Chemical and Electrochemical Oxidation of 7-Hydroxychlorpromazine

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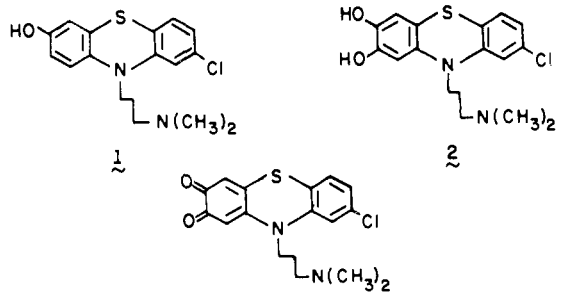
The oxidation of 7-hydroxychlorpromazine, a process associated with several side effects of chlorpromazine therapy, was examined in vitro by electrochemistry and rapid-scanning spectrophotometry. At pH 2, the oxidation results in a quantitative yield of 7,8-dioxochlorpromazine, but several intermediates are observable during the course of the reaction. These include a quinone imine with a half-life of 0.1 s, a monosubstituted benzoquinone with a half-life of ~50 s, and a disubstituted benzoquinone with a half-life of ~5 min. The concentrations of each intermediate were determined quantitatively as a function of time, and a complete oxidation mechanism is proposed. At pH 7, the yield of 7,8-dioxochlorpromazine is less than at pH 2, and an additional reaction pathway involving direct hydroxylation of the quinone imine is observed. The relationship of these reactions to the pharmacology of the hydroxylated chlorpromazine metabolites is discussed.

The chemistry and pharmacology of the hydroxylated derivatives of chlorpromazine have been examined in several different laboratories for at least three reasons. First, the formation of 7-hydroxychlorpromazine (1) and 7,8-dihydroxychlorpromazine (2) comprises a major metabolic route of chlorpromazine both in animals and in humans.[1-4] Second, the 7-hydroxy derivative is compa-



1 2 3

rable to chlorpromazine in pharmacological activity as measured by several animal tests, such as barbiturate sleeping time and motor activity,[5,6] and also has similar effects on biochemical processes, such as serum prolactin levels and mitochondrial calcium accumulation.[7,8] In addition, blood levels of 7-hydroxychlorpromazine have been correlated with improvement of psychotic symptoms in mental patients, a correlation which was not as evident for chlorpromazine sulfoxide or chlorpromazine itself.[9,10] Third, both hydroxy derivatives have been associated with several of the side effects of long-term chlorpromazine therapy, such as skin pigmentation and corneal opacity.[11-13] The mechanism of this toxicity and its relationship to oxidation–reduction reactions of the hydroxylated derivatives have been investigated in several independent studies. In one investigation, it was found that 1 was hydroxylated to 2 and then oxidized to 7,8-dioxochlorpromazine (3) by mushroom tyrosinase, a model enzyme for mammalian tyrosine hydroxylase.[14] It was suggested that oxidation of 1 may eventually lead to melanin-like pigments via the formation of 3. The authors of a related investigation hypothesized that light-induced oxidation of 1 led to quinoid derivatives of phenothiazine, which in turn formed a "pseudo melanin".[11] In another study, it was found that 2 reacts with oxygen to generate cytotoxic hydrogen peroxide and hydroxyl radicals, and it was proposed that this reaction may be responsible for the side effects caused by hydroxylated chlorpromazine metabolites.[15,16]

While these pharmacological hypotheses are largely unproven, they do indicate the potential importance of oxidation of 1 and 2 to the chemistry of these chlorpromazine metabolites in the physiological environment. The viability of these theories, and their relationship to drug activity and side effects, could be better assessed if a detailed knowledge of the oxidation mechanisms of 1 and 2 were available. Accordingly, the present work was directed toward elucidating the products and mechanisms of the electrochemical and chemical oxidation of 7-hydroxy- and 7,8-dihydroxychlorpromazine. The chemical oxidation of 2 to 3 has been reported,[17] but the reaction was used synthetically and not studied in detail. Preliminary electrochemical investigation in our laboratory indicated that the oxidation of 2 is a reversible two-electron process to form 3 but that the oxidation of 1 is much more complex.[18] Thus this report emphasizes the results of a more in-depth examination of the oxidation of 1.

Experimental Section

The electrochemical techniques used here are well established and are explained in detail elsewhere.[19] Electrochemical experiments were performed using potentiostats of conventional designs based on operational amplifiers. Experiments requiring fast voltammetric scan rates (>0.5 V/s) or short electrolysis times (<2 s) were carried out with the aid of a minicomputer interfaced to a conventional potentiostat. In all cases, small-volume (~2 mL) electrochemical cells were used to minimize consumption of chlorpromazine derivatives. A graphite paste working electrode was used for voltammetry and chronoamperometry, while a carbon cloth electrode was necessary for coulometric bulk electrolysis. In all results, potentials are referred to a saturated calomel electrode (SCE).

Absorption spectra were obtained using a custom-built rapid-scanning spectrophotometer interfaced to the minicomputer. The device allowed acquisition of spectra at the rate of 150 nm/s, and up to 30 spectra could be obtained and stored in a period of 2 min. The utility of this system in the present work arose from the ability to obtain a 200-nm wide spectrum within 5 s after a reaction was initiated.

7-Hydroxychlorpromazine (1), 7,8-dihydroxychlorpromazine (2), 7,8-dioxochlorpromazine (3), 7-hydroxychlorpromazine sulfoxide, and 7-hydroxychlorpromazine methiodide were obtained from Dr. A. A. Manian of the Psychopharmacology branch of NIMH and were used without purification. Aniline hydrochloride was obtained from J. T. Baker Chemical Co. After removing the water-insoluble impurities, aniline was extracted into ether from a basic solution. Aniline hydrochloride was precipitated by adding concentrated HCl and was dried under vacuum after filtration, giving a product with a melting point of 196 °C (lit.[20] mp 198 °C). Phosphate buffer (0.2 M) at pH 2 was prepared from reagent grade KH_2PO_4 and HCl. Stock solutions of Ce^{4+} were prepared from $Ce(NH_4)_4(SO_4)_4$ dissolved in 0.5 M H_2SO_4. MacIlvaine buffer at pH 7 was prepared from stock solutions of 0.1 M citric acid and 0.2 M K_2HPO_4. Hydrogen peroxide solutions were standardized with Ce^{4+} using ferroin indicator.[21] Horseradish peroxidase (type VI) was obtained from Sigma Chemical Co.

