of material corresponding to the second and third peaks were identical with those obtained from authentic dimethyl adipate and dimethyl *trans*-1,2-cyclohexanedicarboxylate, respectively. The identities of these components were further checked by addition of authentic esters to the reaction product. Vapor phase chromatography of the new mixture did not show broadening or resolution of the original second and third peaks. Vapor phase chromatography of the individual and mixed authentic dimethyl esters of *cis*- and *trans*-1,2-cyclohexanedicarboxylic acids showed that the retention time of the *cis* diester differed from the *trans* by 0.7 min., thereby excluding the possibility that *cis* diester was present in the mixture of esters arising from ozonolysis.

The Mechanism of Bleaching of Naphthoquinone Imine Dyes in Alkaline Solution

R. L. REEVES AND L. K. J. TONG

Research Laboratories, Eastman Kodak Company, Rochester, New York 14650

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The bleaching of alkali-unstable naphthoquinone imine dyes involves initial base-catalyzed hydrolysis of the azomethine linkage to give a *p*-phenylenediamine and a 1,4-naphthoquinone. The quinone subsequently adds water to form a naphthalenetriol which rapidly reduces unhydrolyzed dye to the corresponding leuco base. The hydrolysis step involves reversible addition of OH^- to the azomethine linkage to form a carbinolamine which then collapses to hydrolysis products in a pH-independent step. At sufficiently high pH, the dye can be almost completely converted to the carbinolamine. The proposed mechanism is supported by product and kinetic studies of the bleaching reaction and by a separate kinetic study of the decomposition of the quinone intermediate.

In an earlier paper,[1] a kinetic study of the alkaline bleaching of some unstable naphthoquinone imine dyes established the following facts of mechanistic significance: (1) the bleaching follows an empirical

$$X + OH^- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Z \xrightarrow{k_3} P \qquad (1)$$

$$X + OH^- \xrightarrow{k_4} P \qquad (2)$$

rate law consistent with a general scheme where X represents dye, Z represents an addition complex, and P denotes the product(s) of the irreversible reaction; and (2) the rate of approach to equilibrium between X, OH^-, and Z is slow enough to measure conveniently. The kinetics of bleaching are equally consistent with a scheme in which $k_4 = 0$ (consecutive mechanism) or where $k_3 = 0$ (parallel mechanism). In the consecutive mechanism, the addition complex is an intermediate in the over-all irreversible path. In the parallel mechanism, the addition complex is merely an inert reserve, whereas all irreversible reaction proceeds by way of direct attack of OH^- to give the product. The kinetics of dye bleaching alone cannot distinguish these two mechanisms, and in the earlier work we considered the two as equally likely and evaluated the rate constants as composite constants.

We now report the results of a detailed study of the bleaching of the water-soluble dye I which was used as one of the models in the earlier kinetic study.[1] This dye was selected because bleaching and formation of Z occur in a pH range convenient for measurement and because the suspected intermediate and most of the products are known compounds. The complete bleaching reaction can be divided into two parts: the primary reaction represented by eq. 1 and a secondary reaction involving reduction of dye by the product(s) from the primary reaction. We shall show that in the primary reaction the irreversible part proceeds through the complex Z (consecutive mechanism), the structure of which is suggested. Evidence is given to implicate the reducing agent responsible for the secondary reaction. Kinetic measurements for both the disappearance of dye and the formation of the products as well as some separate product yields are used to support the proposed mechanism. We refer to bleaching as any dark reaction leading to destruction of the dye chromophore and include in this general term hydrolysis, nucleophilic addition, and reduction.

Results and Discussion

The final products of the bleaching reaction are the *p*-phenylenediamine II, 2-acetyl-3-hydroxy-1,4-naphthoquinone (V), and the leuco base VI corresponding to a two-electron reduction of the original dye. Large-scale product isolation studies from concentrated solutions of a number of other naphthoquinone imine dyes have shown that similar products are obtained in every case.[2] The 3-hydroxyquinone (V) was identified in product mixtures by its polarographic wave which was determined from a pure synthetic sample. The leuco base VI was identified by reoxidation to the original dye and comparison of the absorption curve with that of a known sample, and by the identity of the half-wave potential of VI in the product mixtures with that of a pure synthetic sample and of the original dye. The *p*-phenylenediamine II found in the product mixtures was shown to be identical with a known sample by comparison of the properties of dyes formed by oxidative coupling with several substituted α-naphthols.

