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# Oxidation Kinetics of Phenothiazine and 10-Methylphenothiazine in Acidic Medium

H. ROSEBOOM [x] and J. H. PERRIN [*]

Abstract □ The rate of phenothiazine degradation in an acidic oxygen-saturated medium was studied. 3H-Phenothiazine-3-one and phenothiazine 5-oxide are produced by parallel reactions, and 7-(10'-phenothiazinyl)-3H-phenothiazine-3-one is produced in a more complex manner. The overall phenothiazine degradation rate appears to be pH independent up to pH 7.0. The degradation kinetics of 10-methylphenothiazine were studied after isolation and identification of its degradation products, 10-methylphenothiazine 5-oxide and 3H-phenothiazine-3-one. The main degradation product is 10-methylphenothiazine 5-oxide; but at low pH values and high temperatures, more 3H-phenothiazine-3-one is formed. The degradation rate of 10-methylphenothiazine is pH independent up to pH 7.

Keyphrases □ Phenothiazine and 10-methyl derivative—oxidative degradation in acidic oxygen-saturated medium, effect of pH □ Oxidation—degradation kinetics of phenothiazine and 10-methyl derivative in acidic oxygen-saturated medium, effect of pH □ Degradation kinetics—phenothiazine and 10-methyl derivative, oxidation in acidic oxygen-saturated medium, effect of pH

The oxidative degradation of phenothiazine has been the subject of many investigations (1–7), but little is known about the kinetics of this reaction. Previously, the isolation

of some degradation products and their identification by TLC, melting point, and IR, UV, and mass spectral data were described (8). 7-(10'-Phenothiazinyl)-3H-phenothiazine-3-one was reported as a degradation product for the first time, and its structure has now been confirmed by NMR spectroscopy. The present paper reports the kinetics of the disappearance of phenothiazine (I) and the
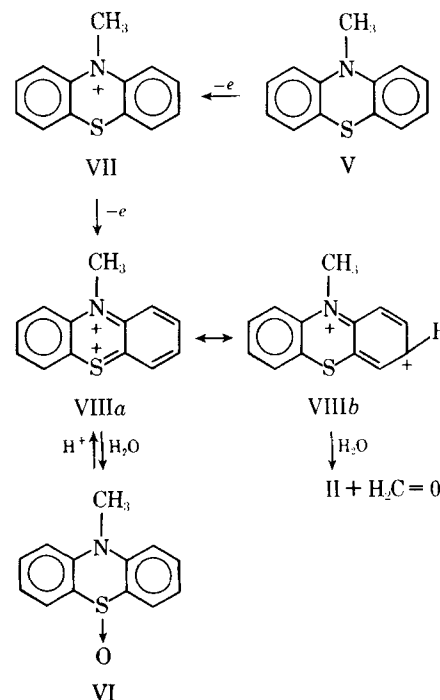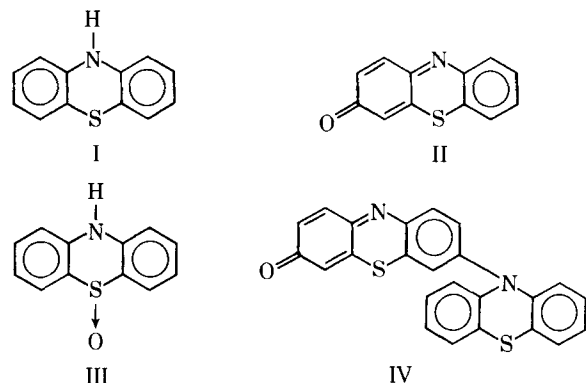
Scheme I

Table I—Apparent First-Order Rate Constants for the Degradation of I (k) and for the Appearance of II (k_3) and III (k_5) in 52.5% Ethanol at pH 2.8 at Various Temperatures

Temperature, °K	k, hr$^{-1} \times 10^3$	k_3, hr$^{-1} \times 10^4$	k_5, hr$^{-1} \times 10^3$
328	1.56	1.30	1.41
338	3.29	4.41	2.75
348	9.08	17.2	6.87
358	19.7	51.0	13.3

Table II—Rate Constants for the Disappearance of I (k) and the Appearance of II (k_3) and III (k_5) in 52.5% Ethanol at 75° at Various pH Values

pH	k, hr$^{-1} \times 10^3$	k_3, hr$^{-1} \times 10^4$	k_5, hr$^{-1} \times 10^3$
1.4	8.95	5.37	8.06
1.7	9.09	4.55	8.18
2.2	9.05	7.24	7.51
2.8	9.08	17.3	6.90
3.2	9.01	18.0	6.49
3.8	9.18	18.4	6.33
4.2	9.15	19.3	6.22
4.7	9.13	20.3	6.21
5.1	9.21	20.3	6.26
5.9	9.11	17.3	6.56
6.9	8.83	9.7	7.32

appearance of some degradation products (II–IV) in an oxygen-saturated acidic medium protected from light.

