Electron Paramagnetic Resonance of Undissociated *p*-Benzosemiquinones

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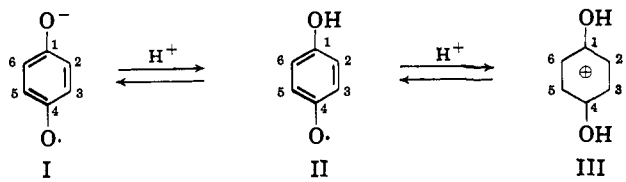
Semiquinones formed by the oxidation of hydroquinones in acidic solution have been studied by paramagnetic resonance absorption spectroscopy with a rapid liquid flow system. A change in the hyperfine pattern has been observed in pH 3–4 solutions which is attributed to the undissociated form of the semiquinones. The pK for proton dissociation of p-benzosemiquinone is estimated at 4.25, and higher pK values are suggested for tolu-p-benzosemiquinone and t-butylbenzosemiquinone.

During the slow reduction of quinone and related compounds, Michaelis, *et al.*,[2] were the first to identify the intermediate semiquinone magnetically by showing that the reduction was accompanied by a diminution of the measured diamagnetic susceptibility, followed by a rise to a final steady level. Recently a considerable number of benzosemiquinone derivatives have been studied in detail by electron paramagnetic resonance (e.p.r.).[3–5] The intermediate semiquinones are usually more stable in basic than in acidic solution because of the formation of the symmetrical semiquinone ion, and most of the studies on semiquinones have been done in alkaline solutions.

The intermediate *p*-benzosemiquinone has also been detected and identified by e.p.r. during the two-step oxidation of hydroquinone catalyzed by enzymes such as peroxidase[6] and laccase.[7] In the regions of low pH where the peroxidase reaction is favorable and the semiquinone is unstable, a continuous flow process was used to maintain a constant radical concentration during the reaction. The hyperfine pattern of the semiquinones at pH 4.8 was the same as that obtained in basic solution, and it was postulated that the semiquinone even at these pH values is dissociated and, therefore, ionic. The proton dissociation and association of *p*-benzosemiquinone can be schematized as



I II III

The negative semiquinone ion I and the hydroquinone cation III are stable owing to their symmetrical structure, and the symmetry in structure is verified by their observed hyperfine patterns.[3,8] The undissociated semiquinone II, however, is not symmetrical and would be expected to show a different hyperfine pattern, owing to a strong coupling at the C-2 and C-6 positions or the C-3 and C-5 positions depending only upon where protonation occurs, and be very unstable.

In order to confirm the existence of II in acidic pH during the oxidation of hydroquinone and its derivatives by 1 equiv. of oxidants, such as Ce^{4+} or peroxidase–H_2O_2, a continuous flow system was adapted to the e.p.r. spectrometer, and the results are reported in this paper.

Experimental

Horse radish peroxidase (E_{403}/E_{270} = 3.0) was purified and crystallized by the method of Kenten and Mann.[9] The concentration of peroxidase was determined by assuming that the milimolar extinction coefficient at 403 mμ is 90 cm.$^{-1}$.

The e.p.r. spectrometer used was a Varian V-4500 x-band instrument, utilizing 100-kc. field modulation and Fieldial® control of the magnetic field. The flow apparatus was a modified version of that described previously.[6] Here, we used two different flow rates; the fast flow rate being about 6 ml./sec. and the slow flow rate about 2 ml./sec. As the volume from mixer to the center of the cavity was 0.135 ± 0.003 ml., the reaction times from mixing to observation at the center of the cavity were kept constant during constant flow and were approximately 0.02 sec. in the fast flow and 0.06 sec. in the slow flow.

Results

1. Hydroquinone. The e.p.r. detection of *p*-benzosemiquinone has been observed in alkaline solutions of hydroquinone or *p*-quinone with addition of oxidants or reductants, respectively. A definite amount of *p*-benzosemiquinone can also be observed in a water solution of quinhydrone at neutral pH.[10] At acidic pH, however, the equilibrium concentration of *p*-benzosemiquinone in a static experiment where the reaction is carried out in a sample tube and then placed in the spectrometer is below the sensitivity of the spectrometer, and continuous flow methods are necessary in order to accumulate a sufficient concentration of semiquinone in the steady state.