Ce^{4+} oxidations of 1 were carried out by the rapid addition of a measured amount of Ce^{4+} from a microburet to a well-stirred solution of 1. To avoid changing the pH of the solution upon addition of the acidic Ce^{4+} reagent, the Ce^{4+} reagent concentration

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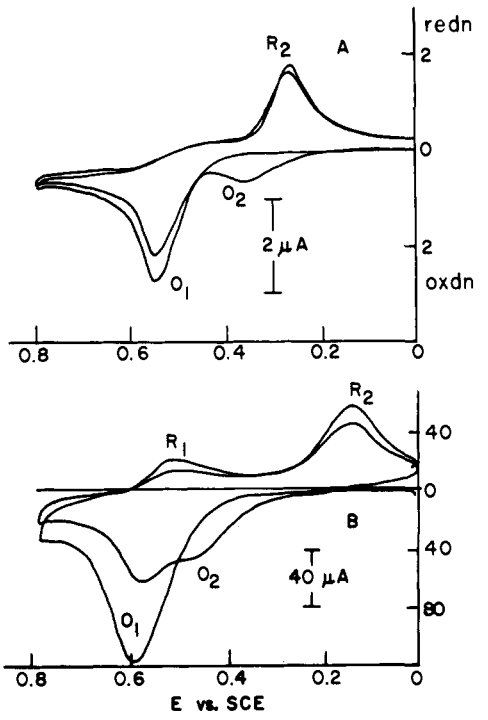


Figure 1. Cyclic voltammograms of 1 in pH 2 phosphate buffer at a graphite paste electrode. A, scan rate = 0.089 V/s; B, scan rate = 20 V/s.

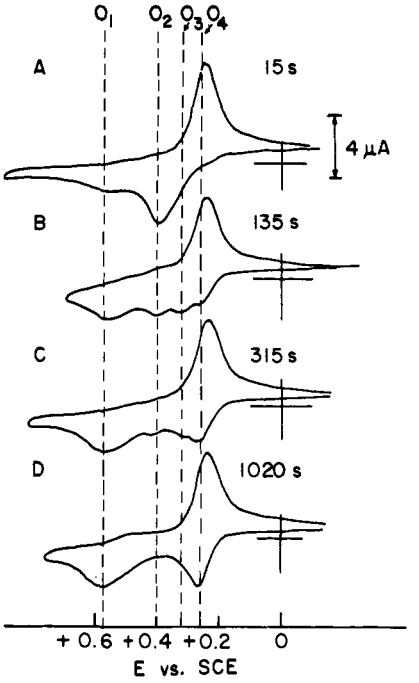


Figure 2. Cyclic voltammograms of 2.17×10^{-4} M 1 in pH 2 phosphate buffer after a two-electron Ce^{4+} oxidation. A, 15 s; B, 135 s; C, 315 s; D, 1020 s after Ce^{4+} oxidation. Graphite paste electrode, scan rate = 0.185 V/s.

was large and the volume of added reagent was small.

Results

The products and mechanisms of oxidation of 7-hydroxychlorpromazine were investigated at pH 2 and at pH 7. All experiments described below were carried out at pH 2 unless indicated otherwise. Two substantially different sets of experiments were carried out before the oxidation mechanism of 1 at pH 2 could be elucidated. In the first, electrochemical oxidation of 1 was performed, and the course of the oxidation was monitored electrochemically and spectrophotometrically. In the second, chemical oxidation was carried out by adding enough Ce^{4+} to solutions of 1 to provide 2 mol of Ce^{4+} per mole of 1. The resulting solution was carefully monitored using the same techniques.

Electrochemical Oxidation. Cyclic voltammograms of 7-hydroxychlorpromazine are shown in Figure 1. Curve A was obtained with a scan rate of 0.1 V/s, initiated in the positive direction at 0.00 V vs. SCE. Peak O_1 corresponds to the initial oxidation of 1 and has been discussed previously.[18] On the reverse scan, the lack of a reverse (reduction) wave for O_1 indicates that the initial product of oxidation is unstable on this time scale and is unavailable for reduction. The appearance of a new couple centered at +0.30 V (R_2/O_2) after the first scan indicates that a new electroactive species is formed from the initial oxidation product. Voltammogram B was obtained at a scan rate of 20 V/s. The shorter time frame allows the observation of R_1, the reduction of the short-lived material generated during O_1. The distortion of the R_2/O_2 couple in curve B is caused by the high scan rate. A half-life for the short-lived oxidation product may be estimated from these data to be 100 ms.

Controlled potential coulometry with an applied potential of +0.70 V required 3.93 electrons per mole of 1 for complete oxidation over a period of 30 min. The UV–visible spectrum and voltammogram of the final product were identical with those of 7,8-dioxochlorpromazine (3). Voltammograms taken during the coulometric oxidation of 1 at pH 2 were qualitatively identical with those at pH 4, reported elsewhere.[18] During the early portion of the electrolysis at pH 2 the solution was purple, before a gradual transition to the red color of 3.

Chronoamperometry allows oxidation to be monitored on a 0.01–1-s time scale, much faster than the tens of minutes required for coulometry. By switching on the potential at a value of +0.75 V in an unstirred solution of 1, the electroactive species immediately next to the electrode may be electrolyzed and the number of electrons involved in the oxidation may be determined from the resulting current.[19] For the first second of electrolysis, the number of electrons per molecule of 1 was a constant 1.91. In order to arrive at this value, it was necessary to assume that the diffusion coefficient of 1 is equal to that of 2, a known two-electron system.[18] Since the range of diffusion coefficients for similar molecules is about ±10%, this experiment establishes that the *initial* step in the oxidation of 1 is a two-electron process.

