(0.01 mol) of sodium acetate was added, and the mixture was refluxed for 2 h. A solution of 5 mL of concentrated H_2SO_4 in 5 mL of H_2O was added, and the mixture was refluxed for an additional 4 h. Water (50 mL) was added to the cooled mixture, and stirring was continued at room temperature for 90 min. The solid was collected and washed with H_2O. The wet cake was taken up in ethyl acetate and extracted twice with 2% $NaHCO_3$ solution. The extracts were washed with hexane, and the bicarbonate solution was adjusted to pH 2.0 with concentrated HCl. After the solution was stirred at room temperature for 1 h, the solid was collected and washed well with H_2O to give 4.2 g (95%) of **24**, mp 203–218 °C dec. Elemental analyses indicated that the product was the monohydrate. In large-scale runs, the decarboxylation step was accomplished without isolation of **24**, using heat and a catalyst, such as thioglycolic acid. A high-yield decarboxylation of **24** is described below.

2-[3-Chloro-4-[(*p*-chlorophenyl)thio]-5-methylphenyl]-*as*-triazine-3,5(2*H*,4*H*)-dione (40). A mixture of 10.6 g (0.025 mol) of **24** in 10 mL of 98% thioglycolic acid was stirred under N_2 at 140 °C for 3 h. The thioglycolic acid was removed at reduced pressure. The residue was quenched with H_2O, granulated for 30 min, filtered, washed with H_2O, and vacuum-dried. The yield of crude **40** was 9.5 g (100%). Recrystallization from glacial acetic acid with activated charcoal treatment, followed by pumping to constant weight, yielded 8.9 g of pale yellow crystals (93.6%), mp 148.5–150.5 °C. In some instances, a combination of a high-boiling solvent (e.g., xylene) and a catalytic amount of thioglycolic acid was used for the decarboxylation.

2-[3-Nitro-4-[(*p*-chlorophenyl)thio]phenyl]-*as*-triazine-3,5(2*H*,4*H*)-dione (35). To a stirred slurry of 132 mg (0.002 mol) of 85% KOH (crushed pellets) in 5 mL of dimethylformamide at 80 °C was added in one portion 419 mg (0.0016 mol) of 2-(3-nitro-4-chlorophenyl)-*as*-triazine-3,5(2*H*,4*H*)-dione. The mixture was stirred for 10 min, and 285 mg (0.0016 mol) of potassium *p*-chlorothiophenolate (**14**) added. The mixture was heated at 90–95 °C for 4.5 h and then was quenched in 200 mL of H_2O. After the mixture was acidified with 10 mL of concentrated HCl, the yellow solid was collected and washed with H_2O. The crude dry yield was 500 mg. Recrystallization from ethanol–acetonitrile with activated charcoal treatment yielded 324 mg (55%) of product, mp 243–245 °C.

3,5-Dibromo-4-[(*p*-nitrophenyl)thio]aniline (Precursor of 36). A solution of 13.5 g (0.07 mol) of stannous chloride dihydrate in 75 mL of concentrated HCl was added to a stirred suspension of 9.5 g (0.015 mol) of bis(2,6-dibromo-4-nitrophenyl) disulfide.[4] The mixture was refluxed for 3 h and then poured into cold water. The solid was collected and suspended in $CHCl_3$. Water was added, and the pH was adjusted to 11 with 40% NaOH solution. After it was washed several times with $CHCl_3$, the alkaline solution was acidified and the mixture was extracted with $CHCl_3$. Evaporation of solvent gave 4.5 g (53%) of crude bis(4-amino-2,6-dibromophenyl) disulfide. The product was combined with 40 mL of hypophosphorous acid and refluxed for 2 h. The mixture was cooled, and the solid that separated was collected and washed with H_2O to yield 4.4 g (89%) of crude 4-amino-2,6-dibromothiophenol.