The quantitative studies of product yields and the kinetic runs were performed on solutions containing I at initial concentrations of 10^{-4} M or less. At higher concentrations, deviations from Beer's law and nonlinear dependence of the bleaching rate on the concentration of I indicated that some aggregation of the dye was occurring. The same three products were obtained from bleaching in the very dilute solutions as were isolated from the concentrated solutions. Because of the low concentrations and sensitivity of II and VI to aerial oxidation, all product and most kinetic studies

(1) R. L. Reeves and L. K. J. Tong, *J. Am. Chem. Soc.*, **84**, 2050 (1962).

(2) D. P. Harnish, unpublished results.

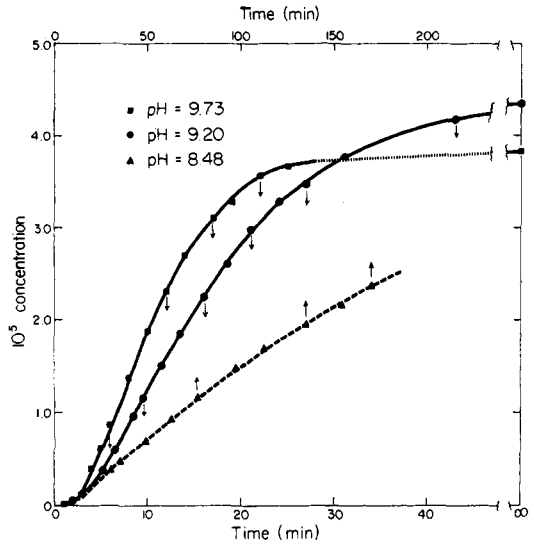


Figure 1.—Rates of formation of the leuco base VI.

were carried out under anaerobic conditions. The good reproducibility of the analyses and rates shows that oxygen was successfully excluded.

Product yields were determined from reactions carried out from pH 8.8, where the concentration of the addition complex is immeasurably small, up to pH 11, where much of the dye is rapidly converted to the complex.[1] The results are summarized in Table I. Duplicate analyses gave reproducible yields to within 2%. At the lower pH values, the *p*-phenylenediamine II and the leuco base VI account for 95 ± 2% of the bleached dye. The product balance from the reaction carried out at pH 11 is not quite so good as that for the lower pH, probably owing to some loss of II by rapid aerial oxidation at the high pH during the handling needed for the analysis. The results show that, at pH around 9, slightly more than 50% of the dye goes to II, while slightly less than 50% goes to the leuco base VI. As the pH is increased to 11, the yields of VI decrease and the yields of II appear to increase. There also seems to be a trend toward decreasing yields of the 3-hydroxyquinone V as the pH of the reaction is increased from 9 to 11, although this is less pronounced than in the case of the leuco base.

TABLE I

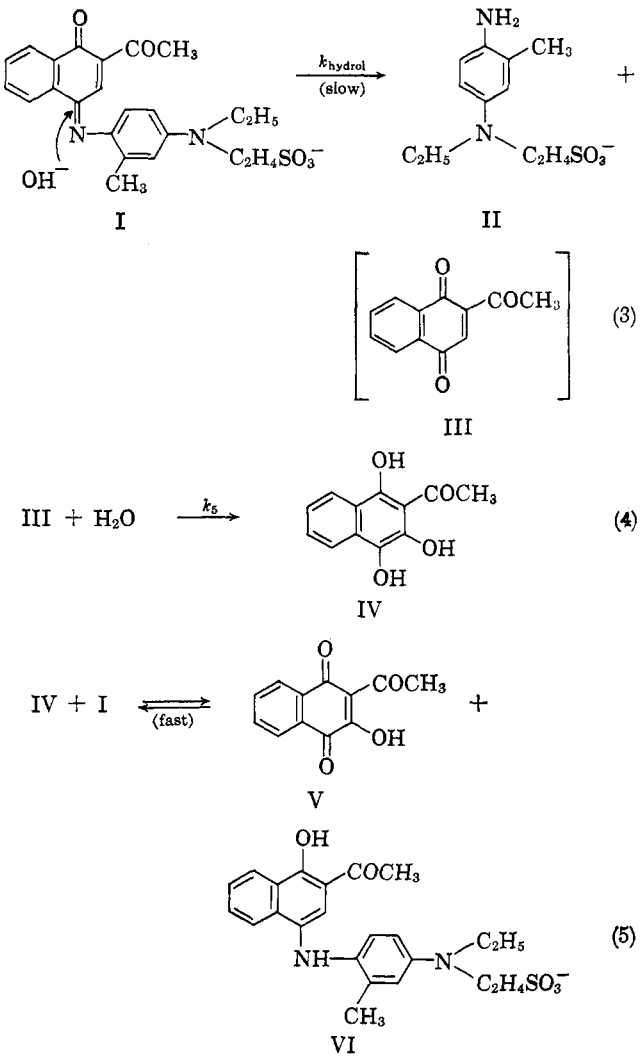
YIELDS OF PRODUCTS FROM BLEACHING OF 10^{-4} *M* SOLUTIONS OF I