Chemical Oxidation. In all experiments discussed below the initial two-electron oxidation of 1 was carried out with a stoichiometric amount of Ce^{4+}. The Ce^{4+} oxidation was rapid and complete, as evidenced by the absence of 1 immediately after addition of the Ce^{4+} reagent. The resulting solution was monitored as a function of time with voltammetry, chronoamperometry, and visible spectrophotometry.

Figure 2 shows the time course of cyclic voltammograms after Ce^{4+} oxidation. In curve A, taken 15 s after Ce^{4+} addition, the only major anodic peak is O_2, the same as the follow-up couple in Figure 1, A, with a potential centered at +0.30 V. After Ce^{4+} oxidation (135 s) (curve B), three more anodic peaks are visible. With time, peaks O_2 and O_3 decrease in size, until after 17 min, only O_1 and O_4 are present (Figure 2, D). No further changes are observed in the voltammograms after 30 min. Peak O_1 has the same potential as the oxidation of 1 shown in Figure 1, A, and indicates a regeneration of 7-hydroxychlorpromazine. The redox couple at +0.24 V (R_4/O_4) in Figure 2, D, is identical with that of 3 determined under the same conditions. One

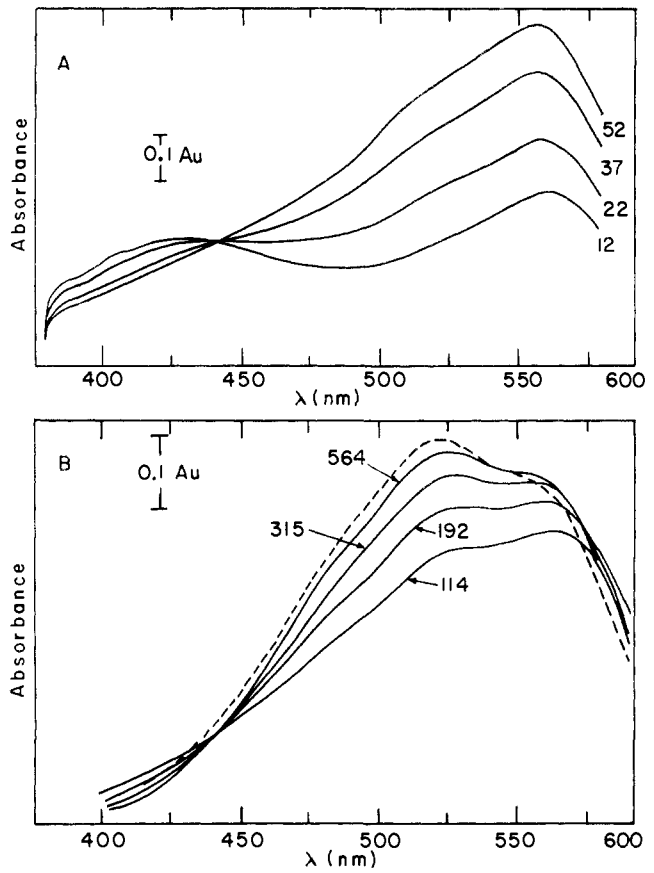


Figure 3. Spectra of 1 in pH 2 phosphate buffer vs. time after Ce^{4+} oxidation. A, 1.95×10^{-4} M initial 1, scan rate = 100 nm/s, scans started 12, 22, 37, and 52 s after Ce^{4+} addition. B, 8.00×10^{-5} M initial 1, scan rate = 20 nm/s, scans started 114, 192, 315, and 564 s after Ce^{4+} oxidation; dashed curve is the spectrum 36 min after Ce^{4+} oxidation.

concludes that the product of Ce^{4+} oxidation is reacting to form **3** and regenerate **1** in a matter of minutes. The final yields of **1** and **3** may be determined voltammetrically by comparing the peak heights of waves O_1 and O_4 with those from solutions of known concentrations of **1** and **3**. Such an analysis indicates a 51% yield of **1** and a 55% yield of **3**, based on the original concentration of **1** before Ce^{4+} oxidation.

During the first 10 min after oxidation, the overlapping voltammetric peaks make quantitation difficult. Assuming that **1** is the only oxidizable species in solution (an assumption which is justified below), chronoamperometry may be used to accurately quantitate the concentration of **1** after oxidation. By comparing the current of a chronoamperometry experiment at +0.75 V with that from solutions of known concentration, the concentration vs. time after oxidation curve for **1** was constructed. As shown in Figure 5, **1** is not present immediately after Ce^{4+} addition, but after about 3 min it reaches a level between 49 and 52% of its original value.

Spectrophotometry was used to monitor the concentrations of other species after Ce^{4+} oxidation. Thirty-six minutes after oxidation the visible spectrum was constant with time and matched the spectrum of **3**. Using the spectral characteristics of authentic solutions of **3** (λ_{max} 508 nm, molar absorptivity = 15 100 M^{-1} cm^{-1}), the yield of **3** was found to be 51%, based on the original concentration of **1**.

Visible spectra as a function of time after Ce^{4+} oxidation are shown in Figure 3. The first spectrum in Figure 3, A, taken 12 s after oxidation of **1**, has two peaks at 424 and

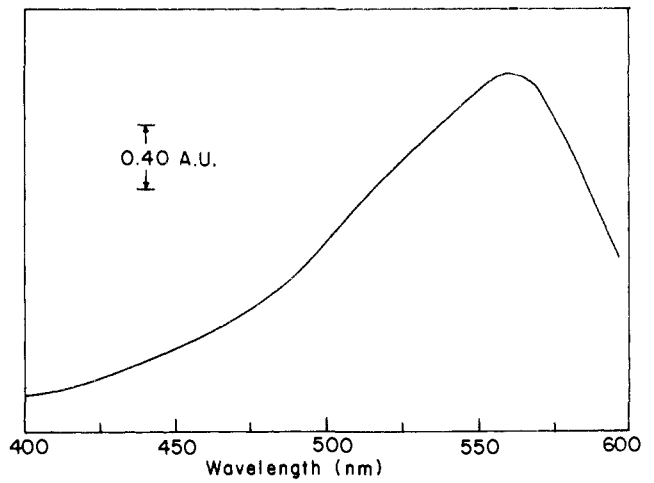


Figure 4. Spectrum taken 55 s after Ce^{4+} addition to **1** in pH 1.3 phosphate buffer. Scan rate = 20 nm/s; 2.88×10^{-4} M original **1**.