The thiophenol (4.4 g, 0.015 mol) was added to a solution of 1.02 g (0.015 mol) of 85% KOH pellets in 25 mL of methanol. The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and the residue was triturated with a little ethyl ether and removed by filtration to give 5.1 g of potassium 4-amino-2,6-dibromothiophenolate. This salt was dissolved in 20 mL of DMF and added to a stirred solution of 2.44 g (0.015 mol) of *p*-chloronitrobenzene in 20 mL of DMF under N_2. The mixture was stirred for 90 min at room temperature. Ice–water (40 mL) was added dropwise, and an oil separated and later solidified. Collected solids were washed with H_2O to give 5.2 g (83%) of 3,4-dibromo-4-[(*p*-nitrophenyl)thio]aniline. This product was not rigorously characterized but was used in the synthesis of **36**, which is well-characterized.

Acknowledgment. We are grateful for the technical assistance of R. Breitenbach, E. H. Fontaine, R. B. James, G. C. Mankiewicz, R. J. Martingano, H. J. Slater, W. W. Windisch, and R. W. Sumner. Mr. Sumner also helped prepare the manuscript.

Side-Chain Effects on Phenothiazine Cation Radical Reactions

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The cation radical of each of the phenothiazine tranquilizers is a likely intermediate in the metabolism of the drugs to at least two of the three major metabolic classes, the sulfoxides and the hydroxylated derivatives. Previous work has shown that the reactions of the radical are highly dependent on the environment, particularly the presence of nucleophiles. The present report discusses the effect of cation radical structure on the formation of sulfoxide and hydroxylated metabolites in vitro. Cyclic voltammetry, spectrophotometry, and liquid chromatography were used to examine reactions of various phenothiazine radicals in aqueous buffers. A radical with a three-carbon aliphatic side chain (e.g., chlorpromazine) forms solely sulfoxide and parent unless amine nucleophiles are present, in which case hydroxylation occurs. A shorter side chain (e.g., promethazine) causes radical dimerization and pronounced hydroxylation, regardless of external nucleophiles. A piperazine side chain (e.g., fluphenazine) promotes hydroxylation, with some sulfoxide observed. The results indicate that a deprotonated amine is necessary for hydroxylation and that the amine may be present in the original drug rather than an external nucleophile. In addition to information about cation radical reactions, the redox properties of several different phenothiazines are presented.

The metabolism of the phenothiazine major tranquilizers is complex and has been studied in detail in several animal species and in humans.[1-3] For chlorpromazine (**1**), at least 77 metabolites have been observed, with 35 of them being identified.[4] While the metabolic profile varies greatly with animal species and dosage regimen,[5] three major pathways are observed: formation of sulfoxide, hydroxylation of the ring, and degradation of the side chain on the 10 position of the phenothiazine nucleus. A phenothiazine cation radical is generally assumed to be a metabolic intermediate in the formation of sulfoxide and hydroxylated products in vivo,[1,2] and work from this laboratory has demonstrated that these metabolites can be formed from cation radical reactions in aqueous buffers.[6,7] It was shown that the

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(2) Forrest, I. S.; Usdin, E. In "Psychotherapeutic Drugs"; Usdin E; Forrest, I. S., Eds.; Marcel Dekker: New York, 1977; pp 709–713, 756–759.
(3) Turano, P; Turner, W. J.; Donato, D. *Adv. Biochem. Pharmacol.* **1974**, *9*, 315.
(4) Turano, P.; Turner, W. J.; Manian, A. A. *J. Chromatogr.* **1973**, *75*, 277.
(5) Reference 2, p 612.
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0022-2623/81/1824-1342$01.25/0

Scheme I

products and mechanism of radical decay are highly dependent on the presence of nucleophiles (often buffer components) in the medium and that the structure of the phenothiazine radical affected the distribution of sulfoxide vs. hydroxylated products after the reaction was complete.[8] The overall objective of these efforts is an appreciation of how radical structure and the surrounding environment affect the formation of sulfoxide and hydroxylated products.