	Yields, % of starting dye		
pH	*p*-Phenylenediamine (II)	Leuco base VI[a]	3-Hydroxyquinone (V)[a]
8.8	...	48.5	34.5
9.2	54	41	36
9.7	...	38	31
9.8	51	39	...
11.0	59	27	29

[a] Replicate determinations from independent runs gave ±2%.

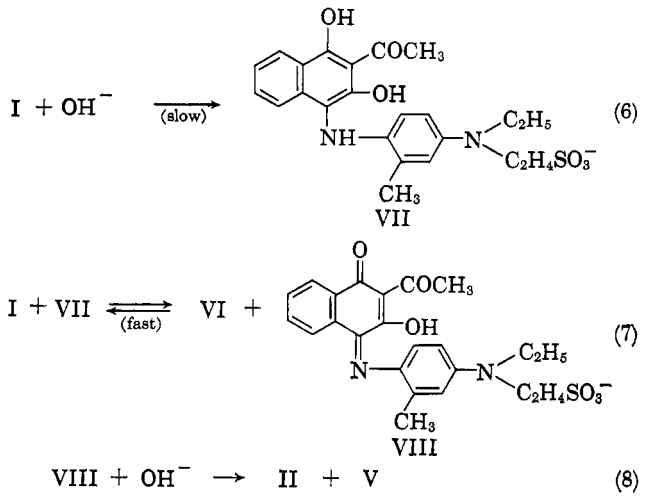
Kinetics and Mechanism.—Qualitatively, the reaction products could be formed by either mechanism given in Scheme I or II. The difference between them involves a fundamental chemical difference in behavior of the dye, *i.e.*, whether the naphthoquinone imine reacts as a typical azomethine by hydrolysis of the C=N linkage, or as a typical quinonoid species so that the OH^- adds to the ring. Both of these possibilities are reasonable, but kinetic considerations readily distinguish between them. First of all, the nearly equal yields of hydrolysis and reduction products at pH 9 require that, in Scheme I, the further decomposition of the quinone III be rapid at this pH compared with hydrolysis ($k_5 > k_{hydrol}$). By the hydrolysis mechanism, the rate of formation of the hydrolysis product II should not show an induction period at those pH values where the complex Z does not accumulate, whereas the rates of formation of V and VI should be parallel and may or may not show induction periods, depending on the relative magnitudes of k_5 and k_{hydrol}. On the other hand, Scheme II requires that the reduction product, VI, be formed without an induction period as an initial product, but the formation of II and V should be parallel and may or may not show induction periods.

SCHEME I



In pH 9.2 borate buffer, the rate of formation of II does not show an induction period. The initial rate of formation of II is equal to the initial rate of disappearance of I in this buffer. The rates of formation of both the leuco base VI (Figure 1) and the hydroxyquinone V have induction periods in this buffer. This clearly shows that II is the initial product of the irreversible reaction and that its formation precedes that of V and VI. At any pH at which induction periods were seen, the curves for V and VI were parallel and dif-

SCHEME II



fered throughout by a constant factor. This factor is the factor by which the final yields of V and VI were found to differ. These facts indicate that V and VI are formed by a common reaction following the initial hydrolysis and make Scheme II untenable. The reason for $d[V]/dt$ not being equal to $d[VI]/dt$, as required by Scheme I, is discussed later. The final product yields also rule out Scheme II, since it is not possible for the yield of II to exceed that of VI by this mechanism—it could only be equal to the yield of VI. At first it was thought that Scheme II could easily be eliminated by the fact that it involves the conversion of one dye to another of different hue, and this should be easily detectable. On preparing VIII, however, we found it to be yellow in polar solvents, so that its formation from I would be interpreted as bleaching.

