Electrochemical Oxidation of Hydroxylated Phenothiazine and Imipramine Derivatives

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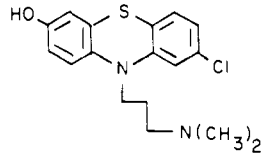
The electrochemical oxidations of several hydroxylated derivatives of promazine, chlorpromazine, imipramine, and 3-chloroimipramine are examined and compared. Oxidation of the monohydroxyphenothiazine derivatives leads to both dihydroxy species and substituted benzoquinones, while oxidation of hydroxylated imipramines leads to only the corresponding benzoquinones. The oxidation potentials of 17 tricyclic psychoactive drugs and metabolites are tabulated and compared. The potential importance of these results to drug activity and side effects is discussed.

There has been substantial pharmacological and chemical interest in the ring-hydroxylated derivatives of phenothiazine and iminobibenzyl drugs for a variety of reasons. The hydroxylated chlorpromazines, including 7-hydroxychlorpromazine (1) and 7,8-dihydroxychlor-



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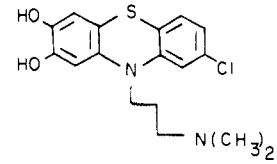

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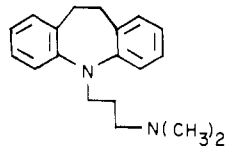

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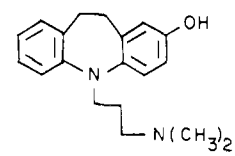

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promazine (2), are metabolites of the widely used antipsychotic drug chlorpromazine in both animals and humans.[1-4] The monohydroxylated derivative is comparable in pharmacological activity to the parent drug in animal tests,[5,6] and blood levels of 1 correlate better with clinical improvement than those of chlorpromazine itself.[7,8] The hydroxylated derivatives have also been associated with several side effects of chlorpromazine treatment, and oxidation of these derivatives is believed to be relevant to these effects.[9-11] In addition, oxidation of 1 is likely to be a route of formation of 2 in vivo, with the oxidation catalyzed possibly by a mixed-function oxidase such as tyrosine hydroxylase.[12]

Biotransformation in humans of tricyclic drugs derived from iminobibenzyl, such as imipramine (3) or its 3-chloro analogue, produces aromatic hydroxylated metabolites with the hydroxyl group para to the ring nitrogen.[13,14] These metabolic products were considered to be inactive until recently, when they were shown to be otherwise and even of possible clinical significance.[15-20] The oxidations are believed to involve the participation of an electron-transport chain and terminate in an oxygen-transferring enzyme, cytochrome P-450.[19] Further metabolic hydroxylation is a possibility but this remains to be established.

Our laboratory has undertaken an examination of the chemical and electrochemical oxidation of several hydroxylated derivatives of phenothiazine-based drugs, particularly 7-hydroxychlorpromazine and two isomeric dihydroxylated species.[21-23] Electrochemistry is well-suited to this type of work, since it allows observation of short-lived intermediates formed upon oxidation, and is sufficiently refined to permit elucidation of complex reaction mechanisms. In addition, electrochemistry can be a microscale technique and permits significant information to be derived from very small amounts of starting material, an aspect of particular importance in the present work, where small quantities precluded product isolation. The relationship between electrochemical results and in vivo findings remains a question, but the intermediates and mechanisms revealed from this in vitro approach provide useful insight into pharmacological mechanisms and metabolic pathways.

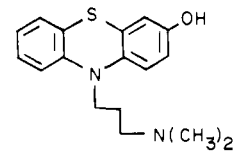
The present work extends this investigation to ring-hydroxylated metabolites of the psychoactive drugs, imipramine, chloroimipramine, and promazine. The chemical and pharmacological ramifications of the work will be discussed.

Experimental Section

All the instrumentation and techniques used in this work are well-established and have been described previously.[22] MacIlvaine buffers made from 0.1 M citric acid and 0.2 M Na_2HPO_4 were used for the entire pH range studied here. All drug derivatives were obtained from the Psychopharmacology Research Branch at the NIMH. All potentials are referred to the saturated calomel electrode (SCE), and +0.246 V need to be added to the cited values to obtain the corresponding potential on a hydrogen scale.