When the nucelophile attacking a phenothiazine cation radical is a carboxylate or phosphate species, the stoichiometry of the reaction is represented by Scheme I where a nucleophilic species acts as a catalyst. A 50% yield of sulfoxide and 50% yield of parent were observed for chlorpromazine cation radical in carboxylate or phosphate buffers in the pH region 2–7.[6,7,8] The buffer was catalytic in the process, forming a radical/buffer adduct as an intermediate. The detailed kinetics of this reaction have been presented previously.[6,8] Phenothiazines with different 2-position substituents, e.g., promazine (2) and trifluoropromazine (3), react by the same mechanism, with the rates differing according to the electron-withdrawing ability of the substitutent. For all phenothiazines having an *n*-propylamine side chain, the reaction of their cation radicals with phosphate or carboxylate nucleophiles yielded 50% sulfoxide and 50% parent.[8]

However, when an amine was present in solution, such as glycine or isopropylamine, quite different products were observed. The yield of sulfoxide was much lower, and hydroxylated products were observed by voltammetry and HPLC. This process is quite complex, but it is clear that 7,8-dioxochlorpromazine, an oxidized form of a hydroxylated metabolite, is formed when chlorpromazine cation radical reacts with amine nucleophiles.[7] Past work also revealed preliminary evidence that the structure of the 10-position side chain had a pronounced effect on the sulfoxide yield of the radical reaction, even in the absence of amine buffers.[8] Side-chain effects on sulfoxide formation were unexpected, since changes in the side chain were at least two carbons removed from the reactive ring system of the radical. The present work was undertaken to clarify the effect of the side-chain structure on the product distribution and therefore reveal the involvement (if any) of the side chain in cation radical reactions. Electrochemistry, HPLC, and UV–vis spectrophotometry were the primary techniques employed because they can provide significant information from the small quantities of radicals and metabolites available. The drugs investigated include those with shorter and longer side chains than chlorpromazine.

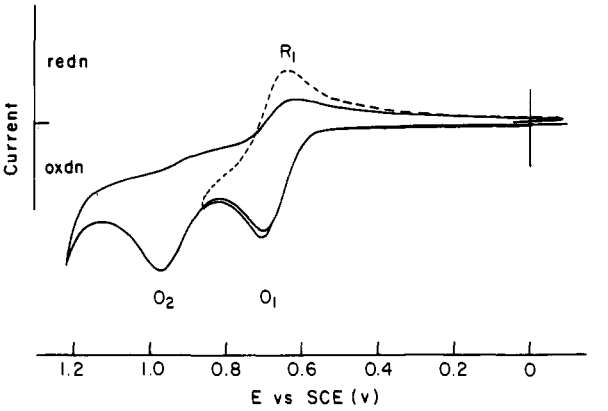


Figure 1. Cyclic voltammogram of 1.0 mM promethazine in 0.1 M HCl. Scan rate = 0.1 V/s. Dashed line results when initial oxidation scan is reversed after O_1 peak.

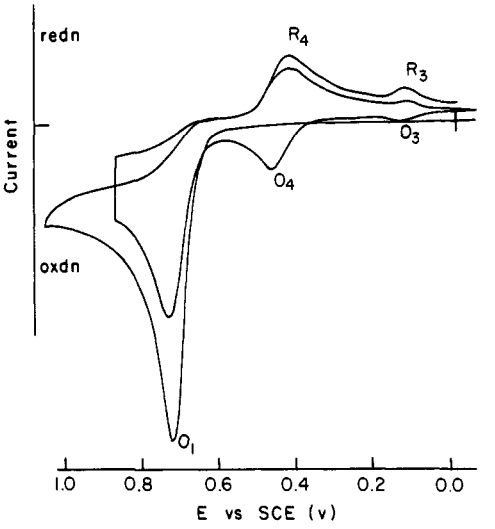


Figure 2. Cyclic voltammogram of 1.0 mM promethazine in pH 3.25 phosphate buffer. Scan rate = 0.1 V/s. Slightly larger R_3 and O_3 peaks result after holding the potential at 0.8 V on the second scan. R_3/O_3 couple has potential within 5 mV of that for 2,3-dihydroxypromazine.