Once the *p*-phenylenediamine II has been established as the first stable product of the initial irreversible step in the over-all dye-bleaching reaction (P in eq. 1), it is possible to establish the role of the addition complex Z in the mechanism. Chemically, the consecutive and parallel mechanisms suggest different structures for Z. In the first case, Z is a reaction intermediate which can react irreversibly to P, suggesting that the OH^- is on the 4-carbon of the naphtho ring. In the second case, Z is an inert complex, suggesting that the OH^- is on a position other than the 4-carbon. The method of distinguishing between the two possibilities involves measurement of P as a function of time at high alkalinity so that the dye is rapidly converted either to Z ($k_4 = 0$) or to a mixture of Z and P ($k_3 = 0$). (Detailed arguments and rate curves calculated for each case were given in the earlier paper.[1]) If $k_3 = 0$, the experimental curve for P in the slower part of the reaction will extrapolate to a finite concentration at zero time determined by the ratio $k_4:k_1$, which, in this case, is 0.36. If $k_4 = 0$, the experimental curve will extrapolate approximately to the origin at zero time since all P must form from Z. The result for pH 11.78 is shown in Figure 2. The calculated curve for dye bleaching at this pH (dashed curve) shows that, within 10 sec. after mixing, 97% of the dye has reacted. The experimental curve for formation of II at this pH extrapolates nearly to the origin, showing that hydrolysis must take place through the

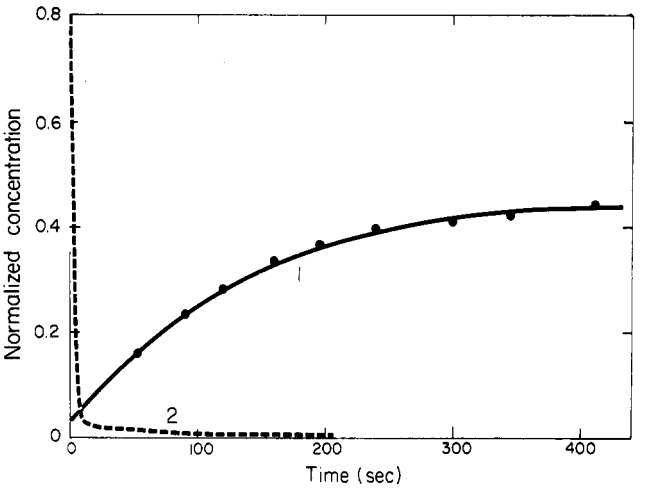
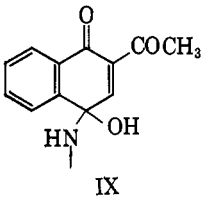


Figure 2.—The formation of *p*-phenylenediamine II from dye at pH 11.78: curve 1, experimental concentration–time curve for II; curve 2, calculated concentration–time curve for the dye.

intermediacy of the addition complex, to which we assign the structure IX. This is the now familiar carbinolamine intermediate proposed for reactions involving formation and hydrolysis of other azomethine compounds.[3-7]



IX

We repeated the measurement of k_1, k_2, and k_3 in buffers (pH 10–11) that were saturated with pure oxygen to eliminate all net reduction to leuco base. The present results support the earlier interpretation but give slightly different numerical values for the constants, presumably owing to the fact that bleaching *via* the reduction path was not completely eliminated in the earlier measurements. With $k_4 = 0$, the new values for k_1, k_2, and k_3 are 70.7 l. mole^{-1} sec.$^{-1}$, 0.0172 sec.$^{-1}$, and 0.00609 sec.$^{-1}$, respectively, compared with 82.0, 0.0127, and 0.00730 for the same constants calculated from the old set of parameters.

First-order plots for bleaching of I in oxygen-free buffers in the pH range 8 to 9 showed slight curvature, with increasing slopes as bleaching progressed. At pH 9.4 and above, the initial rapid bleaching associated with formation of the addition complex Z becomes apparent. Calculation of the concentration of Z at different times[8] shows that at pH 9.4 the concentration builds up to 7% of the initial dye concentration after 2 min. At pH values less than 9 the concentration of Z is immeasurably small and the first-order rate constant for hydrolysis, k_{hydrol}, is a composite constant equal to $k_1k_3[OH^-]/(k_2 + k_3)$, since the steady-state

(3) (a) A. Hantzsch and F. Kraft, *Ber.*, **24**, 3511 (1891); (b) A. Hantzsch and O. Schwab, *ibid.*, **34**, 822 (1901).

(4) O. Dimroth and R. Zoeppritz, *ibid.*, **35**, 984 (1902).

(5) (a) W. P. Jencks, *J. Am. Chem. Soc.*, **81**, 475 (1959); (b) B. M. Anderson and W. P. Jencks, *ibid.*, **82**, 1773 (1960); (c) E. H. Cordes and W. P. Jencks, *ibid.*, **84**, 832 (1962).

(6) R. L. Reeves, *ibid.*, **84**, 3332 (1962).