Results

Figure 1A is a cyclic voltammogram of 2-hydroxyimipramine (4) at pH 4 compared to one of 3-hydroxypromazine (5) under the same conditions. From volt-



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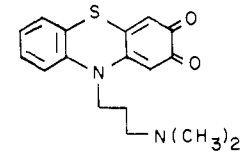

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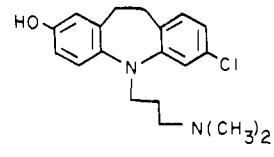

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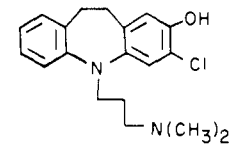

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ammogram 1A, it is apparent that the initial oxidation of

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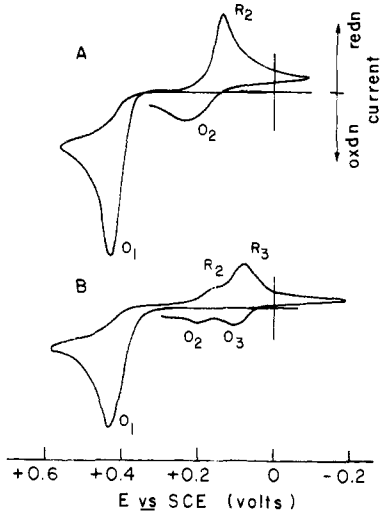


Figure 1. Cyclic voltammograms carried out with a graphite paste electrode at 0.1 V/s in pH 4.0 MacIlvaine buffer: curve A, 2-hydroxyimipramine; curve B, 3-hydroxypromazine.

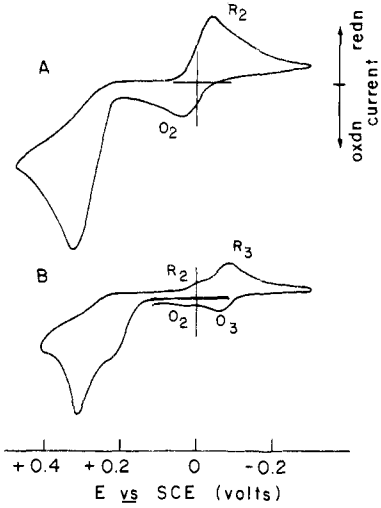


Figure 2. Voltammograms obtained under the same conditions as Figure 1, except with a pH of 7.0: curve A, 2-hydroxyimipramine; curve B, 3-hydroxypromazine.

4 at peak O_1 generates a short-lived product which is not stable enough to generate a reduction peak corresponding to O_1. A new couple (O_2/R_2) is evident after the initial oxidation and is stable on this time scale. The phenothiazine derivative **5** shows similar behavior (Figure 1B), except that two new species are formed (O_2/R_2 and O_3/R_3) after the initial oxidation.

Figure 2 shows voltammograms of **4** and **5** at pH 7, with all other conditions the same as for Figure 1. The voltammetry of **4** is essentially independent of pH, except for the expected shifts in peak potentials. The voltammogram of **5** is distorted at pH 7, probably by adsorption of the sparingly soluble starting material on the electrode. Nevertheless, the two follow-up couples are apparent at pH 7 (O_2/R_2 and O_3/R_3) and the same conclusions about mechanism can be drawn at the higher pH.

Coulometric oxidation of **4** at an applied potential of +0.50 V in pH 7 buffer required 2.17 faradays/mol and resulted in a solution having a voltammetric redox couple identical to O_2/R_2 of Figure 2A. The solution was faintly yellow, with UV–vis absorption peaks at 239, 288, and 450 nm. In contrast, coulometric oxidation of **5** consumed 4.04 faradays/mol at pH 7, and the product was voltammetrically and spectroscopically indistinguishable from 2,3-dioxopromazine (**6**). The yield of **6**, determined spectrophotometrically, was 33%.

