

# Dismutation of a Semiquinone Free Radical of Chlorpromazine

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The dismutation in aqueous solution of a semiquinone free radical of chlorpromazine, R, was studied. Experimental second-order rate constants,  $k_{obs}$ , were determined. In an HCl-KCl buffer system, the  $k_{obs}$  increased with increasing ionic strength in accord with the Brönsted-Bjerrum relationship. However, in the presence of a potassium acid phthalate buffer no ionic strength effect was noted, though an increase in  $k_{obs}$  was noted, apparently due to phthalate ion catalysis. Within the pH range of 0.45 to 3.75 in an HCl-KCl system, the dismutation apparently was independent of  $H^+$  concentration. At pH values considerably above and below this range, however, a marked increase and decrease, respectively, in  $k_{obs}$  was noted. The addition of chlorpromazine (CPZ) to the reaction mixture resulted in a decrease in the  $k_{obs}$ . Chlorpromazine sulfoxide (CPO), under similar conditions, had no effect on the  $k_{obs}$ . Evidence for the formation of a dimer of CPZ was obtained; a  $K$  of dimerization was calculated to be  $1.3 \times 10^4$ .

THE OXIDATION of chlorpromazine (CPZ) to chlorpromazine sulfoxide (CPO) in aqueous media has been shown to be a two-step process (1). The first step is the removal of one electron to yield a semiquinone free radical, R. This is followed by the removal of a second electron to yield a phenazathionium ion, P. This latter species apparently reacts with water to yield CPO. However, under the influence of relatively mild oxidizing agents such as  $Fe^{+3}$ ,  $Mn^{+3}$ , and  $H_2O_2$ , the oxidation of CPZ proceeds only to R (2). Loss of R then occurs *via* a dismutation reaction according to Scheme I (2, 3).

The removal of only one electron from CPZ, and the subsequent dismutation of R is an important pathway for the oxidation of CPZ. Therefore, it was felt that a study of the individual steps of this reaction sequence would be useful in the further elucidation of the over-all process.

This report is concerned with the dismutation step. The availability of R in a pure solid state (4) made possible the investigation of this step without the complicating effect of the simultaneous oxidation of CPZ.

## EXPERIMENTAL

**Materials.**—Chlorpromazine  $HCl^1$  and chlorpromazine sulfoxide  $HCl^2$  were used without purification. All other chemicals were of reagent grade. Deionized water was used for the preparation of all solutions. Nitrogen prepurified (Matheson Co., Inc.) was used for deaeration.

**Equipment.**—A Bausch & Lomb Spectronic 20 with 1.12-cm. cells was used for absorbance measurements at 527  $m\mu$ . The ultraviolet and visible

spectra were determined on a Beckman model DU or model DB spectrophotometer. The kinetic runs were made in a constant-temperature water bath maintained at  $25 \pm 0.05^\circ$ .

**Preparation of the Free Radical.**—The semiquinone free radical of chlorpromazine, R, was prepared by the following procedure (4). Approximately 500 mg. of CPO was dissolved in 10 ml. of 70% perchloric acid and agitated occasionally over a period of 15 min. The solution was chilled to about  $0^\circ$ , 5 ml. of acetone was added, followed by sufficient ether to yield two distinct layers. The mixture, maintained at  $0^\circ$  was agitated gently and then filtered. The residue, the free radical of CPZ, was washed several times with ether and dried at room temperature. This material was used without further purification.

The ultraviolet and visible spectra and the molar absorbance index at 527  $m\mu$ ,  $a_m$ , were determined by dissolving the material in  $\approx 6 M H_2SO_4$ . These solutions followed Beer's law in the concentration range of  $1 \times 10^{-4}$  to  $7 \times 10^{-4} M$ . The spectra were the same as previously reported (4). The  $a_m$  at 527  $m\mu$  was determined to be  $8.44 \times 10^3$ .