559 nm. The absorbance at 424 nm decreases with time, while that at 559 nm increases, corresponding to a color change from yellow to purple. Figure 3, B, shows additional spectra taken from 114 to 564 s after Ce^{4+} addition. The peak at 559 nm gradually decreases, while a new peak at 508 nm grows to become the final spectrum of **3**, 36 min after oxidation (dashed curve).

These data permit the observation of three products of the chemical oxidation of **1**. The yellow material (λ_{max} 424 nm) is formed initially and corresponds to the first redox couple (O_2/R_2) formed in voltammograms 1, A, and 2, A. This yellow material reacts to form the purple substance (λ_{max} 559 nm), which eventually is transformed into **3** as evidenced by the spectra and voltammograms. During this period **1** is regenerated, and final yields of 50 ± 2% are observed for both **1** and **3**. Notice that the generation of the red color of **3** (λ_{max} 508 nm) occurs significantly later than the regeneration of **1**, indicated by chronoamperometry.

Although several mechanistic conclusions may be drawn from the qualitative time dependence of the spectra, a quantitative analysis of the spectral and electrochemical data was carried out. To determine the molar absorptivity for the purple chromophore, the reaction was repeated at pH 1.3. At this pH, the conversion of the 424-nm to the 559-nm chromophore is fast, relative to the generation of **3**, and a spectrum taken 55 s after oxidation shows the 559-nm chromophore with very little interferences from other species (Figure 4). Spectra taken after Figure 4 indicate that the formation of the 559-nm chromophore was essentially complete after 55 s, so its molar absorptivity was estimated to be 17 200 M^{-1} cm^{-1} at 559 nm and 11 200 M^{-1} cm^{-1} at 508 nm. Combining these data with the known spectral characteristics of **3**, a two wavelength simultaneous analysis[22] at 508 and 559 nm yielded concentration vs. time data for **3** and the 559-nm chromophore, shown in Figure 5 and Table I. The concentration results should be considered approximate due to uncertainty in the molar absorptivity of the 559-nm chromophore. Regardless of their accuracy, the trends apparent in Figure 5 are valid and confirm the conclusions drawn by inspection of Figure 3. Again, note that the formation of **3** occurs significantly later than both the formation of the 559-nm chromophore and the regeneration of **1**.

As will be apparent below, it was useful to examine the effect of aniline on the reactions following Ce^{4+} oxidation. When 0.5 mol of aniline hydrochloride per mole of original **1** was added to the solution immediately after oxidation,

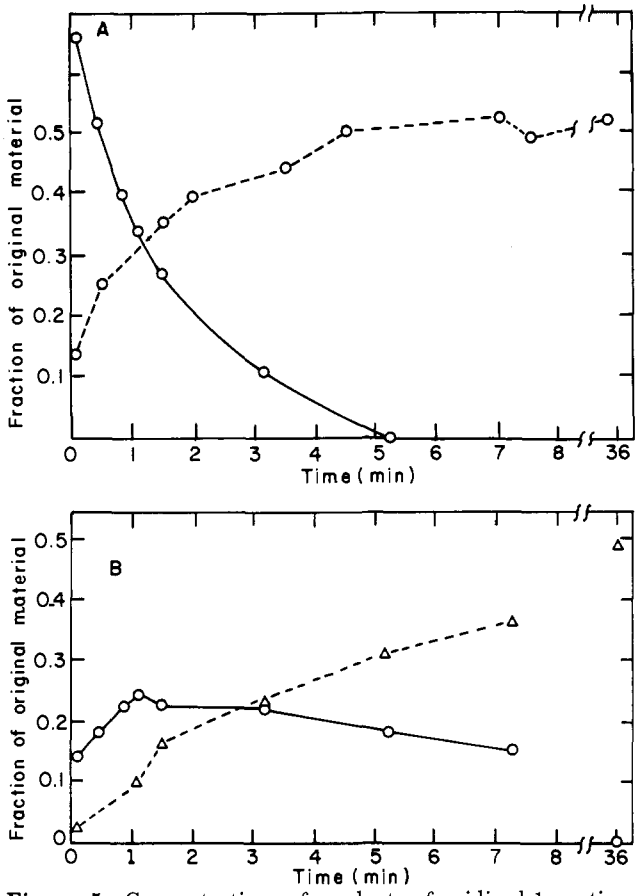


Figure 5. Concentrations of products of oxidized 1 vs. time, expressed as a fraction of original 1. A, solid curve 5, dashed curve 1. B, solid curve 6, dashed curve 3. Concentrations determined as described in the text (pH 2).

Table I. Concentrations of Intermediates as a Function of Time, Expressed as a Fraction of the Initial Concentration of 1

Time, s	1	6	3	5[a]
12	0.18	0.14	0.02	0.66
27	0.25	0.18	0.05	0.52
51	0.30	0.22	0.08	0.40
66	0.32	0.24	0.10	0.34
90	0.35	0.22	0.16	0.27
192	0.44	0.22	0.23	0.11
315	0.51	0.18	0.31	0
440	0.52	0.15	0.36	−0.03
2160	0.52	0	0.48	0

[a] Determined by difference.

a purple color (λ_{max} 556 nm) formed instantly. This chromophore was stable for more than 8 h but did eventually form a material having the same spectrum and cyclic voltammogram as **3**. 40% of original 1 was regenerated when aniline was added to the solution immediately after Ce^{4+} oxidation.