It is reasonable to assume that, certainly at low pH, the oxidation of hydroquinone by 1 equiv. of oxidants goes in two simple steps,[6] namely, the formation of

(1) (a) Hokkaido University; (b) Varian Associates.
(2) (a) L. Michaelis, M. P. Shubert, R. K. Reber, J. A. Kuck, and S. Granick, *J. Am. Chem. Soc.*, **60**, 1678 (1938); (b) L. Michaelis and S. Granick, *ibid.*, 70, 624 (1948).
(3) B. Venkataraman and G. K. Fraenkel, *ibid.*, 77, 2707 (1955).
(4) B. Venkataraman, B. G. Segal, and G. K. Fraenkel, *J. Chem. Phys.*, 30, 1006 (1959).
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(7) T. Nakamura, "Free Radicals in Biological Systems," M. S. Blois, *et al.*, Ed., Academic Press, Inc., New York N. Y., 1961, p. 169.
(8) J. R. Bolton and A. Carrington, *Proc. Chem. Soc.*, 385 (1961); *Mol. Phys.*, **5**, 161 (1962); **6**, 169 (1963).
(9) R. H. Kenten and P. J. A. Mann, *Biochem. J.*, **57**, 347 (1954).
(10) H. S. Mason, G. Narni, and I. Yamazaki, Abstracts, International Biophysics Congress, Stockholm, 1961, p. 328.

semiquinones and their subsequent decay. If the decay velocity (V_d) of the semiquinone is fast enough, for example, if the velocity constant for dismutation is around 10^8, the concentration of the semiquinone will reach the steady state (velocity of free-radical formation is equal to V_d) within less than 0.1 sec. The dominant mechanism of decay of the semiquinones is a simple dismutation reaction provided that the hydroquinone concentration is high and the dismutation constant for the semiquinone is large.[6] Under these circumstances, $V_d = 2K_d(SQ\cdot)^2$ and $(SQ\cdot)_s = (V_f/2k_d)^{1/2}$, where k_d and $(SQ\cdot)_s$ are the dismutation constant and the steady-state concentration of semiquinone, respectively. The steady-state concentration of semiquinone is proportional then to the square root of the rate of semiquinone formation. Since the formation rate is proportional to the hydroquinone concentration, reasonable steady-state semiquinone concentrations are obtained only when the hydroquinone concentration is kept as high as possible. The oxidation of hydroquinone by ceric ion is very fast even at acidic pH, and the reaction is over within several seconds when 0.01 *M* ceric solution is mixed with an equal volume of 0.24 *M* hydroquinone solution. The disappearance of reactants causes the decrease in the velocity of formation and consequently in the steady-state concentration of semiquinone.

Figure 1 shows the hyperfine pattern of *p*-semiquinone obtained during a continuous slow flow. At pH 5.2, the pattern is the same as that observed at neutral or alkaline pH with a splitting constant of 2.3 gauss and an intensity ratio of 1:4:6:4:1. As the pH decreases, a remarkable change in the intensity ratios is observed; 1:1.9:4.5:1.9:0.87 at pH 4.2 and 1:0.85:4.4:0.82:1 at pH 3.9. The quintet appears to be giving way to a triplet. In addition to the ceric system, the peroxidase system was also used as the oxidant at pH 3.7. Peroxidase has one heme iron per molecule as its active site, and its concentration is less than 1 μM in the reaction mixture. As shown in Figure 2 the different oxidizing systems give no essential change in the hyperfine pattern. The intensity ratio is 1:0.61:3.9:0.54:1 in the ceric system and 1:0.61:4.5:0.54:1 in the peroxidase system. This enzyme is inactive at the extreme acidic pH values, and pH 3.7 is almost the lower limit in which it can be used as a catalyst. As shown in Figure 2, a fast continuous flow, 6 ml./sec., gives a slightly larger signal than that of a slow flow. This is due to the fact that the formation is over in the mixing chamber, and the semiquinone is decaying in the time from mixing to observation. Fast flow cuts this time down but does not allow enough time to sweep the entire spectrum because of limited volumes in the flow system. The intensity ratio of the first three lines is 1:0.53:3.7. The dependency of the second line which is a part of the original quintet upon pH is quite remarkable, and at pH 3.2 it almost disappears (Figure 3). However, the splitting between the first and central lines remains constant over the entire pH range. The intensity ratios of the first three lines are 1:0.38:4.2 at pH 3.5 and 1:0.06:4.4 at pH 3.2. The poor S/N ratio of these signals is due to the low stationary concentration of the semiquinone and also because of the short response times (usually 0.001 sec.) required in order to sweep the field as rapidly as possible.