**Procedure.**—Samples of R, ranging from about 30–40 mg., were dissolved in water or the acid portion of the respective solvent systems. This solution was added to the rest of the buffer in a 100-ml. volumetric flask. Water was added to bring the solution to volume. All solvents were equilibrated at  $25^\circ$  prior to addition of R to avoid excessive loss of the free radical during temperature equilibration. The solution was transferred to a 250-ml. conical flask secured in the constant-temperature bath. Deaeration of the solution was initiated at this time and continued throughout the experiment. The first aliquot (time zero) was withdrawn by a pipet about 3 min. after the start of deaeration. Additional aliquots were withdrawn at 3–5-min. intervals. The reaction was quenched in all cases by permitting the pipet to drain into 50-ml. flasks containing exactly 20 ml. of 2.5  $N HCl$  previously chilled to  $\approx 4^\circ$ . This quenching procedure was shown to quench the reaction satisfactorily over the period of time necessary to determine the absorbance of the sample. In addition, it was demonstrated that neither CPZ nor CPO, in the concentrations present, were converted to the free radical under these conditions.

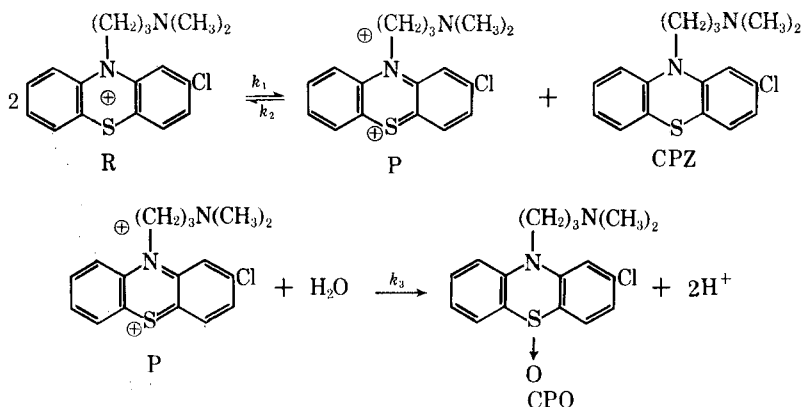
The absorbance of each dilution was determined

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<sup>1</sup> Marketed as Thorazine HCl by Smith Kline & French Laboratories, Philadelphia, Pa. Obtained through the courtesy of Dr. R. Blythe.

<sup>2</sup> Obtained through the courtesy of Dr. R. Blythe, Smith Kline & French Laboratories, Philadelphia, Pa.



at 527  $m\mu$ . The concentration of the radical was calculated by use of Eq. 1 after application of the appropriate dilution factor.

$$C = A/a_m b \quad (\text{Eq. 1})$$

where

$C$  = concentration, moles L.<sup>-1</sup>  
 $A$  = absorbance scale reading  
 $b$  = cell light path, cm.

The experimental second-order rate constants,  $k_{\text{obs}}$ , were calculated from the slopes of the plots of the reciprocals of concentration *versus* time in accordance with

$$(1/C) - (1/C_0) = k_{\text{obs}} t \quad (\text{Eq. 2})$$

where

$C_0$  = concentration, moles L.<sup>-1</sup>, at time zero  
 $C$  = concentration, moles L.<sup>-1</sup>, at time  $t$   
 $k_{\text{obs}}$  = calculated second-order rate constant, L. mole<sup>-1</sup>sec.<sup>-1</sup>

The  $k_{\text{obs}}$  were determined as a function of pH, ionic strength, and CPZ concentration in HCl-KCl and potassium acid phthalate-HCl buffer systems. Ionic strength was adjusted where necessary with KCl.

## RESULTS AND DISCUSSION

**Effect of pH on  $k_{\text{obs}}$ .**—Runs were made in HCl-KCl solutions at a constant ionic strength and different pH values. The calculated  $k_{\text{obs}}$  are given in Table I. Values at pH 6.9 and less than zero are included as comparisons.

Under the conditions of these experiments, the  $k_{\text{obs}}$  does not change significantly from an average value of 10.4 L. mole<sup>-1</sup> sec.<sup>-1</sup> over the pH range of 0.45 to 3.75. However, an abrupt change occurs at very low pH values, where  $k_{\text{obs}}$  approaches

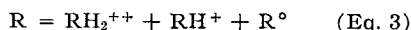
TABLE I.—EXPERIMENTAL RATE CONSTANTS FOR THE DISMUTATION OF R IN HCl-KCl SOLUTIONS AT 25°C.,  $\mu = 0.5$ , AND DIFFERENT pH VALUES

pH	$k_{\text{obs}}$ L. moles <sup>-1</sup> sec. <sup>-1</sup>
$\ll 0^a$	$\approx 0$
0.45	10.9
0.71	10.3
1.00	10.4
3.75 <sup>b</sup>	11.1
6.90 <sup>c</sup>	111.0

<sup>a</sup> R was dissolved in concentrated hydrochloric acid. No loss of R was noted over a 30-min. observation period. <sup>b</sup> No hydrochloric acid was used in this run. <sup>c</sup> The pH was adjusted with 2 N sodium hydroxide solution.

zero, and again at about pH 6.9, where the  $k_{\text{obs}}$  approaches very large values.