Since many commercial phenothiazines have side chains at the N-10 position, 10-methylphenothiazine (V, Scheme I) was synthesized and its degradation was investigated as a model compound. The oxidation potential of V was reported to be 150 mv higher than that of phenothiazine (2, 9), with 10-methylphenothiazine 5-oxide (VI) and 3H-phenothiazine-3-one (II) as the degradation products (10). The quantitative aspects of this degradation were not investigated.

In the current work, these products were synthesized and the influence of pH and temperature on the degradation rate was investigated after the degradation products were identified.

EXPERIMENTAL

Materials—Phenothiazine[1] gave a single spot using several TLC solvent systems and was used as supplied. Compound II, phenothiazine 5-oxide (III), and 7-(10′-phenothiazinyl)-3H-phenothiazine-3-one (IV) were prepared as previously described (8).

Compounds V (10, 11) and VI (10, 12) were synthesized as described in the literature. Compound V was recrystallized from ethanol, mp 100–101.5° [lit. (10, 11) mp 100.5–101.4°]. Compound VI was recrystallized from water, mp 194–196° [lit. (10, 12) mp 194–196°]. The structures were confirmed by IR and NMR spectroscopy.

All other materials were reagent grade, and deionized water was used throughout this study.

Isolation and Identification of Degradation Products—Compound V was dissolved in 52.5% ethanol, buffered to pH 4.7 (13), and stored in the dark at 85° for 5 days after saturation with oxygen. The products were extracted with carbon tetrachloride and isolated following TLC as described previously (8). Three zones, A, B, and C, were seen at R_f 0.80, 0.65, and 0.25, respectively.

Zone A was compared with V on the following TLC systems: silica gel GF$_{254}$ and ether–petroleum ether (1:3), silica gel GF$_{254}$ and acetone–6 N ammonia (50:1), and silica gel GF$_{254}$ and chloroform. In all cases, V and Zone A behaved similarly; they had similar IR and NMR spectra, and the mixed melting point was 99–101°.

Zone B was compared with II in the same chromatographic systems. In all cases, Zone B and II behaved similarly; they had similar IR and NMR spectra, and the mixed melting point was 159–162°.

Zone C was compared with VI in the following TLC systems: silica gel GF$_{254}$ and acetone–6 N ammonia (50:1), silica gel GF$_{254}$ and ether–petroleum ether (1:3), silica gel GF$_{254}$ and acetone–chloroform (3:2), and basic aluminum oxide and chloroform. In all cases, Zone C and VI behaved identically; they had similar IR and NMR spectra, and the mixed melting point was 193–196°. Compounds II and VI were the only degradation products of V found.

Methods—Phenothiazine was dissolved to a concentration of 500 μg/ml in a mixture of ethanol and a sodium acetate–hydrochloric acid buffer (13). The pH of this solution was measured[2] at 75°. The solution was placed in small screw-capped bottles, saturated with oxygen, and kept in an oxygen atmosphere in the dark at a fixed temperature.

Compounds I, II, and IV were determined spectrophotometrically after extraction from the reaction mixture with carbon tetrachloride and separation on a column of acidic alumina as previously described (14).

Compound III could be determined polarographically without isolation from the reaction mixture (14). The influence of temperature, pH, and ethanol concentration (weight per weight) on the degradation rate was followed in this manner.

The degradation kinetics of V were studied similarly. In this case, II was determined quantitatively as previously described (14) but without the chromatographic stage; V was determined by high-pressure liquid chromatography[3] using a silica[4] column and a UV detector. The solvent was a mixture of methanol and water (65:35) with a flow rate of 1 ml/min, and phenothiazine was the internal standard. The only other degradation product, VI, was determined by difference.

RESULTS AND DISCUSSION

Phenothiazine Degradation—*Temperature*—Under all circumstances, the degradation was always first order with respect to phenothiazine. Table I gives the observed rate constants at various temperatures for the oxidation in 52.5% ethanol at pH 2.8. The Arrhenius plot gives an E_a of 20.4 kcal/mole. This energy of activation includes a term for the variation of the oxygen solubility with temperature. The decreased solubility of oxygen with increasing temperature is partially compensated for by the increased solubility due to increased oxygen pressure above the solution as the temperature is raised.