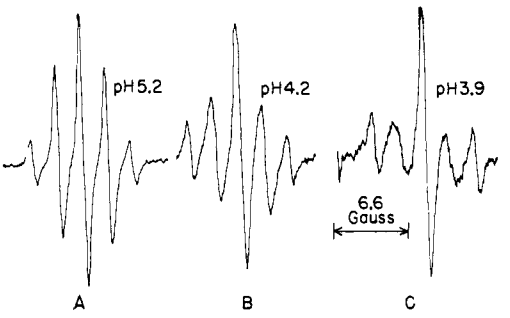


Figure 1. E.p.r. spectra of *p*-benzosemiquinone in solution, pH 5.2 (A), 4.2 (B), and 3.9 (C) during slow flow.

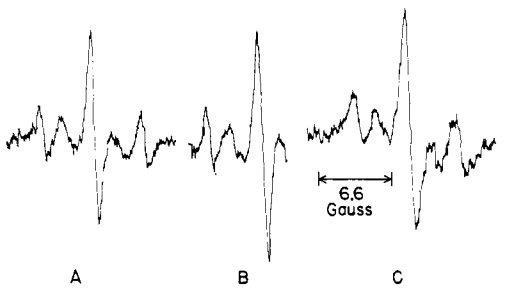
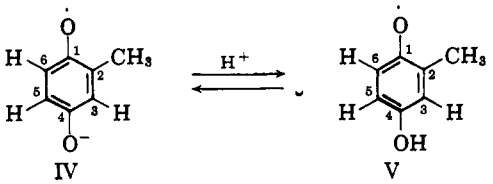


Figure 2. E.p.r. spectra of *p*-benzosemiquinone in solution, pH 3.7, of 420 m*M* hydroquinone mixed with 10 m*M* Ce^{4+}; slow flow (A) and fast flow (B). A solution of 200 m*M* hydroquinone and 1.6 μM peroxidase was mixed with 50 m*M* hydrogen peroxide; slow flow (C).

2. Toluhydroquinone. The e.p.r. of tolu-*p*-benzosemiquinone has been observed by Venkataraman and Fraenkel[3,4] in an alkaline alcoholic solution. Using the flow system as above, the same hyperfine pattern is obtained at neutral pH. The pattern is explained by a strong interaction with the protons of the methyl group and a different coupling with the three ring protons. The methyl coupling is almost equivalent to that of the single proton on C-6. As shown in Figures 4 and 5, with decreasing pH, the hyperfine pattern again changed remarkably and finally only a weak quintet is observed at pH 3.7. The spectrum might be interpreted by assuming that the hyperfine interaction is with the three equivalent methyl protons and again the single proton on C-6 as shown in the structure V.



IV V

3. t-Butylhydroquinone. The e.p.r. spectrum of *t*-butyl-*p*-benzosemiquinone at neutral pH gives an eight-line pattern of approximately equal intensity resulting from the nonequivalence of the three ring protons and essentially zero coupling of the *t*-butyl protons (Figure 6). At pH 4.0, the eight-line hyperfine pattern disappears and a broad doublet takes its place. The doublet can only be explained if one assumes the structure for the undissociated *t*-butylbenzosemiquinone to be VII. Because of the low solubility of *t*-butylhydroquinone at low pH, 4.0 was as low a pH as we

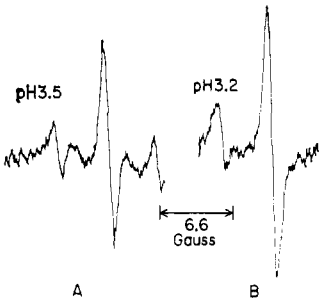


Figure 3. E.p.r. spectra of *p*-benzosemiquinone in solution, pH 3.5 (A) and 3.2 (B); 240 m*M* hydroquinone was mixed with 10 m*M* Ce^{4+}; fast flow.