Experimental Section

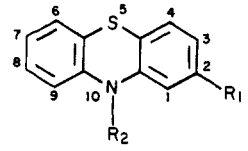
Except for the small solution volumes employed (~3 mL), the electrochemical and chromatographic techniques were conventional and have been previously.[8,9] All redox potentials are reported vs. the saturated calomel electrode (SCE) and a graphite past working electrode was used in all cases. Radical perchlorate salts were synthesized from the parent drugs by an established procedure.[6] Chlorpromazine (SKF), fluphenazine (Squibb), perphenazine (Schering), prochlorperazine (SKF), promethazine (Wyeth) and trifluoroperazine (SKF) were donated by their manufactures. Structures of the drugs examined are given in Table I. 2,3-Dihydroxypromazine, 2,3-dihydroxyperphenazine, and other hydroxylated species were gifts from A. A. Manian, Psychopharmacology Research Branch, NIMH. UV-vis spectra were obtained on a custom-built rapid-scanning spectrophotometer, capable of acquiring a 200-nm wide spectrum every 4 s. The chromatographic analyses were carried out as described previously,[8] using a reversed-phase column and an ethanol/H_2O/acetic acid eluent containing 0.01 M heptanesulfonic acid as an ion pairing reagent.

Results

Below pH 3, the cyclic voltammograms of promethazine have the appearance of Figure 1, taken in pH 1 HCl. Two

(7) Cheng, H. Y; Sackett, P. H.; McCreery, R. L. *J. Med. Chem.* 1978, *21*, 948.
(8) Sackett, P. H.; McCreery, R. L. *J. Med. Chem.* 1979, *22*, 1447.
(9) Adams, R. N. "Electrochemistry at Solid Electrodes"; Marcel Dekker: New York; 1969; Chapter 5.

Table I. Structures and Dissociation Constants of Phenothiazine Drugs



name	no.	R_1	R_2	pK_a of side chain [a]
chlorpromazine	1	Cl	$CH_2CH_2CH_2N(CH_3)_2$	9.3
promazine	2	H	$CH_2CH_2CH_2N(CH_3)_2$	9.4
triflupromazine	3	CF_3	$CH_2CH_2CH_2N(CH_3)_2$	9.2
promethazine	4	H	$CH_2CH(CH_3)N(CH_3)_2$	9.1
chlorphenethazine	5	Cl	$CH_2CH_2N(CH_3)_2$	9.1 [b]
fluphenazine	6	CF_3	$CH_2CH_2CH_2$-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	3.9, 8.1
perphenazine	7	Cl	$CH_2CH_2CH_2$-c-$N(CH_2CH_2)_2N$-CH_2CH_2OH	3.7, 7.8
prochlorperazine	8	Cl	$CH_2CH_2CH_3$-c-$N(CH_2CH_2)_2N$-CH_3	3.7, 8.1 [c]
trifluoperazine	9	CF_3	$CH_2CH_2CH_2$-c-$N(CH_2CH_2)_2N$-CH_3	3.9, 8.1

[a] From ref 12 unless noted otherwise. [b] Estimated to be equal to promethazine. [c] From ref 16.