If II–IV are formed by parallel reactions, then their appearance should satisfy the following equation (15):

$$A_i = A_0 + \frac{k_i}{k} P_0 (1 - e^{-kt}) \qquad \text{(Eq. 1)}$$

where A_i is the concentration of the degradation product at time t; P_0 and A_0 are the initial concentrations of phenothiazine and the degradation product, respectively; k is the first-order rate constant for phenothiazine degradation; and k_i is the first-order rate of appearance of A. A plot of A_i against $(1 - e^{-kt})$ should give a straight line if parallel re-
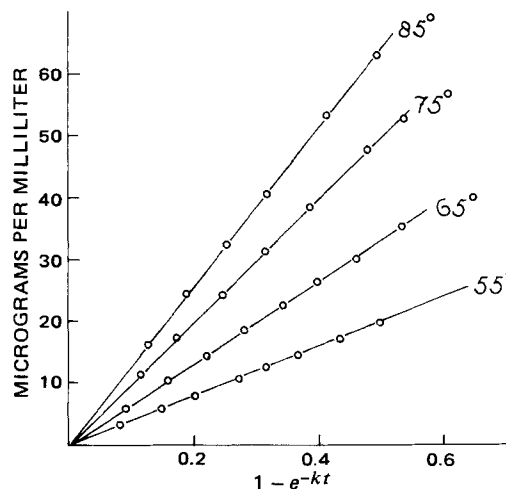

Figure 1—*Amount of II formed as a function of time at various temperatures in 52.5% ethanol at pH 2.8.*
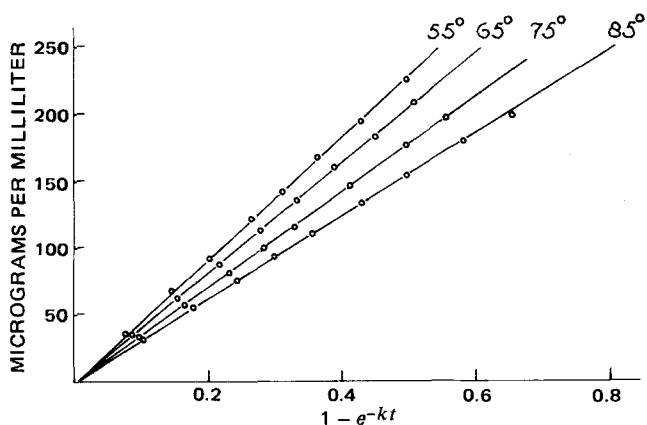
Figure 2—*Amount of III formed as a function of time at various temperatures in 52.5% ethanol at pH 2.8.*

action kinetics are obeyed, the value of the slope allowing k_i to be calculated.

Figures 1–3 show the plots for II, III, and IV, respectively, as a function of temperature. Compounds II and III seem to be formed via parallel reactions whereas IV does not; an intermediate product probably is involved in this latter case. The effects of temperature on the slopes in Figs. 1 and 2 are clearly very different, indicating that temperature greatly influences the amount of II and III formed.

The derived reaction rate constants for the appearance of II and III as a function of temperature are given in Table I. The Arrhenius plot gives an E_a of 17.9 kcal/mole for III and 29.2 kcal/mole for II. Table I also shows that III is the major degradation product.

Influence of pH—Table II lists the k, k_5, and k_3 values at different pH levels at a constant temperature of 75°. The values for k_3 at pH 1.4 and 1.7 are inaccurate because of the instability of II at these pH values; the values are calculated from the initial slope of the A_3 versus $(1 - e^{-kt})$ plot, because the plot is only a straight line in the early stages of the degradation at these pH values. Table II shows that phenothiazine degradation is pH independent, but at pH 4.7 there is a maximum in the formation of II and a minimum in the formation of III. This result could be due to the formation of II from III at this pH, so a solution of III in 52.5% ethanol at pH 4.7 was saturated with oxygen and assayed at 24, 48, 72, 96, and 120 hr. The recovery of III was 101, 101, 100, 100, and 99%, respectively, which clearly shows that the minimum in the formation of III is not due to its own instability.