This observed pH- $k_{\text{obs}}$  relationship can be satisfied by assuming the existence of three different species of R as shown in Scheme II and Eq. 3.



In the pH range of 0.45 to 3.75, the single protonated species,  $RH^+$ , would predominate, assuming the  $pK_a$  of the quaternary salt of R is not appreciably different from that of 9.22 reported for CPZ (5). At very low pH values, it is reasonable to expect that both the ring and alkyl nitrogen are protonated (6), and the predominant species would then be the doubly protonated form,  $RH_2^{++}$ . Above pH 6.9, the completely nonprotonated species,  $R^\circ$ , would be present in significant concentration.

Each of these species would be expected to have a different  $k$  of dismutation,  $k_d$ . The value of  $k_d$  would be expected to be an inverse function of the extent of protonation, since protonation would

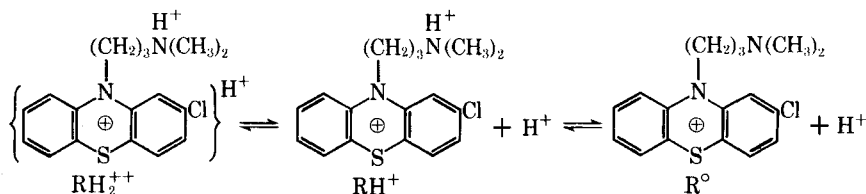


TABLE II.—EXPERIMENTAL RATE CONSTANTS FOR THE DISMUTATION OF R IN HCl-KCl BUFFER AT 25°C., pH 1.96, AND DIFFERENT IONIC STRENGTHS

$\mu$	$k_{obs}$ L. moles <sup>-1</sup> sec. <sup>-1</sup>
0.0125	2.07
0.0625	3.73
0.1000	4.56
0.1875	7.72

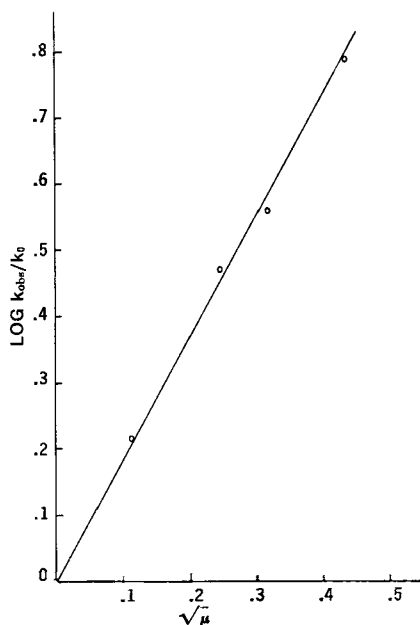


Fig. 1.—Effect of ionic strength,  $\mu$ , on  $k_{obs}$  in an HCl-KCl buffer, pH 1.96.

both reduce the tendency of a species to give up an electron and increase the repulsive forces between adjacent molecules.

The  $k_{obs}$  would then be a function of (a) the proportion of each species making up R. This in turn would be a function of the pH of the solution and the pKa's of the two protonated species and (b) the  $k_d$ 's of the species present.

**Effect of Ionic Strength on  $k_{obs}$ .**—Runs were made in HCl-KCl buffer at pH 1.96 and different ionic strengths. The calculated values of  $k_{obs}$  are given in Table II.

The Brönsted-Bjerrum relationship as given in Eq. 4 predicts that  $k_{obs}$  will increase with increasing ionic strength for a reaction involving two similarly charged species. This effect can be seen in Fig. 1. The increasing slope is as would be expected for the reaction of two like-charged species. The calculated slope of 1.60, however, is higher than that of 1.02 predicted by Eq. 4 for the interaction of two singly charged species.

$$\log \frac{k_{obs}}{k_0} = 1.02 Z_a Z_b \sqrt{\mu} \quad (\text{Eq. 4})$$

where

- $k_{obs}$  = the observed rate constant
- $k_0$  = the rate constant at  $\mu = 0$
- $Z_a Z_b$  = the charges on the two reacting species
- 1.02 = the constant for a dilute aqueous system at 25°

**Effects of a Potassium Acid Phthalate-HCl Buffer System.**—In the presence of 0.025 M potassium acid phthalate at pH 2.00, changes in ionic strength from 0.055 to 0.178 had no apparent effect on the  $k_{obs}$ . Thus, it appears that R, even at pH 2.00, behaves as if it were an uncharged species in the presence of phthalate. Since it was previously postulated that reduction of the effective charge on R would result in an increase in  $k_d$ , such an increase should be seen in the presence of phthalate. This effect was demonstrated in the next experiment.