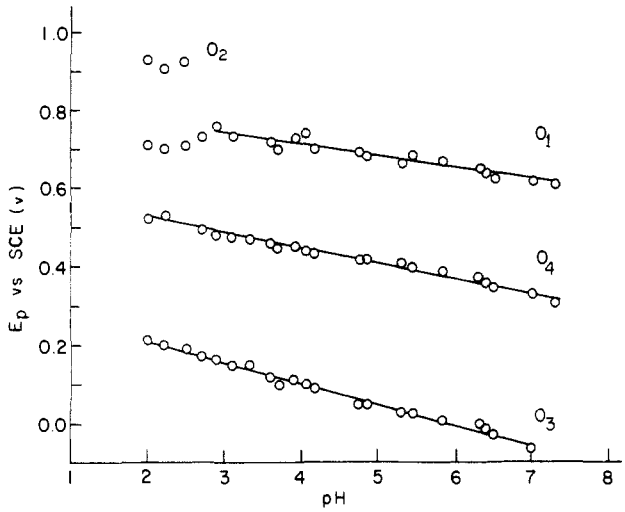


Figure 3. Anodic peak potentials for promethazine as a function of pH. Citrate/phosphate buffer is used throughout the indicated pH range. Slopes of the lines are as follows: O_1, 29 mV/pH unit; O_3, 53 mV/pH unit; O_4, 37 mV/pH unit.

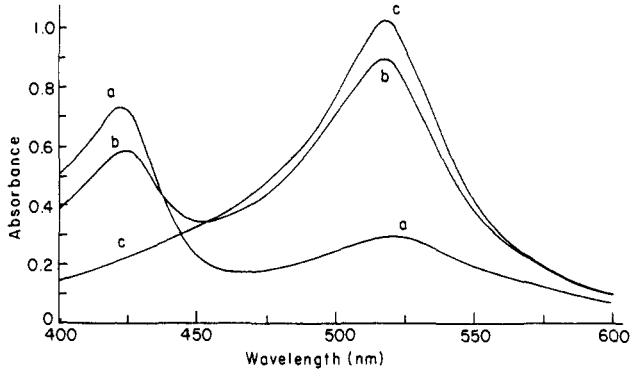


Figure 4. Visible spectra of promethazine radical cation taken 25 s after dissolution of the radical perchlorate salt in phosphate buffer. Curve a, pH 2.40; curve b, pH 1.45; curve c, pH 0.8. Scan rate = 150 nm/s.

oxidation waves are observed, corresponding to the formation of a cation radical (O_1) and a dication (O_2). Like chlorpromazine, the promethazine radical is fairly stable on a voltammetric time scale at low pH, as shown by the presence of a reduction wave (R_1) on the reverse scan. As the pH in increased, the position of the O_2 peak shifts in a negative direction until a pH of 3 is reached, at which point the two oxidation waves coalesce. Figure 2 is a cyclic voltammogram at pH 3.25, showing a single oxidation wave (O_1) on the initial scan, which was shown to be a two-electron wave by chronoamperometry. Two new couples (O_3/R_3 and O_4/R_4) are apparent at pH 3 after the initial scan, implying formation of new compounds from the oxidation products formed during O_1. These couples were apparent at lower pH, but with smaller amplitude, indicating their formation is accelerated at higher pH. The variations in peak potential for all four anodic peaks with changes in pH are shown in Figure 3. The O_1 peak varies at a rate of 29 mV/pH unit for the pH range 3–8, unlike the corresponding peak for chlorpromazine, which is pH independent. The O_3 peak potential varies at a rate of 53 mV/pH unit, and the magnitude of the observed potentials are within a few millivolts of those observed for 2,3-dihydroxypromazine. The behavior of the R_4/O_4 couple is similar to that of 7-hydroxychlorpromazine in both magnitude and slope.[10] Chromatograms of reaction products of promethazine cation radical in phosphate buffer show no sulfoxide, but several peaks are observed eluting before the parent. One of these peaks has spectral characteristics identical with 2,3-dioxopromazine.

The spectral characteristics of the promethazine radical were observed by dissolving radical perchlorate salt in solutions buffered at values below pH 3, where the radical is sufficiently stable to examine using rapid-scanning spectrophotometry. At low pH (e.g., 0) or low radical concentration, a peak at 519 nm was observed with a molar absorptivity of 8350. These values are quite comparable to those for promazine radical (518 nm and 6507 M^{-1} cm^{-1}).[8] As the pH of the concentration is increased, a new peak at 422 nm appears, and the relative contribution of the 422-nm peak increases with pH (Figure 4) or total radical concentrations (Figure 5). Both peaks decay on a time scale of minutes at a pH above 0; so accurate absorbances for the two components were obtained by extrapolating sequential spectra to $t = 0$. A plot of the log of the absorbance at 519 nm vs. that at 422 nm is shown in Figure 5, for varying total radical concentration at a pH of 2.50. The slope of the least-squares line through these points is 2.01.