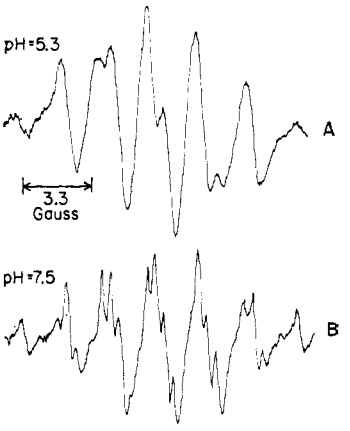


Figure 4. E.p.r. spectra of tolu-*p*-benzosemiquinone in solution, pH 5.3 (A) and 7.5 (B); 100 m*M* toluhydroquinone was mixed with 10 m*M* Ce^{4+}; slow flow.

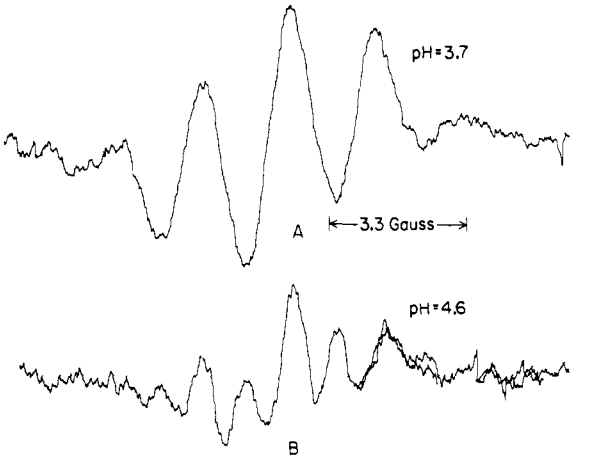


Figure 5. E.p.r. spectra of tolu-*p*-benzosemiquinone in solution, pH 3.7 (A) and 4.6 (B); 100 m*M* toluhydroquinone was mixed with 10 m*M* Ce^{4+}; slow flow.

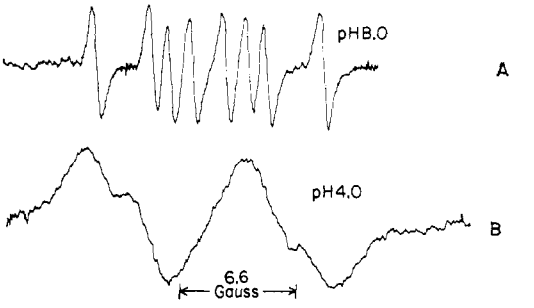
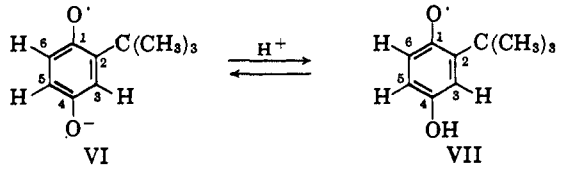


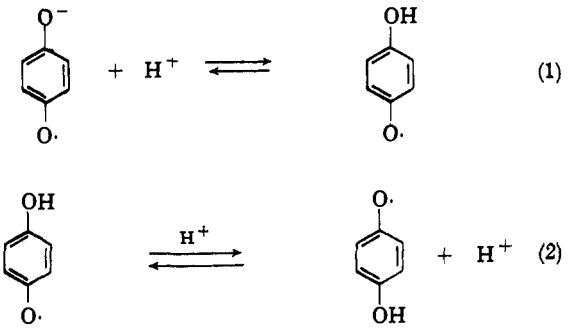
Figure 6. E.p.r. spectra of *t*-butyl-*p*-benzosemiquinone in solution at pH 8.0 (A) and 4.0 (B); 30 m*M* *t*-butylhydroquinone was mixed with 5 m*M* Ce^{4+}; slow flow.

could go. The doublet results from a strong coupling to the single ring hydrogen on the C-6 carbon.