The voltammograms of 3-chloro-8-hydroxyimipramine (**7**) and 3-chloro-2-hydroxyimipramine (**8**) were qualitatively identical to those in Figures 1A and 2A, with the peak potentials tabulated in Table I. At pH 7, coulometric oxidation of **7** required 1.99 faradays/mol, while **8** required 1.94 faradays/mol, again indicating overall two-electron oxidations. The UV–vis spectra of the products of oxidation of **7** were similar to those of the oxidation product of **4**, with a very weak visible absorption band.

Voltammograms of **1** at pH 7 were similar to those of **5**, with slightly different peak potentials (Table I). Coulometric oxidation of **1** at pH 7 required 3.79 faradays/mol and resulted in a 33% yield of 7,8-dioxochlorpromazine.

Voltammograms of a series of substituted phenothiazines and iminobibenzyls were obtained, and a composite tabulation of oxidation potentials is shown in Table II. The values are expressed as half-peak potentials and serve as a comparison of ease of oxidation. The relationships between voltammetric half-peak potentials and the thermodynamic $E^{\circ\prime}$ values depend on charge-transfer

Table I. Summary of Electrochemical Data for Oxidations at pH 7

compd	half-peak potent. of init oxidat	faradays/mol required for complete coulometric oxidat
7-hydroxychlorpromazine (**1**)	0.245	3.79
3-hydroxypromazine (**5**)	0.230	4.04
2-hydroxyimipramine (**4**)	0.270	2.17
3-chloro-8-hydroxyimipramine (**7**)	0.320	1.99
3-chloro-2-hydroxyimipramine (**8**)	0.290	1.94

reversibility and other factors,[24] but for these types of compounds the two values are usually within 20 mV of each other.[21] Several pharmacologically related compounds are included in Table II for comparison.

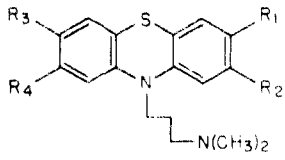
Discussion

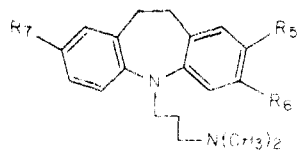
As demonstrated in earlier papers,[22,23] phenothiazines with a hydroxyl group para to the thiazine nitrogen can undergo a two-electron oxidation to the corresponding quinone imine. These quinone imines may reversibly hydrolyze to substituted benzoquinones, thus degrading the phenothiazine nucleus, or be attacked by hydroxide ion, leading to ortho dihydroxy derivatives. The present results show that these general reaction pathways are also applicable to the compounds studied at neutral pH but with some important differences.

As one would predict, 7-hydroxychlorpromazine and 3-hydroxypromazine are very similar in their behavior upon oxidation, leading eventually to the corresponding dioxo compounds at pH 7.0. This reaction sequence is shown in Scheme I for 3-hydroxypromazine. The incomplete yield of **6** indicates that the quinone **9** may react to other species, which remain to be elucidated. It was shown earlier that quantitative yields of 7,8-dioxochlorpromazine were obtained from 7-hydroxychlorpromazine below pH 5, but the yield decreased at higher pH.[23] Similar behavior was observed for 3-hydroxypromazine in this work. Given the mechanism depicted in Scheme I, the two new redox couples apparent in Figures 1B and 2B correspond to the redox system associated with **6** (O_3/R_3) and that associated with **9** (O_2/R_2).