(7) Y. Inoue and D. D. Perrin, *J. Chem. Soc.*, 2648 (1963), and references cited therein.

(8) See eq. 6, ref. 1.

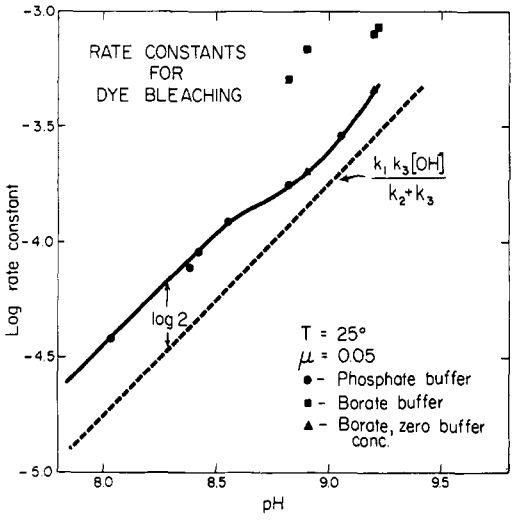


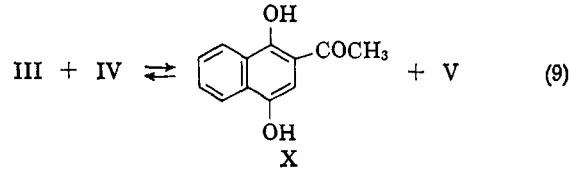
Figure 3.—Comparison of rate constants for total bleaching with rate constants for hydrolysis alone.

approximation for Z here is valid. The pH–rate profile for hydrolysis, calculated from the experimental values of the constants, is shown as the dashed line in Figure 3. The observed initial rate constants (for the sum of hydrolysis and reduction rates), measured in phosphate buffers over the pH range 8.0–8.6 are found to be nearly twice the calculated hydrolysis rate. This fact, plus the nearly equal yields of hydrolysis product II and reduction product VI obtained in this pH range (Table I), are in accord with Scheme I where a slow hydrolysis is followed by a rapid decomposition of one of the hydrolysis products to a reducing agent. Thus, in this pH region, each mole of hydrolysis immediately gives a second mole of reduction, and the observed rate of dye bleaching is twice the rate of hydrolysis. In the pH region 8.8–9.2 the observed rate constants for total bleaching are less than twice the hydrolysis rate constants and tend to approach the latter values more closely. This fact and the finding that the ratio of yields II:VI increases with pH suggest that, as the pH is raised, the rates of hydrolysis and decomposition of the quinone III become more nearly the same. This could only occur if the rate of hydrolysis of the dye shows a higher dependence on OH^- concentration than the rate of quinone decomposition. This has been shown to be the case. In principle, it should be possible to go to a sufficiently high pH that the rate of decomposition of III would be negligible compared with the hydrolysis rate so that the observed rate of bleaching would then equal the hydrolysis rate. It was not possible to do this because of the kinetic complication associated with formation of the addition complex Z and failure of the steady-state approximation for Z. In addition, we found indications that the dependence of the rate of decomposition of III on the OH^- concentration changed at higher pH values. Table I does show, however, that the ratio of reduction product to hydrolysis product decreases progressively up to pH 11.

The rates of formation of the leuco base VI were measured over the pH range 8.5 to 9.7, covering the pH range where a changing ratio k_{hydrol}/k_5 is indicated. The rate curves for several pH values are plotted in Figure 1. The importance of the induction period is seen to decrease as the pH is lowered until, at pH 8.5, it has practically disappeared. This is the expected behavior if the ratio k_{hydrol}/k_5 decreases progressively as the pH is lowered.

Rates of total bleaching in borate buffers over the pH range 8.8–9.2 are greater than the rates in phosphate buffers at the same pH and ionic strength (Figure 3). The bleaching rate was found to vary with the borate buffer concentration over the range of concentrations from 0.05 *M* to 0.2 *M*. Extrapolation of linear plots of k_{obsd} against the total borate concentration to zero buffer concentration gave the uncatalyzed rate constants. These agree well with those measured in phosphate buffers (Figure 3) and indicate that the phosphate salts show negligible catalytic activity. Variation of the borate buffer concentration at two pH's showed that it is the basic component of the buffer that is acting as the catalyst.