The formation of IV was complex, and no rate constant for the formation could be measured; however, some idea of the influence of pH could be obtained by determining the amount formed during one half-life of phenothiazine as a function of pH. Figure 4 shows a maximum in the
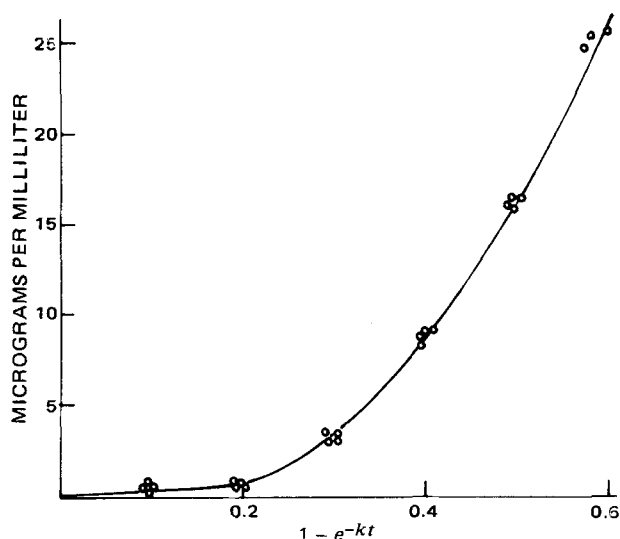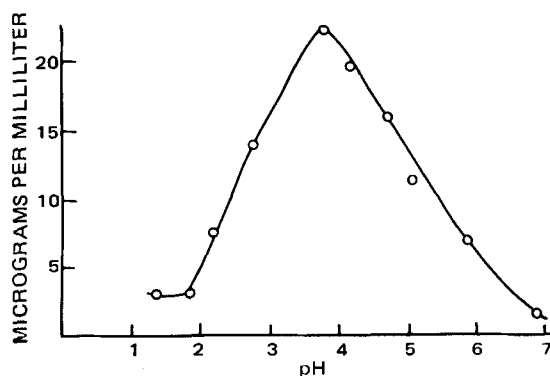

Figure 4—*Amount of IV formed during one half-life of I as a function of pH in 52.5% ethanol at 75°.*

formation at about pH 3.8.

Influence of Ethanol Concentration—Table III lists the k and k_3 values and the amounts of IV formed during one half-life of phenothiazine at pH 4.7 and 75° as a function of the ethanol concentration. The overall disappearance rate decreased with an increasing amount of ethanol in the solution, paralleling the decrease in oxygen solubility in the solvent. Both k_3 and the amount of IV produced increased with increasing ethanol concentrations. Above 62% ethanol, all changes were very small. Changes in ethanol concentration did not appear to alter significantly the amount of III, the major degradation product, formed.

These results show that II and III are formed from I by parallel reactions but do not elucidate the mechanism for the formation of the dimer. The dimer can be formed by at least two mechanisms: a coupling between I and II and a dimerization of I and its subsequent oxidation. An attempt to elucidate the total mechanism of degradation is reported in a companion paper (16).

Degradation of V—Under all conditions studied, V appears to degrade by a first-order process. Compounds II and VI, the only degradation products, seem to appear by parallel reactions. Treating the kinetic data as described for I gives the rate constants for the degradation of V (k) and for the formation of II (k_3) and VI (k_5) in 52.5% ethanol at pH 4.7 (Table IV). The pH has no influence on the degradation rate of V but does influence k_3 and k_5 and thus the relative amounts of degradation products formed (Table V).

Scheme I gives a probable mechanism for the degradation of V. It is oxidized in two steps to the phenazothionium ion that exists in two mesomeric forms, VIIIa and VIIIb, which hydrolyze to VI and II, respectively. No hydrogen ions are involved in the formation of VIII from V, so the degradation is pH independent (Table V) and there is probably no general acid–base catalysis. The hydrolysis of the ion VIIIa to VI, however, is reversed by hydrogen ions. Therefore, at lower pH values, some II is formed; but at pH 6.9, this amount was negligible. Table V shows that at pH 1.7 less II is formed than at pH 3.8; however, II is unstable at the lower pH.