Cyclic voltammograms of perphenazine exhibit two oxidation waves on the initial scan, similar to those shown in Figure 1 and those observed for chlorpromazine. Throughout the pH range 3–7, two new couples are observed after the first scan, qualitatively similar to those (O_3 and O_4) in Figure 2. The peak potentials for the

(10) Neptune, M; McCreery, R. L. *J. Med. Chem* **1978**, *21*, 362.

perphenazine oxidation are summarized in Figure 6, for voltammograms obtained in phosphate/citrate buffer. O_1 and O_2 are the pH-independent oxidation waves observed on the first scan, and O_3 and O_4 are the new couples observed after initial oxidation. The redox behavior for authentic 2,3-dihydroxyperphenazine was identical with that for the O_3/R_3 couple observed for the parent drug and is included in Figure 6 for comparison.

The voltammetric behavior of fluphenazine was very similar to that of perphenazine, with the same follow-up couples being observed. In contrast to promethazine, the initial oxidation peaks (O_1, O_2) for perphenazine and fluphenazine were not pH dependent, although the other two couples had behavior similar to promethazine. For all derivatives with three carbon side chains, the initial oxidation was pH independent, indicating that the side-chain pK_a was not altered significantly by oxidation of the ring. The perphenazine radical spectrum was not dependent on pH or concentration and had the usual radical cation absorption band at 525 nm. After reactions of the perphenazine radical at either pH 2 or 6 in phosphate buffer, a 23% yield of sulfoxide and 43% yield of parent were observed as determined by HPLC.[8] Two additional unidentified chromatographic peaks were observed, which elute in the region expected for hydroxylated species.

Voltammograms of prochlorperazine and trifluoperazine did not show the O_3 and O_4 couples observed for promethazine and perphenazine, indicating that the products responsible for these waves were not formed in significant amount in the time scale of a cyclic voltammogram (~5 s). Upon reaction of the radical salts with pH 7 phosphate buffer (0.3 M) a 63% yield of parent was observed for trifluoperazine and 61% for prochloroperazine, determined coulometrically. These compounds were not amenable to HPLC analysis, but the same coulometric analysis on the reaction of chlorpromazine radical, which is known to yield 50% parent, indicated a 51% yield of parent.

A summary of the product distributions for reactions of various combinations of radicals and nucleophiles is given in Table II. When no amine nucleophile was added, the "amine pK_a" is that of the parent drug side chain (the thiazine nitrogen is not protonated under these conditions). An "S" under "products" indicates that a quantitative yield of 50% sulfoxide and 50% parent was observed for the radical reaction. An "H" indicates that some hydroxylated products were identified by HPLC, voltammetry, spectrophotometry, or all three. For prochlorperazine and trifluoperazine, the "(H)" indicates that more than 50% of the parent was formed, indicating behavior different from chlorpromazine, but that hydroxylated products were not identified definitively.

Table II. Reaction Products of Phenothiazine Cation Radicals in Various Aqueous Solutions (pH 7.0)

radical derived from	added amine	pK_a of amine [a]	buffer	products [b]
1	none	9.3	none	H [c]
1	none	9.3	acetate	S [e]
1	none	9.3	phosphate	S [d]
1	isopropylamine	10.7	none	H [c]
1	isopropylamine	10.7	acetate	S [c]
1	isopropylamine	10.7	citrate	S [c]
1	Mes [g]	6.2	acetate	H [c]
1	Mes	6.2	citrate	H [c]
2	none	9.4	phosphate	S [e]
3	none	9.2	phosphate	S [e]
4	none	9.1	phosphate	H [e,f]
5	none	9.1	phosphate	H [e]
6	none	3.9	phosphate	H [e,f]
7	none	3.7	phosphate	H [e,f]
8	none	3.7	phosphate	(H) [f]
9	none	3.9	phosphate	(H) [f]