Since chlorpromazine sulfoxide has been identified as an oxidation product of chlorpromazine, it was necessary to find whether sulfoxidation of 1 could be involved in its oxidation pathway. A cyclic voltammogram of 7-hydroxychlorpromazine sulfoxide at pH 2 revealed an initial oxidation at +0.82 V and a reversible follow-up couple at +0.34 V. None of these waves was present in the voltammograms of 1, either before or after oxidation, and, furthermore, voltammetry of the electrolysis products of 7-hydroxychlorpromazine sulfoxide showed that they are different from those of 1. Sulfoxidation of 1 can thus be ruled out in these experiments.

Methylation of the terminal nitrogen of the side chain of 1 results in the quaternary salt, 7-hydroxychlorpromazine methiodide, which showed voltammetric behavior identical with that of 1 after correction for current resulting from iodide. It is therefore unlikely that the dimethylaminopropyl side chain is involved in the reactions of oxidized 1. Finally, electron spin resonance spectra of the oxidized solutions revealed no observable radicals at any time after oxidation. Several attempts to obtain NMR spectra of intermediates were made, but the small amount of 1 available (<200 mg), coupled with the short lifetimes of the intermediates, precluded success. An attempt to isolate the product of the reduction of the 424-nm chromophore was made, but its instability prevented success.

A similar examination of the oxidation of 1 in the pH range from 3 to 7 was carried out. Cyclic voltammograms of 1 in this pH range have been reported previously[18] and indicate the immediate generation of two new redox systems after the initial oxidation of 1, rather than the single couple observed at pH 2. The more negative of the two couples corresponds in potential and shape to the redox reaction of 2 to form 3. Chronoamperometry of 1 at pH 7 is a four-electron oxidation on a 1-s time scale, and coulometric oxidation at 0.6 V at pH 7 required 4.14 electrons over a 30-min period. The yield of 3 from the coulometric oxidation was 31%, assessed spectrophotometrically.

A two-electron chemical oxidation of 1 at pH 7 with the peroxidase/H_2O_2 system was complete in a few seconds, yielding 22% of 3, again determined spectrophotometrically. Analyses of concentrations of intermediates vs. time, such as those performed at pH 2, could not be carried out at pH 7 because of the speed of the reactions at the higher pH. However, when the pH was lowered immediately after chemical oxidation at pH 7, the purple intermediate 6 could be observed spectrophotometrically. Sulfoxidation as well as involvement of the side chain was ruled out at pH 7 by a process analogous to that described above at pH 2.

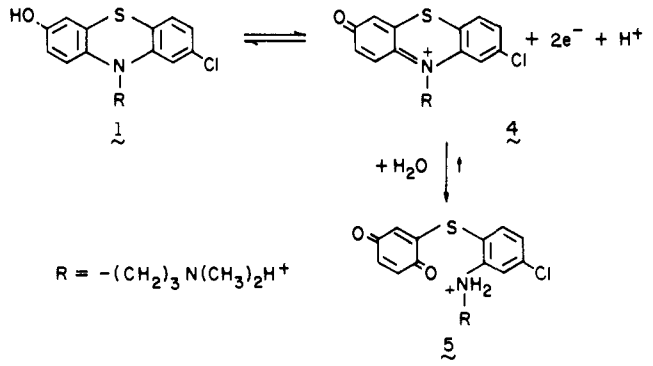
Discussion

Oxidation at pH 2. On a short time scale, the electrochemical data at pH 2 indicate that the oxidation of 7-hydroxychlorpromazine at +0.75 V is a two-electron process generating a reactive intermediate with a half-life of about 100 ms. This intermediate decays to form a yellow chromophore (λ_{max} 424 nm) which is stable on a time scale of a few tens of seconds. This chromophore has an absorbance maximum in the common range for 1,4-benzoquinones and has a redox couple (R_2/O_2 in Figure 1, A, and 2, B) with a potential close to that for hydroquinone/quinone in the same medium (+0.32 V vs. SCE).[23] Furthermore, sulfur substituents on quinones have been shown to have little effect on their redox potentials.[24] Given that quinone imines commonly hydrolyze to quinones,[25,26] the mechanism of Scheme I can be concluded for the initial oxidation of 1 carried out chemically by Ce^{4+} or electrochemically at +0.75 V.

The quinone 5, which is in the 2+ oxidation state (two electrons removed) relative to 1, eventually reacts to form 50% yields of both 1 (zero oxidation state) and 3 (4+ oxidation state), as shown in Scheme II. Scheme II does not explain the time-resolved data, however, since the regeneration of 1 is almost complete before 3 appears in significant concentration.

It is apparent from Figure 3 that the 424-nm chromophore, attributed to 5, is unstable and reacts to form the 559-nm chromophore within 3 min. As can be seen in

Scheme I



Scheme II

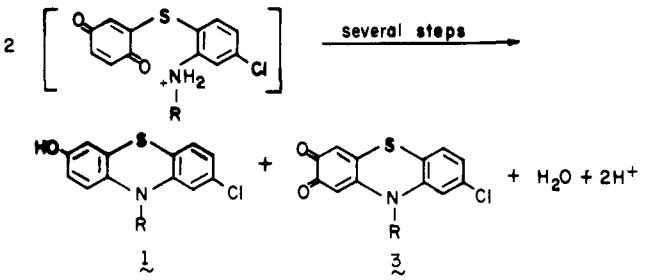


Figure 5, 1 is being regenerated during this period, such that nearly 50% of 1 has been restored at the point where 5 is virtually gone. At times below 1 min, approximately one molecule of the 559-nm chromophore (denoted 6) is produced for every molecule of 1 which is regenerated. In addition, the decay of the 559-nm chromophore is concurrent with the generation of 3. Thus Scheme II must be altered to include the purple (559 nm) intermediate, as shown in reactions 1 and 2.