At the lower pH, a small amount of 7-hydroxy-$3H$-phenothiazine-3-one is formed. The energy of activation for the degradation of V is 16.9

Figure 3—*Amount of IV formed as a function of time at various temperatures in 52.5% ethanol at pH 2.8.*

Table III—**Rate Constants for the Degradation of I (k) and the Appearance of II (k_3) and the Amounts of IV Formed during One Half-Life of I at 75° at pH 4.7 at Various Ethanol Concentrations**

Ethanol, %	k, hr^{-1} × 10^3	k_3, hr^{-1} × 10^3	IV, μg/ml
47.5	17.4	3.31	11.3
52.5	9.08	2.00	16.2
62.0	6.40	1.60	34.5
72.7	6.19	1.55	32.5

Table IV—**Rate Constants for the Degradation of V and the Appearance of II (k_3) and VI (k_5) in 52.5% Ethanol at pH 4.7**

Temperature, °K	k, hr^{-1} × 10^4	k_3, hr^{-1} × 10^4	k_5, hr^{-1} × 10^4
358	14.3	7.1	7.2
353	10.6	4.5	6.1
348	7.5	2.9	4.6
343	5.1	1.4	3.7

Table V—Rate Constants for the Degradation of V and the Appearance of II (k_3) and VI (k_5) in 52.5% Ethanol at 85°

pH	k, hr^{-1} × 10^3	k_3, hr^{-1} × 10^3	k_5, hr^{-1} × 10^3
1.7	1.37	0.58	0.79
3.8	1.30	0.75	0.55
4.7	1.43	0.70	0.73
6.9	1.27	—	1.27

kcal/mole; for the formation of VI and II, it is 10.3 and 26.7 kcal/mole, respectively. This large difference results because VIIIb is much less favored than VIIIa, as in the degradation of I.

Comparison of the rate constants for the degradation of I and V under the same conditions shows that V is less easily oxidized. This finding is unexpected because the introduction of a methyl group should increase the electron density in the ring system and, therefore, favor the loss of an electron. This explanation can be a steric effect. Phenothiazines are bent around the nitrogen–sulfur axes (17), resulting in two possible conformations. In one conformation, the lone pair on the nitrogen can easily interact with π electrons of the ring system; in the other, the lone pair is forced outside the system. In I itself, the configuration is such that interaction is possible; however, in V, the lone pair is forced outside the system. The methyl group thus decreases the electron density in the aromatic system and increases the stability.

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Mechanism for Phenothiazine Oxidation

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Abstract □ The mechanism of phenothiazine degradation was studied by following the degradation of 3,10'-diphenothiazine in ethanol–water mixtures as well as the electrochemical oxidation of phenothiazine. A mechanism, including the formation of an oxidized dimer and some polymers, is suggested.

Keyphrases □ Phenothiazine—electrochemical oxidation degradation in ethanol–water mixtures, mechanism suggested □ Oxidation—phenothiazine degradation in ethanol–water mixtures, mechanism suggested □ Degradation—phenothiazine in ethanol–water mixtures, mechanism suggested

The isolation and identification of some degradation products of phenothiazine were described previously (1), as were the kinetics of the oxidative degradation in an acidic medium (2). The degradation has been the subject of many investigations (3–9); however, no definite mechanism was established, particularly since 7-(10'-phenothiazinyl)-3H-phenothiazine-3-one was not recognized as a degradation product until recently (1, 2).

In this paper, further experiments to elucidate the mechanism of formation of 7-(10'-phenothiazinyl)-3H-phenothiazine-3-one (XIV) are described, and a mechanism for the overall oxidation is proposed (Scheme I).

EXPERIMENTAL

Materials—All materials were as previously described (2).

Phenothiazine (I) Oxidation in Presence of 3H-Phenothiazine-3-one (VII)—In a mixture of ethanol and water buffered with sodium acetate and hydrochloric acid, 500 μg of I/ml and 50 μg of VII/ml were dissolved. The solutions were saturated with oxygen, placed in screw-capped bottles, and stored in the dark at 75°. The solutions were assayed for I, VII, and XIV as described previously (10).

Oxidation of 3,10'-Diphenothiazine (IXb)—Solutions of 100 μg of IXb/ml were prepared and stored as described. The solution was extracted with carbon tetrachloride; the products were separated by TLC, using silica gel GF$_{254}$ and developing with ether–petroleum ether (1:2). The products were assayed as described previously (10).

Electrochemical Oxidation of I—After dissolving 100 μg of I/ml in the buffered ethanol–water and flushing with nitrogen, a current–voltage curve[1] was recorded at various pH values, using a rotating platinum electrode and a silver–silver chloride reference electrode. Then I was oxidized at a constant potential[2], using a platinum working and auxiliary electrode and a silver–silver chloride reference electrode. The potential of the working electrode was 400 mv throughout this study.

The solution was stirred by bubbling nitrogen continuously through it. After completion of the reaction, the solution was allowed to stand in the dark in a nitrogen atmosphere until a permanent color was obtained.