Additional evidence for Scheme I was sought by separate study of reactions 4 and 5. 2-Acetyl-1,3,4-naphthalenetriol (IV) was quantitatively formed *in situ* by reduction of the 3-hydroxyquinone V with sodium borohydride. Treatment of the solution of IV with an equal concentration of I gave rapid and quantitative conversion to V and VI. Reaction 4 is reported to proceed as indicated.[9] In the absence of other reducible species, the triol IV is oxidized by unchanged quinone as it forms. The yields of X and V should be equal and should amount to half the

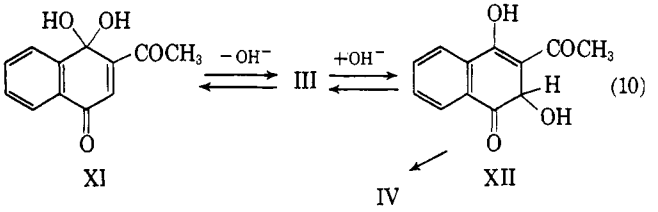


quinone used. Treatment of a solution of IV with an equivalent amount of III at pH 9.2 gave rapid and quantitative conversion to V and X. When, however, III alone was decomposed at pH 9.2, the yields of V and X were only 28% and 42%, respectively, instead of the theoretical 50%. The same results are obtained, regardless of whether one starts with purified III or forms it *in situ* by oxidation of X. The evidence indicates that reaction 4 does not occur exactly as written but that III reacts with water to give more than one reducing agent, IV being one of them. We were unable to detect a cathodic wave in the potential region 0 to −0.6 v. that would correspond to the oxidized form of an unknown reducing agent. The fact that the product ratio V:X from the decomposition of III alone is close to the ratio V:VI obtained from the dye-bleaching reaction supports the assumption that III is an intermediate in the latter reaction. Failure to obtain the ratio V:VI predicted by Scheme I is due to complications in the decomposition of the quinone and supports rather than argues against the correctness of the main features of the proposed mechanism.

Both polarographic and spectrophotometric evidence show that the quinone III reacts rapidly in aqueous buffers to give intermediates or complexes that slowly give products. Thus no cathodic wave could be seen at the expected potential for III within 30 sec. after

(9) C. J. P. Spruit, *Rec. trav. chim.*, **46**, 655 (1947).

injection of an acetone solution into the buffer.[10] On the other hand, the appearance of V and X occurred over a period of about 10 min. Spectra obtained on flowing solutions at different reaction times up to 1 sec. show that at least two species must intervene between III and the products (Figure 4). These rapid reactions must be largely reversible because the known products account for 70% of the decomposed quinone. It is possible that species such as XI and XII are important. The slow product-forming step could conceivably be the conversion of XII to IV.



The rate of the slow step in the decomposition of III is the only one of importance in the dye-bleaching mechanism, and this was measured by following the formation of the two products, V and X, in buffers containing only III initially. Plots of log $(C_\infty - C_t)$ *vs.* time were linear for both products and gave the same derived rate constants. These constants were independent of pH over the pH range 8.6 to 9.6, with a value for the first-order rate constant of 7.3×10^{-3} sec^{-1}. Thus III fulfills the requirements of Scheme I that its rate of decomposition be greater than that of hydrolysis of dye over this pH range, and that it be pH independent.

The mechanism and the values of the rate constants predict that addition of the quinone III to a solution of dye should greatly accelerate dye bleaching. At low pH the observed rate of bleaching with added quinone should be a measure of the rate of decomposition of III. Treatment of a solution of I at pH 8.9 with a twofold excess of III gave accelerated bleaching with an initial rate constant of 3.8×10^{-3} sec^{-1}. The fact that the measured rate was about half the rate of decomposition of III is due to the fact that, with both dye and quinone present initially, the IV that is formed from III will reduce both I and unchanged III about equally, since the potentials of both substances are so close. To the extent that III is reduced instead of I, the rate of bleaching of I will be reduced.

All the data presented are qualitatively consistent with the proposed mechanism, but two other facts need to be considered. The mechanism and the rate constants require that in the pH range 8.5–9.0, the rate of dye bleaching follow first-order kinetics except during the earliest stages of the reaction. We find that small deviations from first-order kinetics occur during the late stages of bleaching, indicating that some small additional path for bleaching is operative. At 80% reaction, the additional bleaching accounts for about 4% of the original dye. Considering that only 95% of the dye is accounted for by the isolated products, that the dye sample could not be prepared in an absolutely pure state, and that the decomposition of the quinone intermediate is not completely understood, we believe that the small kinetic discrepancy

(10) The expected half-wave potential for III is the same as that for X, which could be determined.