[a] pK_a of added amine, unless none was added, in which case the lowest pK_a of the phenothiazine side chain is given. [b] H denotes at least partial hydroxylation; S denotes only sulfoxide formation. [c] Data from ref 7. [d] Data from ref 6. [e] Data from ref 8. [f] Data from present work. [g] MES = 2-(*N*-morpholino)ethanesulfonic acid (pK_a = 6.2).

Discussion

Since the sulfoxides and hydroxylated metabolites represent two of the three major classes of metabolites for the phenothiazines (the third being side-chain degradation), it is of interest to consider what factors affect their formation. It is clear from previous reports that amine nucleophiles promote the formation of hydroxylated products from chlorpromazine cation radical; however, in a medium containing only carboxylate or phosphate nucleophiles, only sulfoxide and parent are formed.[7] Previous work indicated that modifications to the 10-position side chain can reduce sulfoxide yield, but the details and cause of this process were not revealed. The present results demonstrate that 2,3-dioxopromethazine, indicating hydroxylation of the radical accompanying the reduced sulfoxide yield. Thus, for radicals with shorter side chains (e.g., 4 and 5) or those containing a piperazine ring (8 and 9), hydroxylation occurs even in the absence of an amine buffer. From Table II it is clear that fluphenazine and chlorpromazine have quite different behavior in the same phosphate buffer, and promethazine shows still different behavior, as indicated from its voltammetry. The basic question to address is why structural changes in the side chain, three carbons removed from the reactive ring system, should have a pronounced effect on the distribution of hydroxylated or sulfoxide products.

The pH dependence of the initial oxidation of promethazine was unexpected, since similar processes for chlorpromazine are completely independent of pH above a value of 0. The variation of the O_1 potential for promethazine (29 mV/pH unit) implies it is a two-electron oxidation accompanied by the loss of one proton above pH 3.[11] The proton cannot be related to the thiazine nitrogen, since the pK_a of this nitrogen is in the region of −2, and it will not be protonated above pH 0. The proton must be lost from the amine of the side chain, since the nitrogen will be protonated in the parent drug (pK_a = 9.1).[12] Apparently, the proximity of the highly charged ring system in the oxidized form lowers the pK_a of the side-chain amine to cause deprotonation. The pH dependence of the oxidation potential of promethazine has been noted before[13] and was attributed to electronic interaction between the ring system and the amine. The slope of the O_1 potential vs. pH plot of Figure 3 indicates that oxidation of the ring lowers the pK_a of the side-chain amine by at least 5 units, resulting in deprotonation of the amine in the oxidized form. The formation of mono- and dihydroxylated species is indicated by the voltammograms and supported by the electrochemical and spectral similarity of the O_3 couple with 2,3-dihydroxypromazine, which has the same ring system as dihydroxypromethazine. Above pH 3, the voltammograms of promethazine indicate a two-electron oxidation to a product which reacts to form hydroxylated products on a time scale of less than 1 s. If the radical is

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(12) Newton, D. W.; Klaza, D. B. *Drug Intell. Clin. Pharm.* **1978**, *12*, 546.
(13) Kabasakalian, P.; McGlotten, J. *Anal. Chem.* **1959**, *31*, 431.

dissolved in a medium with pH >3, thermodynamics dictate that it disproportionate into promethazine and the two-electron oxidized product, which then hydroxylates. The radical can either hydroxylate directly or disproportionate and then hydroxylate. This behavior is in sharp contrast to chlorpromazine for which a disproportionation to a two-electron product was rigorously ruled out.[6] The difference is caused by the deprotonation of the side-chain amine in promethazine, which lowers the O_2 potential and stabilizes the two-electron species.