Discussion

For each of the semiquinones studied, it was observed that, as the pH is decreased, the observed hyperfine pattern in alkaline and neutral pH values changes dramatically to much simpler patterns. This change seems to be associated with a change in the coupling of the unpaired electron to the various ring hydrogens as a result of protonation of the oxygen. A five-line spectrum with a splitting of 2.3 gauss is observed for the dissociated *p*-benzosemiquinone and a three-line spectrum with a splitting of 4.6 gauss for the undissociated molecule (pH 3.2). The triplet pattern is assumed to result from a coupling with the two hydrogens on C-2 and C-6 or C-3 and C-5 depending upon which oxygen is protonated. Since this coupling is twice that observed for the dissociated semiquinone and assuming the total spin density in the ring is constant, the spin density at these carbons must now be twice what it was for the dissociated semiquinone. This is easily understood by simply assuming that the dissociated semiquinone ion has twice as many resonant structures as the undissociated one. Atherton, *et al.*,[11] have calculated the spin density in undissociated *p*-benzosemiquinone by the LCAO–MO treatment and assigned a density of 0.50 to the *ortho* carbon atom which is almost twice the spin density reported for the four equivalent carbon atoms of the *p*-benzosemiquinone ion. However, an intensity ratio of the signal at pH 3.2 observed here is 1:4.4:1, which is quite different from the theoretical ratio of a simple triplet spectrum of 1:2:1. This deviation from a simple 1:2:1 triplet can be explained by assuming that either there is an exchange taking place both between the ion



and the protonated specie (reaction 1) or between the two different oxygens in the protonated species as indicated in reaction 2. In the region of slow exchange

(11) N. M. Atherton, E. J. Land, and G. Porter, *Trans. Faraday Soc.*, **59**, 818 (1963).

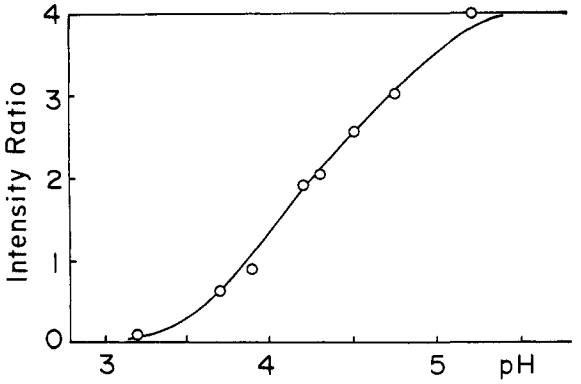


Figure 7. pH dependency of the intensity ratio of the second line to the first line of the e.p.r. signals of the *p*-benzosemiquinone.

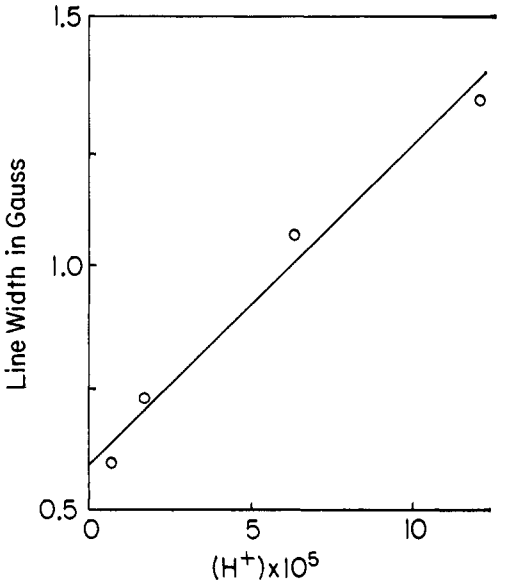


Figure 8. Line-width dependence of *p*-benzosemiquinone ion on (H^+). Slope gives rate of H^+ ion exchange between semiquinone ion radical and semiquinone radical.