The effect on the  $k_{obs}$  of a potassium acid phthalate-HCl buffer system at different pH values was determined. These runs were made at a constant potassium acid phthalate concentration and constant ionic strength. Typical second-order plots are given in Fig. 2. The calculated  $k_{obs}$  are given in Table III.

The rapid increase of  $k_{obs}$  with increase in pH (Fig. 3) in the presence of the phthalate buffer must be attributed mainly to phthalate ion catalysis. Hydroxyl ion catalysis is apparently not

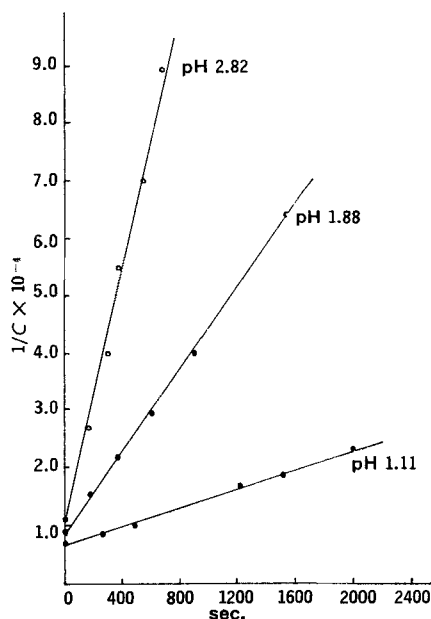


Fig. 2.—Typical second-order plots for the dismutation of R in a potassium acid phthalate buffer at an ionic strength of 0.12 and different pH values.

TABLE III.—EXPERIMENTAL RATE CONSTANTS FOR THE DISMUTATION OF R IN POTASSIUM ACID PHTHALATE-HCl BUFFER<sup>a</sup> AT 25°C.,  $\mu = 0.12$ , AND DIFFERENT pH VALUES

pH	$k_{obs}$ L. moles <sup>-1</sup> sec. <sup>-1</sup>
1.11	8.3
1.48	11.1
1.88	28.1
2.10	36.1
2.31	40.4
2.65	77.5
2.82	116.0

<sup>a</sup> The concentration of potassium acid phthalate was maintained at 0.05 M. The HCl concentration was adjusted to obtain the different pH values.

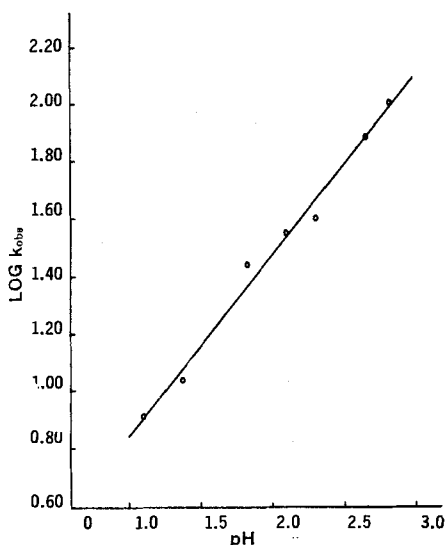


Fig. 3.—Effect of pH on the log  $k_{obs}$  for the dismutation of R in a potassium acid phthalate (0.05  $M$ )–HCl buffer at ionic strength of 0.12.

involved since  $k_{obs}$  was previously shown to be independent of pH in the range of 0.45 to 3.75. Biphthalate catalysis appears to be insignificant, since  $k_{obs}$  at pH 1.11 (where phthalate ion concentration is extremely low) approaches that observed in HCl–KCl solutions of the same ionic strength.

The log  $k$ –pH relationship, as shown in Fig. 3, is not surprising under these circumstances, since the phthalate ion concentration would be expected to be approximately proportional to pH.