In the case of the three imipramine derivatives **4**, **7**, and **8**, the degradative reactions were better defined. By

Table II. Anodic Half-Peak Potentials of Phenothiazine and Imipramine Derivatives[a]

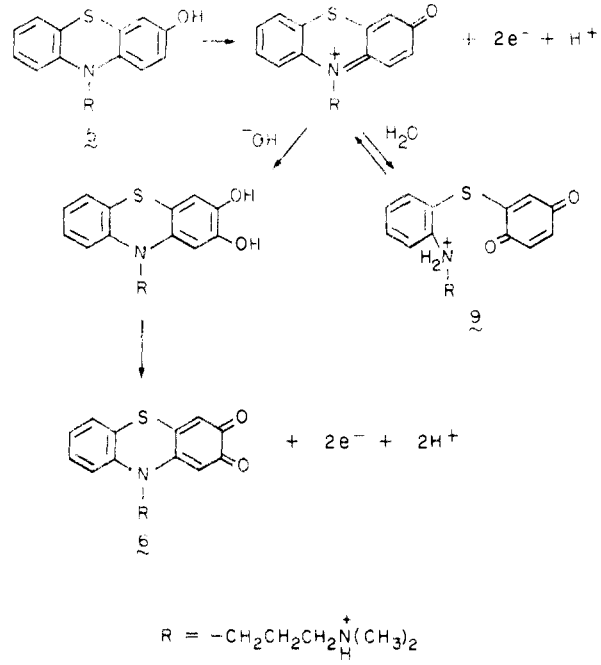




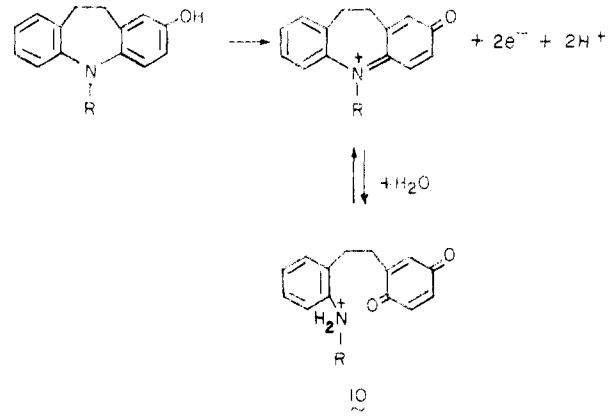
compd	R_1	R_2	R_3	R_4	R_5	R_6	R_7	$E_p/2$
chlorpromazine	H	Cl	H	H				+0.620
3-hydroxychlorpromazine	OH	Cl	H	H				+0.205
7-hydroxychlorpromazine	H	Cl	OH	H				+0.245
8-hydroxychlorpromazine	H	Cl	H	OH				+0.255
3,7-dihydroxychlorpromazine	OH	Cl	OH	H				+0.125
3,8-dihydroxychlorpromazine	OH	Cl	H	OH				+0.135
7,8-dihydroxychlorpromazine	H	Cl	OH	OH				-0.060
3,7,8-trihydroxychlorpromazine	OH	Cl	OH	OH				0.125
promazine	H	H	H	H				-0.520
2-hydroxypromazine	H	OH	H	H				+0.200
3-hydroxypromazine	OH	H	H	H				+0.230
2,3-dihydroxypromazine	OH	OH	H	H				-0.100
imipramine					H	H	H	+0.780
2-hydroxyimipramine					OH	H	H	+0.270
3-chloroimipramine					H	Cl	H	+0.840
3-chloro-8-hydroxyimipramine					H	Cl	OH	+0.320
3-chloro-2-hydroxyimipramine					OH	Cl	H	+0.290

[a] pH = 7.0; scan rate = 0.10 V/s; graphite paste electrode.

Scheme I



Scheme II



analogy to the phenothiazines, the initial oxidation must be the formation of a quinone imine, requiring two electrons. After this, only one new redox couple is formed, as shown in Figures 1A and 2A, and the overall oxidation required two electrons in all cases (Table I). If the quinone imine were hydroxylated and subsequently oxidized to a dioxo derivative, the overall reaction would require a total of four electrons and can therefore be ruled out. Furthermore, the dioxo derivatives, if formed, would be expected to have a strong visible chromophore, similar to that of **6**; this was not observed for the imipramine derivatives. One concludes that the quinone imines formed from **4**, **7**, and **8** undergo rapid hydrolyses to substituted benzoquinones, as shown in Scheme II for compound **4**. These quinone products are stable on a time scale of the tens of minutes required for coulometric oxidation. However, authentic samples of the quinones were not available for comparison, and insufficient quantities of starting material precluded product isolation. It should be noted that other reaction pathways can be envisioned which consume a total of 2 faradays/mol of charge upon coulometric oxidations. However, an evaluation of yields of the quinone products indicate they are the major ones, and other possible reaction pathways are comparatively minor.