of reaction 1 and with the equilibrium to the left, one would predict merely a broadening of the second and fourth peaks of the quintet of the ion assuming the total spin densities on the ring carbons nearly unchanged. However, during intermediate exchange between the ion and the monoprotonated species, one would predict a triplet of intensity 1:4:1. In the region of very fast exchange of the hydrogen between the two oxygens of the protonated specie or between two protonated species, the spectrum should be a 1:4:6:4:1 quintet again. These spectra would result no matter whether the splitting of the C-2 and C-6 hydrogens resulting from protonation at the C-1 oxygen was zero or unchanged from that of the ion. It is most likely, however, that the C-2 and C-6 splitting is very close to zero. In the *t*-butylhydroquinone radical the splitting of the broad doublet is less than twice that of the large doublet splitting arising from the C-6 hydrogen of the ion. This smaller splitting in the radical and the breadth of the lines are further evidence to indicate that an intermediate exchange rate is being observed and that the splittings of the C-3 and C-5 hydrogens are smaller for the radical but probably not zero.

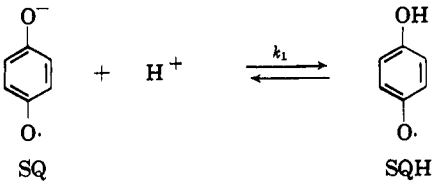
If the intensity ratio of the second line to the first line is plotted against pH for hydroquinone, a typical dissociation curve is obtained as shown in Figure 7 and yields a pK of 4.25. Although it is much more difficult to estimate the pK for tolu-*p*-benzosemiquinone and *t*-butyl-*p*-benzosemiquinone, it is reasonable to assume slightly higher pK values than 4.25 judging from the pH dependence of the e.p.r. pattern for their respective semiquinones.

It is interesting to note that this e.p.r. method is the only way to determine on which oxygen protonation first occurs in unsymmetrical semiquinone ions. In the case of the toluhydroquinone if the proton had gone on the oxygen on carbon C-1, the resulting hyperfine pattern of this undissociated molecule would have been a triplet or possibly a doublet of doublets instead of a quintet. Also, in the case of *t*-butylhydroquinone the result is even more convincing. If the proton had gone on the oxygen or C-1 in this case, the spectrum would again have been a triplet instead of a doublet as observed.

A consideration of steric hindrance and the strong inductive interaction by substitution of bulky groups on C-2 would predict protonation in the substituted molecules on the oxygen furthest removed from the substituted groups.

Rate of Protonation. Not only does the hyperfine pattern change with pH but, as mentioned above, the line widths of certain lines of the dissociated semiquinone are observed to broaden with pH and finally disappear. In the region of slow exchange and when the equilibrium of reaction 1 lies to the left, we can estimate the rate of protonation by monitoring the change in line width.

We can write a rate equation for the rate of protonation of the semiquinone from the equation



$$\frac{d(SQ)}{dt} = k_1(SQ)(H^+) \qquad (3)$$

$R/(SQ) = k$ where $R = d(SQ)/dt$, and $k_1(H^+) = k$ is the pseudo-first-order rate constant.

$$\frac{R}{(SQ)} = k = \frac{1}{\tau} = \frac{\sqrt{3}\,\Delta H\gamma}{2} - \frac{1}{T_2} \qquad (4)$$

where τ is the mean resonance lifetime of species (SQ), ΔH is the line width at half-maximum in gauss, and $(T_2)^{-1}$ is the natural line width in the absence of protonation. Equation 4 becomes

$$\frac{\sqrt{3}\Delta H\gamma}{2} = k_1(H^+) + \frac{1}{T_2} \qquad (5)$$

A plot of ΔH *vs.* (H^+) should yield a straight line, the slope of which is $2k_1/\sqrt{3}\,\gamma$. Figure 8 shows a plot of eq. 5 in which k_1 is found to be $1.5 \times 10^{10}\ M^{-1}$ sec.$^{-1}$.