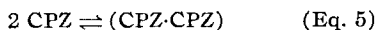
**Effect of Added CPZ and CPO on  $k_{obs}$ .**—Since both CPZ and CPO are products of the dismutation reaction, it was of interest to determine their effect on  $k_{obs}$ .

Dismutation of R was studied at pH 1.97,  $\mu$  0.175, in the potassium acid phthalate–HCl buffer to which different concentrations of CPZ and CPO were added. The addition of CPO under these conditions had no apparent effect on the  $k_{obs}$ . However, the addition of CPZ had a marked effect on the  $k_{obs}$ . The calculated  $k_{obs}$  in the presence of added CPZ are given in Table IV.

The data from this experiment appear to support the reaction scheme as represented by Scheme I. Under these experimental conditions the first step in the dismutation, yielding CPZ and P, apparently is reversible, while the interaction of P with water yielding CPO is not.

A plot of  $k_{obs}$  versus CPZ concentration added is given in Fig. 4. The change in the slope of the curve which occurs at CPZ  $> 1.6 \times 10^{-4} M$  is apparently due to the significant dimerization of CPZ that occurs at this concentration.

Assuming that the concentration of CPZ and its dimer (CPZ·CPZ) are equal at the inflection point (*i.e.*, when the total concentration of CPZ is  $1.6 \times 10^{-4} M$ ), a dimerization constant,  $K_d$ , can be calculated from:



$$K_d = \frac{(\text{CPZ} \cdot \text{CPZ})}{(\text{CPZ})^2} \quad (\text{Eq. 6})$$

$$K_d = \frac{8 \times 10^{-5}}{(8 \times 10^{-5})^2} = 1.3 \times 10^4 \quad (\text{Eq. 7})$$

This value is in good agreement with the value of  $K_d \approx 10^4$  determined by Borg and Cotzias using potentiometric redox titrations (2).

By calculating the  $k_{obs}$  early in the reaction, *i.e.*, before any significant amounts of CPZ are produced, it can be assumed that in the presence of added CPZ the concentration of CPZ in solution is a constant. Then from Scheme I:

$$\frac{-dR}{dt} = 2k_1(R)^2 - 2k_2(P)(\text{CPZ}) \quad (\text{Eq. 8})$$

$$\frac{dP}{dt} = k_1(R)^2 - k_2(P)(\text{CPZ}) - k_3(P)(\text{H}_2\text{O}) \quad (\text{Eq. 9})$$

Assuming steady-state conditions for P, *i.e.*,  $dP/dt = 0$  and letting  $k_3' = k_3(\text{H}_2\text{O})$ .

$$P = \frac{k_1(R)^2}{k_2(\text{CPZ}) + k_3'} \quad (\text{Eq. 10})$$

Substituting Eq. 10 in Eq. 8 yields

$$\frac{-dR}{dt} = \left[ 2k_1 - \frac{2k_1k_2(\text{CPZ})}{k_2(\text{CPZ}) + k_3'} \right] (R)^2 \quad (\text{Eq. 11})$$

Since (CPZ) under the conditions of the experiment is constant,

$$k_{obs} = 2k_1 - \frac{2k_1k_2(\text{CPZ})}{k_2(\text{CPZ}) + k_3'} \quad (\text{Eq. 12})$$

This equation can be treated in two ways to evaluate the rate constants.

(a) By assuming

$$k_3 > k_2(\text{CPZ}) \quad (\text{Eq. 13})$$

Eq. 12 simplifies to:

$$k_{obs} = 2k_1 - \frac{2k_1k_2(\text{CPZ})}{k_3'} \quad (\text{Eq. 14})$$

The plot in Fig. 4 represents this form of the equation. The slope equals  $2k_1k_2/k_3'$  and the intercept  $2k_1$ .