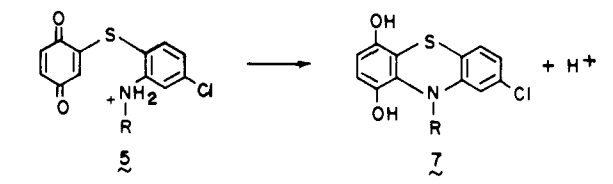
$$5 + 5 \rightarrow 1 + 6 \quad (1)$$

$$6 \rightarrow 3 \quad (2)$$

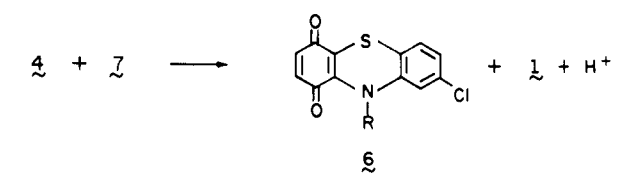
In this scheme, reaction 1 has a half-life of about 1 min, while (2) has a half-life of ~5 min. Direct information about the structure of 6 was difficult to obtain because of the short time scale of these reactions. A few of its characteristics can be inferred, however, from careful consideration of the results. The only likely mechanism for regeneration of 1 depends on the reversibility of the quinone imine hydrolysis shown in Scheme I. If a small amount of 4 were present in equilibrium with 5, it would be available to oxidize some other solution component and as a result be reduced back to 1. The final 50% yield of 1 indicates that half of the quinone 5 has been reduced, via 4, to original 7-hydroxychlorpromazine. The other half of 5 must have been oxidized to a species which is in a 4+ oxidation state relative to 1. Therefore, both 6 and 3 must be in 4+ oxidation states relative to 1.

Further evidence concerning the structure of the purple intermediate may be deduced with the aid of the experiments involving the addition of aniline to the reaction mixture immediately after the Ce^{4+} oxidation. The aniline reaction, although faster, qualitatively mimics the process in the absence of aniline. A nearly identical purple chromophore is formed, and 40% of the original 1 is regenerated. One may conclude that the aromatic amine of quinone 5 must be involved in formation of the 559-nm chromophore in the absence of aniline. Barring cleavage of a sulfur–carbon bond, only two possibilities for amine involvement exist: cyclization to the quinone imine via Schiff's base formation or a Michael addition to the

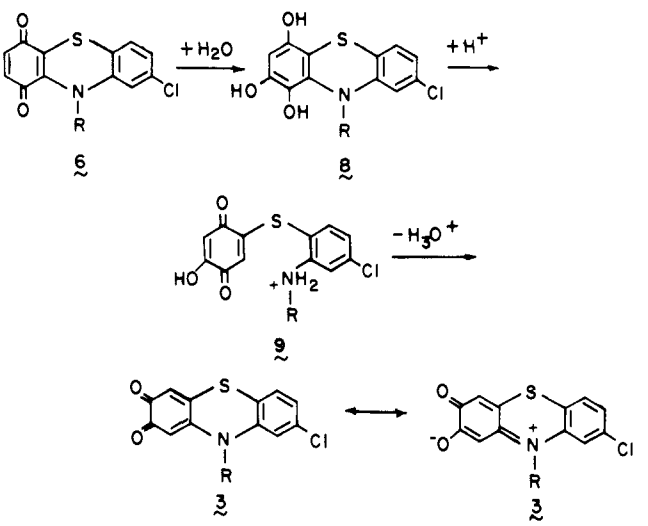
Scheme III



Scheme IV



Scheme V



quinone ring. Since quinone imine formation is a reversible equilibrium favoring the quinone, it will not result in measurable concentrations of quinone imine.[27,28] So the Michael addition reaction to form 6,9-dihydroxychlorpromazine (7) is most likely, as shown in Scheme III. 7 would be expected to have a redox potential comparable to or lower than that of 5, so it would be oxidized by the quinone imine 4 in equilibrium with 5, as shown in Scheme IV. The proposal of 6,9-dioxochlorpromazine (6) as the 559-nm chromophore is supported by several considerations. It is in the 4+ oxidation state relative to 1, as required by the time dependence shown in Figure 5. Both sulfur- and nitrogen-substituted quinones have visible absorptions shifted to significantly longer wavelengths compared to unsubstituted quinones.[29,30]

It is important to note that this sequence of reactions results in 1 mol of 5 being converted to 0.5 mol each of 6 and 1, the 0.5 mol of 1 being stable under these conditions and appearing as a final product. Thus the aromatic amine of quinone 5 undergoes both Michael addition and Schiff's base formation, with the latter reaction supplying the oxidizing agent for the Michael adduct.

The externally added aniline is unlikely to form a Schiff's base adduct to any significant extent, since the resulting *N*-phenylquinone imine would hydrolyze rapidly under these conditions.[27,28] A more likely reaction is a Michael addition of aniline to the quinone ring, a very well-characterized reaction,[31,32] the result in this case being a sulfur/amine-substituted quinone with spectral characteristics similar to those of 6.

The final step in the process is the reaction of 6 to form 3, a reaction with a half-life of about 5 min and which is

complete within 30 min. A plausible route for this reaction involves hydroxylation of 6, followed by a reverse Michael reaction, to form a substituted hydroxyquinone, as shown in Scheme V.