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Kinetics of the Thallic Ion Oxidation of Olefins. I. Salt Effects on the Oxidation of Ethylene

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The oxidation of ethylene, $Tl^{+3} + CH_2{=}CH_2$ $(H_2O) \rightarrow CH_3CHO + (CH_2OH)_2$, is first order in ethylene, first order in thallic ion, and strongly accelerated by increasing salt concentration. This acceleration is of the type previously observed for the mercuration of benzene; i.e., the rate constant is an inverse function of the activity of water. This reaction is thus another example of the activation of metal ions by dehydration. The order of magnitude of the effect in various media, perchlorate > sulfate > nitrate, can be explained by deactivation of the metal ion by ion-pair formation. A mechanism for the reaction is proposed in terms of π- and σ-bonded intermediates, and the possible identity of the step of the mechanism most affected by the rate acceleration is discussed.

Recently, aqueous thallic ion was reported to oxidize olefins to glycols and carbonyl compounds,[1] whereas, in acetic acid and methanol, 1,1- and 1,2-disubstituted as well as allylic oxidation products are found.[2-4] Although the mechanism of the allylic oxidation is uncertain, the evidence suggests that the other products arise from the oxidative decomposition of a σ-bonded oxythallation adduct analogous to the stable product of oxymercuration of olefins.[5]

This paper describes a kinetic study of the oxidation of ethylene in aqueous solution. Of special concern are the large salt effects observed for this reaction. Rate acceleration of the same magnitude has been observed for some reactions of aqueous mercuric ion with unsaturated organic molecules.[6,7]

Perrin and Westheimer[8] have demonstrated that for mercuration of benzene the rate acceleration is not related to acidity but rather is an inverse function of the water activity.

Results

The reaction was studied in aqueous sulfuric, nitric, and perchloric acids as well as in mixtures of perchloric acid and sodium perchlorate. All rates were measured by ethylene uptake and most runs were made at a constant ethylene pressure of 1 atm.

Order of Reaction and Products. Plotting the data at atmospheric pressure as first-order reactions, presuming the decrease in thallic ion concentration is proportional to the ethylene uptake, gave linear plots indicating that the reaction is first order in thallic ion. The final reaction mixtures contained no thallic ion and the number of moles of ethylene taken up in excess of solubility was equal to the number of moles of thallic ion reduced.

The order of ethylene was determined by running several reactions in a constant volume reactor.[9] When the data were plotted as a reaction first order in ethylene and first order in thallic ion, linear plots were obtained, proving the validity of the assumption. The second-order rate constants agreed well with those obtained in atmospheric pressure runs (see Table I).

Table I. Kinetic Data. Perchlorate System

$HClO_4$, *M*	$NaClO_4$, *M*	Solubility of ethylene, m*M*	k_2,[a] M^{-1} sec.$^{-1}$	−Log a_{H_2O}
0.35	...	4.72	0.45	0.006
0.50[b]	...	4.64	0.63	0.009
0.85	...	4.45	0.75	0.015
1.35	...	4.30	0.97	0.026
0.5	1.0	3.40	1.35	0.029
1.5	...	4.25	1.08	0.030
1.85	...	4.14	1.53	0.039
1.15	1.12	2.85	2.50	0.045
0.5	2.0	2.70	2.67	0.048
2.25	...	4.01	2.17	0.052
2.5	...	3.93	3.08	0.061
2.5[b]	...	3.93	2.97	0.061
0.35	3.0	2.15	4.84	0.067
1.15	2.12	2.20	4.67	0.070
0.5	3.0	2.05	6.17	0.071
2.75	...	3.85	4.50	0.072
3.0	...	3.76	5.59	0.082
0.5	3.5	1.82	8.51	0.087
3.25	...	3.69	7.18	0.094
0.5	4.0	1.60	13.0	0.103
3.5	...	3.61	8.25	0.106
3.75	...	3.53	9.34	0.119
0.5	5.0	1.08	25.5	0.134
4.0	...	3.45	12.1	0.135
4.25	...	3.37	13.2	0.156
0.5	5.5	0.9	40.0	0.159

[a] Most of these values are an average of at least two runs. [b] Run in constant volume reactor.

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