Using the portion of the curve prior to the inflection point, *i.e.*, where CPZ dimerization is negligible, these constants can be calculated to be:

$$2k_1 = 29.0 \text{ L. mole}^{-1} \text{ sec.}^{-1}$$

$$\frac{k_2}{k_3'} = 1.7 \times 10^3 \text{ L. mole}^{-1}$$

TABLE IV.—EXPERIMENTAL RATE CONSTANTS FOR THE DISMUTATION OF R IN POTASSIUM ACID PHTHALATE–HCl BUFFER AT 25°C.,  $\mu = 0.175$ , pH = 1.97, AND DIFFERENT CONCENTRATIONS OF ADDED CPZ

CPZ Concn. added $\times 10^6$	$k_{obs}$ L. moles <sup>-1</sup> sec. <sup>-1</sup>
100	17.0
80	19.0
50	21.0
30	21.5
10	24.5
6	26.4
3	27.4
1.5	28.0
0	29.0

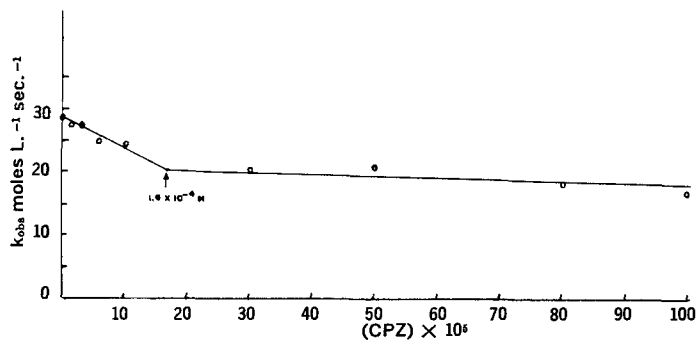


Fig. 4.—Effect of CPZ on the  $k_{obs}$  for the dismutation of R in a potassium acid phthalate-HCl buffer at pH 1.97 and ionic strength 0.175.

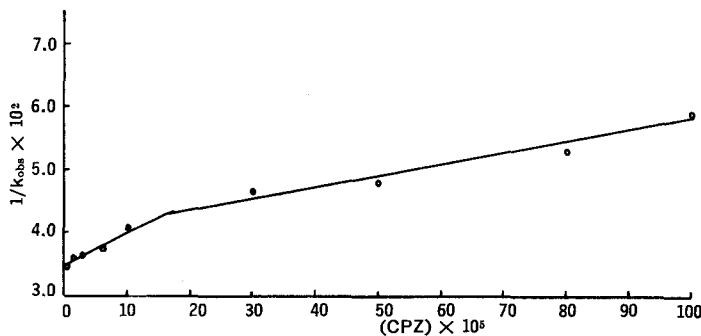


Fig. 5.—Effect of CPZ on the  $k_{obs}$  for the dismutation of R in a potassium acid phthalate-HCl buffer at pH 1.97 and ionic strength 0.175.

(b) In order to avoid the assumption made in Eq. 13, Eq. 12 can be manipulated to yield:

$$k_{obs} = \frac{2k_1k_3'}{k_2(\text{CPZ}) + k_3'} \quad (\text{Eq. 15})$$

Taking reciprocals, Eq. 15 yields:

$$\frac{1}{k_{obs}} = \frac{1}{2k_1} + \frac{k_2(\text{CPZ})}{2k_1k_3'} \quad (\text{Eq. 16})$$

A plot of  $1/k_{obs}$  versus (CPZ), as shown in Fig. 5, yields a straight line with a slope of  $k_2/2k_1k_3'$  and an intercept of  $1/2k_1$ . Determination of  $2k_1$  and the ratio of  $k_2/k_3'$  gives the same values as reported above. Thus, it appears the assumption made in Eq. 13 is valid.

## SUMMARY

A solid free radical of chlorpromazine, R, was prepared, and experimental second-order rate constants for its dismutation were determined in aqueous media. The ionic strength effect on the observed rate constants,  $k_{obs}$ , was found to be indicative of a reaction between two like-charged species. Within the pH range of 0.45 to 3.75,  $k_{obs}$  was independent of  $H^+$  concentration. At pH 6.9, a marked increase in the  $k_{obs}$  was noted, and at

pH values considerably below zero the value of  $k_{obs}$  approached zero. The existence of three species of R, differing from one another by extent of protonation and  $k$  of dismutation, was postulated to explain this pH dependency. An increase in  $k_{obs}$  was noted in the presence of potassium acid phthalate apparently due to phthalate rather than biphthalate ion catalysis. The addition of chlorpromazine (CPZ) to the reaction mixture served to decrease the value of  $k_{obs}$ , while the addition of the sulfoxide (CPO) did not. This effect apparently is related to the ability of CPZ, and not CPO, to take part in a back reaction step. Evidence for the formation of a dimer of CPZ was obtained, and a  $K$  of dimerization was calculated to be  $\approx 1.3 \times 10^4$ .

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