (1951).

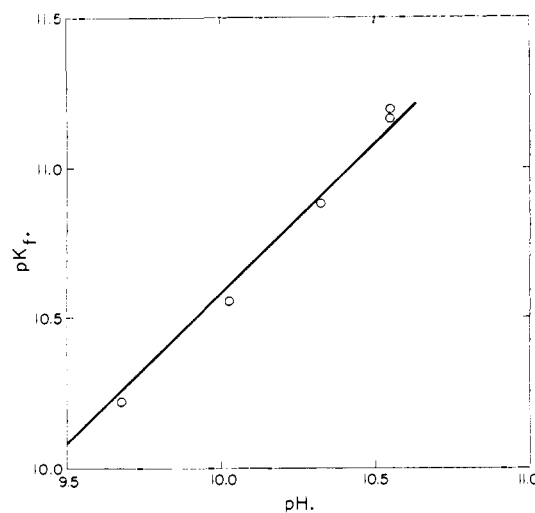


Fig. 6.— $pK_f$  vs. pH; points, data; curve, theoretical slope for  $K_f$  proportional to  $(H^+)$ .

The equilibrium constants of Schwarzenbach and Heller<sup>13</sup> for formation of the hydroxy complexes also predict a decrease in slope of the plot of the III-II oxidation-reduction potential vs. pH for pH values above about 9. These authors apparently did not extend their potential measurements to high enough pH to confirm this prediction. Kolthoff and Auerbach,<sup>14</sup> however, did not find such a decrease in slope for a plot of polarographic half-wave potential vs. pH extending to pH 10.9 (25°). With their  $E_{1/2}$  values for pH 9 and 10.9, it can be shown that, in this pH range,  $K_f$  should be proportional to  $(H^+)$ . Unfortunately, the significance of this result is open to question, since the polarographic reduction at pH 10.9 is reported to be irreversible.

An approximate value for  $pK_f$  can be calculated using half-wave potentials. At pH 10 and 25°,  $E_{1/2} = 0.014$  v. vs. S.C.E. for R<sup>15</sup> and -0.27 v. for III (interpolated from the data of Kolthoff and Auerbach<sup>14</sup>). The calculated  $pK_f$  value is 10, in approximate agreement with the data of Fig. 6.

**Acknowledgment.**—The author wishes to thank Dr. L. K. J. Tong, of these Laboratories, for suggestions helpful in interpretation of the data.

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(14) I. M. Kolthoff and C. Auerbach, *THIS JOURNAL*, **74**, 1452 (1952).

(15) D. B. Julian and W. R. Ruby, *ibid.*, **72**, 4719 (1950).