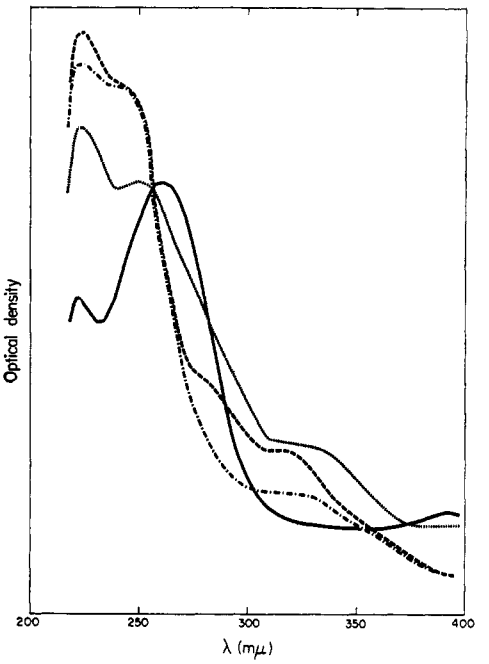


Figure 4.—Rapid reaction of 2-acetyl-1,4-naphthoquinone (III) at pH 9.2: solid curve, 2-acetyl-1,4-naphthalenediol (X); broken curves, oxidized X after 12.2 msec. (– – –), 58.9 and 139 m sec. (— · —), and 736 msec. (----).

$$III + VI \rightleftharpoons X + I \qquad (11)$$

must be attributed to an unknown side reaction and should not be taken as an argument against the main features of the mechanism. In addition, the oxidation–reduction equilibrium between reaction products (reaction 11) is rapidly established and further complicates a rigorous kinetic analysis. The closeness of the half-wave potentials of I, VI, and X (Table II) indicates that the equilibrium constant for reaction 11 will be close to unity. We mixed equal concentrations of I and X at pH 9.2 and from the equilibrium concentration of I determined $K = 0.27 = [VI][III]/[I][X]$. The occurrence of this equilibrium means that, as the leuco base accumulates, the steady-state concentration of III will continually be reduced to levels below those predicted by reactions 3 and 4 alone, and the concentration of I will be slightly higher than that predicted.

TABLE II

HALF-WAVE POTENTIALS OF DYE AND PRODUCTS AT PH 9.2

	Structure			
	I	VI	V	X
$E_{1/2}$ (*vs.* s.c.e.), v.	−0.199	−0.201	−0.44 to −0.49[a]	−0.156
Wave type	Cathodic	Anodic	Cathodic	Anodic

[a] The value varied with concentration.

Experimental

Materials.—The preparation and isolation of dye I were described previously.[1] It was further purified by conversion to the barium salt and recrystallization from methanol.

Anal. Calcd. for $C_{46}H_{46}BaN_4O_{10}S_2 \cdot H_2O$: C, 53.4; H, 4.6; Ba, 13.2. Found: C, 53.8; H, 5.0; Ba, 13.6.

As the absorptivity of the dye sample was 7% lower than that of the oxidized leuco base, the dye was assumed to be only 93% pure. The appropriate correction factor was applied when product yields were determined from weighed dye samples.

The leuco base VI was obtained by treating 1 g. of the dye barium salt in 250 ml. of deaerated 50% ethanol–water with small portions of neutralized ascorbic acid in 50% ethanol until the blue color just disappeared. The filtered solution was evaporated under vacuum until the barium salt of VI precipitated. The product was filtered (suction) in a stream of nitrogen and the cake was washed with cold, deaerated isopropyl alcohol. Rapid removal of moisture was essential to prevent reoxidation. The recrystallization was repeated in the presence of a trace of ascorbic acid until replicate carbon and hydrogen analyses agreed to within 0.1%.

Anal. Calcd. for $C_{46}H_{50}BaN_4O_{10}S_2 \cdot H_2O$: C, 53.2; H, 5.0. Found: C, 53.2; H, 5.2.

Analytically pure samples of 2-acetyl-1,4-naphthoquinone (III), 2-acetyl-1,4-naphthalenediol (X), and 2-acetyl-3-hydroxy-1,4-naphthoquinone (V) were prepared by published procedures.[9]

Analytical Methods.—Dye could be determined either spectrophotometrically or polarographically. Beer's law was followed in the concentration range 0–5 × 10^{-5} *M* but slight deviations from linearity were found at the higher concentrations. As the association of the dye at the higher concentrations gave poor polarograms, the spectrophotometric method was the one of choice.