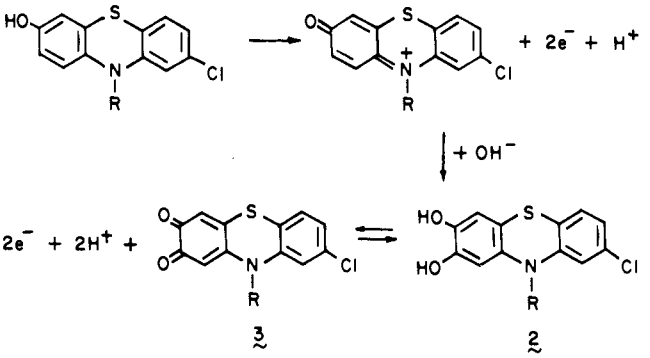
This overall mechanism accounts for all the features apparent in the voltammograms of Figure 2 and the spectra of Figure 3. Since the voltammetric scans of Figure 2 are started at potentials negative of 0.0 V, all species immediately next to the electrode surface are forced to be in their reduced forms at the beginning of the scan. Three of the four anodic waves of Figure 2, B, have been accounted for; O_1 is the oxidation of regenerated 1, O_2 is the oxidation of the reduced form of quinone 5, and O_4 is the anodic wave for the oxidation of 2, the reduced form of the final product 3. O_3 has the same time course as the 559-nm chromophore and can be attributed to the redox couple associated with quinone 6. Due to differences in charge-transfer reversibility for O_2, O_3, and O_4, their reduction waves are not resolved and are all contained in the single cathodic peak. Its amplitude changes little with time as a result. While these voltammetric data are a useful analytical probe for this system, it should be kept in mind that the reductions of various species at the beginning of the scan affect only the solution immediately next to the electrode and do not perturb the reaction in the bulk solution.

The chronoamperometry experiment used to determine the time course of the concentration of 1 relied on the assumption that 1 was the only species present which could be oxidized at +0.75 V. Despite the possible presence of reduced forms of the other species, this assumption is justified by the existence of the Schiff's base equilibrium between quinone 5 and quinone imine 4. Thus whenever quinone 5 is present, the strong oxidizing agent 4 is also present, albeit in small equilibrium concentration. Any reduced materials besides 1 which are formed are rapidly oxidized during the regeneration of 1. Therefore the chronoamperometry experiment measures only the concentration of 1. It is useful to note that the chronoamperometrically determined concentrations of 1 parallel the increase of the amplitude of the O_1 peak in Figure 2, which is due to 1. The voltammetry does not require the assumption just made above, but the chronoamperometry data were used in Figure 5 because of their greater accuracy.

The voltammetric and spectroscopic data do not reveal any more than four species present in appreciable concentration, the four measurable species being 1, 5, 6, and 3. Other species which are not detected by these methods may be present in nonnegligible concentration, although the possibility is remote. It is useful to consider the case where only four species are present. The concentration of 5 may then be determined by subtracting the sum of the concentrations of 1, 6, and 3 from the original concentration of 1 before Ce^{4+} oxidation. The results are shown in Figure 5 and Table I. The concentrations of 5 determined by difference are in agreement with the trend observed in the spectra of Figure 3, and the last three entries of Table I indicate good internal consistency of the data. As mentioned above, however, the trends in concentrations of intermediates are obvious, regardless of the quantitative accuracy of the results in Table I.

The only pronounced difference between the reaction of externally added aniline with the quinone 5 and the intramolecular cyclization of Scheme III is the stability of the product. The purple adduct from added aniline was stable for days, although it did eventually form 3, while the intramolecular analogue lasted only minutes. The difference can be attributed to the site of attack of the aromatic amine on quinone 5. The intramolecular amine can only add ortho to the sulfur, while the external aniline may also add para. If the aniline adds para, the resulting quinone (after oxidation) cannot be hydroxylated para to the sulfur, as depicted in Scheme V, and cannot easily rearrange to form 3.

Scheme VI

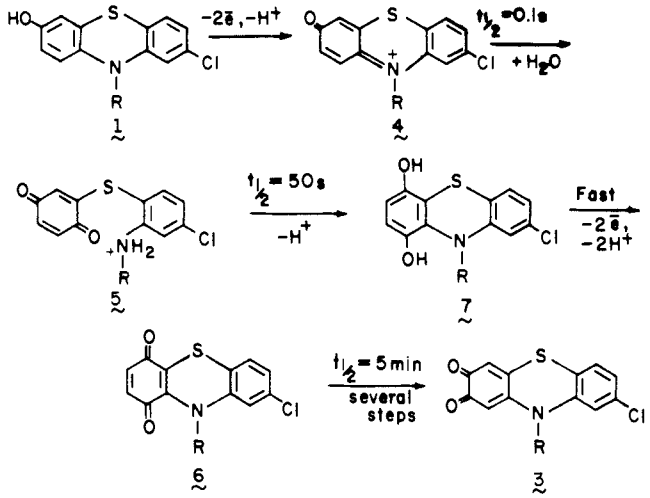


Oxidation at pH 7. From the pH 7 results, certain important conclusions can be drawn about the oxidation of 1 at physiological pH. First, the immediate formation of 3, demonstrated by cyclic voltammetry and chronoamperometry, indicates direct hydroxylation of the quinone imine 4, according to Scheme VI. The immediate formation of the characteristic 508-nm chromophore upon chemical oxidation of 1 supports a direct hydroxylation route as well. However, after four electrons have been removed by complete electrolysis of 1, the yield of 3 is only 31%, leaving a majority of the starting material unaccounted for at pH 7. The formation of 3 results from both the direct hydroxylation and quinone imine hydrolysis routes, since the purple intermediate can be observed spectrophotometrically after oxidation at either pH 2 or 7. The relative importance of each route at pH 7 cannot be assessed from these results because of the very short time frame of the reactions.

Since direct hydroxylation of 4 can only account for less than half of the products at pH 7, the remainder must result from hydrolysis to quinone 5, as shown in Scheme I. The fate of quinone 5 at pH 7 is very likely to involve the mechanism of Scheme VI, but not all of the quinone produces the dioxochlorpromazine product 3. However, the disubstituted quinone 6 does form at pH 7 and is a likely oxidation product in vivo. The complete mechanism worked out at pH 2 provides insight into the reactions at pH 7, and the construction of a complete scheme at physiological pH is presently under way. The essential conclusions from the available data are that both the hydrolysis (Scheme VII) and hydroxylation (Scheme VI) mechanisms occur at pH 7 and that the quinone imine 4, the quinone 5, and the quinone 6 are formed at both pH 2 and 7 upon oxidation of 1.