While hydroxylation of the quinone imines derived from the imipramine derivatives was not observed, the quinone imine of 3-hydroxypromazine did hydroylate to eventually form 2,3-dioxopromazine. Since the only structural difference between **4** and **5** is the ethylene vs. sulfur bridge, the different products must be caused solely by this feature. The rate of quinone formation from the quinone imine is probably greater for **4** and **5** due to ring strain in the seven-membered ring, thus allowing less time for attack by hydroxide. Perhaps more importantly, the condensation of the quinone to re-form the tricyclic nucleus is probably slower for the imipramine derivatives, again due to the seven-membered ring. Thus, a smaller equilibrium concentration of quinone imine will exist, hindering hydroxylation. Finally, the difference in inductive effects of ethylene vs. sulfur bridges may differentially affect the rates of hydroxylation of the quinone imines.

In the case of 3-chloro-2-hydroxyimipramine, it is possible that the chlorine atom may be substituted by hydroxyl, leading to a 2,3-dioxoimipramine, but requiring only an overall two-electron oxidation. Since this product should have a visible chromophore analogous to **6**, the formation of a dioxoimipramine from **8** is highly unlikely.

As shown in Table II, the oxidation potentials of this series of compounds fall into three principle groups. The parent drugs promazine, chlorpromazine, imipramine, and 3-chloroimipramine have fairly high oxidation potentials, above 0.5 V vs. SCE. The multihydroxy compounds listed here, including 7,8-dihydroxychlorpromazine, have very low potentials in the region of −0.1 V vs. SCE. The remaining hydroxylated phenothiazine and imipramine derivatives have potentials in the region of 0.1–0.3 V. Except for a few cases, such as phenothiazine anthelmintics[25] and 6-hydroxydopamine,[26] redox potentials have not been correlated with biological activity. However, the possibility of formation of toxic species, such as hydrogen peroxide and hydroxyl radical from easily oxidized species, has been established, and the pharmacological importance of these findings discussed.[27,28] As shown in Table II, the multihydroxy derivatives are most likely to be easily oxidized in vivo.

Conclusions about the biological importance of these in vitro results are necessarily indirect, but several important observations about the metabolism of the drugs may be made. First, the electrochemical oxidations used here lead to products which are observed with chemical[22] and enzymatic[12] oxidations, as well as those which are generated in vivo.[4] 7,8-Dioxochlorpromazine and its dihydroxy precursor serve as examples, as well as the formation of the sulfoxide from chlorpromazine.[29] This similarity of electrochemical and metabolic products leads to the conclusion that electrochemically produced species serve as likely possibilities for metabolic generation. The quinone products of both the phenothiazine and imipramine systems which are proposed here are likely metabolites which have not yet been considered. Second, a dihydroxylated or *o*-dioxo derivative of imipramine does not form by the oxidative route examined here, and we consider it unlikely to form in vivo. Third, the tricyclic nucleus is severed during oxidation of both the phenothiazine and imipramine systems, potentially leading to diphenyl metabolites. Finally, it should be noted that of the at least 77 metabolites of chlorpromazine, the drug studied in most detail of the four mentioned here, 42 remain unidentified.[4] Several of the unknown species do not have intact phenothiazine ring systems, as is the case with some of the electrochemical products.

In the final analysis, the pharmacological utility of electrochemical results depends on the correlations between metabolic and electrochemical oxidations. It is unlikely that the metabolic process is identical to that elucidated here, but the intermediates and products observed electrochemically are likely candidates for metabolic involvement. In some cases, such as the benzoquinone derivatives, the electrochemical results revealed intermediates which had not been suggested previously.

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