The leuco base VI, the 2-acetyl-1,4-naphthalenediol (X), and the 2-acetyl-3-hydroxy-1,4-naphthoquinone (V) were all determined polarographically. Linear calibrations were obtained for all three compounds for all conditions used. The same current–concentration calibration constant was obtained at several pH's. The half-wave potentials are given for pH 9.2 in Table II. The half-wave potential of the 3-hydroxyquinone V was shifted to slightly more negative values in the presence of the *p*-phenylenediamine II than when measured alone at the same pH. The half-wave potential of V in a product mixture was the same as that of a synthetic mixture of V and II. The current–concentration calibration constant for V was the same in the presence and absence of II. The leuco base VI and the hydroxyquinone V did not interfere with their respective analyses. Thus, a synthetic mixture containing 5 × 10^{-5} *M* V and VI gave, by polarographic analysis, 4.95 × 10^{-5} and 4.89 × 10^{-5} *M* V and VI, respectively. Therefore, the low yields of V found in the product analyses cannot be due to an interference in the analysis by any of the known products.

The *p*-phenylenediamine II was determined by oxidative coupling with 1-hydroxy-N-ethyl-3′,5′-dicarboxy-2-naphthanilide (XIII) in pH 7 buffer containing 1% Triton X-100. The low pH and the surfactant were used to ensure that, in the kinetic runs, the hydrolysis of the dye would be immediately quenched and Beer's law would be followed. Thus, 10 ml. of a reaction solution containing II in concentrations between 0 and 1 × 10^{-4} *M* was added to 1.0 ml. of 3 × 10^{-3} *M* XIII in the buffer–Triton solution. One milliliter of 4.2 × 10^{-3} *M* ferricyanide solution in water was added to oxidize II and form the blue dye with XIII. At the same time any leuco base present was oxidized to I so that, at this point, the solution contained two blue dyes plus a purple coloration from oxidation of XIII by the excess oxidant. The color due to I and oxidized XIII was immediately discharged by addition of 1 ml. of 0.02 *M* Na_2SO_3, leaving only the dye formed from II and XIII. The formation constant for the sulfite complex[1] of I is greater by six orders of magnitude than that for the dye from XIII. Calculation showed that the concentration of sulfite used for the analysis would completely convert I to its colorless complex without converting measurable amounts of the dye from XIII. A linear calibration was obtained for the method.

Kinetic Measurements.—Most of the runs were made at 25°; a few were also made at 30°. The analytical methods were calibrated for both temperatures. The ionic strength of most of the solutions was 0.05 and was determined solely by the buffer concentration. In those runs where the borate buffer concentration was varied, the ionic strength was 0.2 and was determined by the buffer and the necessary amount of potassium chloride. The total rate of bleaching (hydrolysis plus reduction) was measured in solutions that had been purged of air by bubbling with nitrogen that had first been passed through Fieser's solution.[11] All transfers were made in syringes that had been flushed several times with nitrogen. The dye was first dissolved in 50% ethanol–water in a concentration such that after initiation of a run by dilution with buffer the final solution contained 1% ethanol and dye concentrations between 5 × 10^{-5} and 1 × 10^{-4} *M*.

The rate of formation of leuco base VI was measured by following the rate of increase of the anodic diffusion current at a fixed potential. No other species arising from decomposition of the dye gave anodic waves. In following the rate of formation of the 3-hydroxyquinone V it was necessary to rapidly scan complete polarograms periodically, since the disappearing dye also gave a cathodic wave. This was done by rapidly knocking the mercury drops from the capillary with an accessory to the Metrohm Type E261R polarograph and increasing the rate of change of potential. In this way a complete wave could be obtained in 15 sec.

The study of the decomposition of 2-acetyl-1,4-naphthoquinone (III) was carried out either by injection of an acetone solution of III into the buffer, or by forming III by oxidation of the correresponding hydroquinone X. Similar results were obtained by either method. When the former method was used, some decomposition in the acetone solution occurred unless the solvent was first purified and dried. Rates of formation of X were determined from the increase in anodic diffusion current at the appropriate potential. Plots of log $(C_\infty - C_t)$ *vs.* time were linear for both V and X and the derived rate constants were the same. The spectrophotometric study of the decomposition of III was carried out by mixing equal volumes of the hydroquinone X in the buffer with 2 equiv. of ferricyanide in water in the flow machine[12] and scanning the ultraviolet absorption curves under different steady-state conditions (reaction times). The hydroquinone was oxidized within 12 msec.

(11) L. F. Fieser, "Experiments in Organic Chemistry," 2nd Ed., D. C. Heath and Co., New York, N. Y., 1941, p. 395.

(12) C. A. Bishop, R. F. Porter, and L. K. J. Tong, *J. Am. Chem. Soc.*, **85**, 3991 (1963).