The elucidation of the oxidation scheme of 1 in vitro allows several inferences about the pharmacology of the hydroxylated phenothiazine metabolites to be drawn. First, the oxidation of 7-hydroxychlorpromazine leads to cleavage of the phenothiazine ring system. Several metabolites of chlorpromazine do not yield phenothiazine color reactions on TLC plates[4] and may result from such ring cleavage. Second, at least two intermediates in the oxidation are quinones and may be involved in the formation of "pseudo melanin" suggested by other workers.[11,14] Third, the eventual formation of 7,8-dioxochlorpromazine is likely to involve two redox systems with the 2,4,5-trihydroxy substitution pattern of 6-hydroxy-

Scheme VII



dopamine, as shown in Scheme V. The cytotoxic mechanism of 6-hydroxydopamine remains controversial but is known to involve redox reactions; the 2,4,5-trihydroxy-substituted intermediate may have similar cytotoxic activity.

While these pharmacological hypotheses are largely unsubstantiated, the interactions of the products of 7-hydroxychlorpromazine oxidation with their physiological environment may explain some of the side effects of long-term chlorpromazine therapy. In any case, these new molecules are likely to form upon oxidation of 1 in vivo and should be considered in the overall scheme of chlorpromazine metabolism.

Conclusion

The overall electrochemical and chemical oxidation of 7-hydroxychlorpromazine at pH 2 is represented by Scheme VII. When the initial two-electron oxidation is carried out by a stoichiometric amount of Ce^{4+}, the resulting quinone **5** reacts via a multi-step pathway to form a 50% yield of 7,8-dioxochlorpromazine, and 50% of the original 7-hydroxychlorpromazine is regenerated. Intermediates in the process include at least one molecule which does not retain the phenothiazine ring system and at least two derivatives of 1,4-benzoquinone. In addition to the reactions depicted in Scheme VII, direct hydroxylation of the quinone imine (Scheme VI) occurs at pH 7. At the higher pH, however, complete reaction to 7,8-dioxochlorpromazine does not occur, and any additional products or mechanisms which may be present at physiological pH are currently being examined.

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References and Notes

(1) V. Fishman and H. Goldenberg, *Proc. Soc. Exp. Biol. Med.*, 112, 501 (1963).
(2) J. W. Daly and A. A. Manian, *Biochem. Pharmacol.*, 16, 2131 (1967).
(3) A. A. Manian, D. H. Efron, and S. R. Harris, *Life Sci.*, 10 (part I), 679 (1971).
(4) P. Turano, W. J. Turner, and A. A. Manian, *J. Chromatogr.*, 75, 277 (1973).
(5) J. Buckley, M. Steenberg, H. Barry, and A. A. Manian, *J. Pharm. Sci.*, 62, 715 (1973).
(6) A. A. Manian, D. H. Efron, and M. Goldberg, *Life Sci.*, 4, 2425 (1965).
(7) H. Y. Meltzer, U. S. Fang, M. Simonovich, and S. Paul, *Eur. J. Pharmacol.*, 41, 431 (1977).
(8) S. A. Tjioe, A. A. Manian, and J. J. O'Neill, *Biochem. Biophys. Res. Commun.*, 48, 212 (1972).
(9) A. V. P. Mackay, A. F. Healey, and J. Baker, *Br. J. Clin. Pharmacol.*, 1, 425 (1974).
(10) G. Sakalis, T. L. Chan, S. Gershon, and S. Park, *Psychopharmacologia*, 32, 279 (1973).
(11) T. L. Perry, C. A. Culling, K. Berry, and S. Hanson, *Science*, 146, 82 (1964).
(12) M. H. Van Woert, *Nature (London)*, 219, 1054 (1968).
(13) H. R. Adams, A. A. Manian, M. L. Steenberg, and J. P. Buckley in "Phenothiazines and Structurally Related Drugs", I. S. Forrest, C. J. Carr, and E. Usdin, Ed., Raven Press, New York, N.Y., 1974, p 281.
(14) T. A. Grover, L. H. Piette, and A. A. Manian in ref 13, p 561.
(15) R. E. Heikkila, G. Cohen, and A. A. Manian, *Biochem. Pharmacol.*, 24, 363 (1975).
(16) S. Tjioe, A. A. Manian, and J. J. O'Neill, *Psychopharmacol. Commun.*, 1, 373 (1975).
(17) A. Zirnis, J. K. Suzuki, J. W. Daly, and A. A. Manian, *J. Heterocycl. Chem.*, 12, 239 (1975).
(18) R. L. McCreery, *J. Pharm. Sci.*, 66, 357 (1977).
(19) R. N. Adams, "Electrochemistry at Solid Electrodes", Marcel Dekker, New York, N.Y., 1969.
(20) F. Ullman, *Ber. Dtsch. Chem. Ges.*, 31, 1968 (1898).
(21) H. Willard, H. Furman, and C. Bricker, "Elements of Quantitative Analysis", 4th ed, Van Nostrand, Princeton, N.J., 1956, p 243.
(22) C. N. Reilley and D. T. Sawyer, "Experiments for Instrumental Methods", McGraw-Hill, New York, N.Y., 1961, p 122.
(23) Reference 19, p 366.
(24) E. R. Brown, K. T. Finley, and R. L. Reeves, *J. Org. Chem.*, 36, 2849 (1971).
(25) D. Hawley and R. N. Adams, *J. Electroanal. Chem.*, 10, 376 (1965).
(26) J. F. Corbett, *J. Chem. Soc. B*, 213 (1969).
(27) D. W. Leedy, Ph.D. Thesis, University of Kansas, Lawrence, Kan., 1968.
(28) Reference 19, p 342.
(29) H. J. Tuber and M. Hasselbach, *Chem. Ber.*, 92, 674 (1959).
(30) J. M. Snell and A. Weissberger, *J. Am. Chem. Soc.*, 61, 450 (1939).
(31) H. Suida and W. Suida, *Justus Liebigs Ann. Chem.*, 416, 113 (1918).
(32) R. M. Acheson and B. F. Sansom, *J. Chem. Soc.*, 4440 (1955).