Below a pH of 3, the radical of promethazine does not degrade to hydroxylated products as rapidly and can be examined spectrophotometrically. The existence of two spectroscopically distinct forms of the promethazine radical below pH 3 is explained by reversible aggregation of two or more radicals. Consider reaction 1 which has the

$$nM \rightleftharpoons P_n \quad (1)$$

$$K = \frac{[P_n]}{[M]^n} \quad (2)$$

equilibrium constant indicated in eq 2, where M is monomer action radical and P_n is some aggregate. After taking the log of eq 2 and substituting concentration with absorbance (1-cm cell), eq 3 results, where A_p is the ab-

$$\log A_p = n(\log A_M) - [n(\log \epsilon_M) - \log \epsilon_p - \log K] \quad (3)$$

sorbance of the aggregate, A_M is the absorbance of the monomer, and ϵ's are molar absorptivities. In the case of promethazine, A_M was measured at 519 nm and A_p at 422 nm, while the total concentration of radical was varied. A plot of log A_p vs. log A_M has a slope of 2.01 (Figure 5), so n must be 2 and the aggregate is a dimer. Note that the term in parentheses in eq 3 is a constant independent of concentration and that n may be determined without knowing the molar absorptivities or K. At higher concentration or pH, the dimer is favored, implying the loss of a proton upon dimerization, as shown in eq 4. The

$$2(MH^{+2}) \rightleftharpoons (M_2H)^{+3} + H^+ \quad (4)$$

monomer is the protonated radical cation having an overall +2 charge. The equilibrium constant for this reaction can be estimated from the data in Figure 5 by varying the total radical concentration. The value is 125 ± 10 and is a constant over a factor of 8 in total radical concentration.[14]

The dimerization and pH-dependent redox behavior of promethazine radical greatly complicate examination of its reactions, but two points are clear. First, spectroscopic, electrochemical, and chromatographic evidence indicate formation of hydroxylated species from the reactions of the radical. Second, there is a strong interaction between radicals in solution which involves the side-chain amine. The stoichiometry of this interaction implies loss of a proton from the side chain of one radical, followed by reversible attack of the amino onto another radical forming a dimer. Either above or below pH 3, the behavior of promethazine differs substantially from that of chlorpromazine, except at very low pH (i.e., 0). Whether the promethazine radical dimerizes or disproportionates, the differences in its behavior from that of chlorpromazines result from the deprotonation of the side-chain amine.

Voltammograms of perphenazine and fluphenazine show hydroxylation occurring during the time scale of a cyclic voltammogram, and in the case of perphenazine, one product has identical electrochemical behavior with authentic 2,3-dihydroxyperphenazine. No evidence of a dimer was found for the radicals of these drugs, since the radical spectrum had behavior expected for a single species and was not dependent on concentration. As noted by others,[13] the pH independence of the initial oxidation of drugs with three carbon side chains indicates that three carbons are sufficient to insulate the side-chain amine from the effects of ring oxidation.

The data in Table II allow conclusions to be drawn about the factors which determine whether hydroxylation will be observed. Hydroxylation will occur if an amine from some solution component can act as a nucleophile. Consider the chlorpromazine cation radical in plain water or in a solution containing isopropylamine. Both the side-chain amine and isopropylamine have pK_a values above 9, so the amine is mostly protonated at pH 7 or below. However, if no other nucleophiles are present, the small amount of unprotonated amine will cause hydroxylation. Other nucleophiles (e.g., acetate) can easily exceed the rate of the amine reaction, since the deprotonated amine is in such low concentration. As the pK_a of the amine is lowered, as with α-(*N*-morpholino)ethanesulfonic acid (Mes; pK_a = 6.2), a much higher fraction is in the deprotonated form, and hydroxylation is observed even in the presence of other nucleophiles. Citrate is the fastest carboxylate nucleophile studied, yet Mes competes favorably with citrate to yield hydroxylated species.

For chlorpromazine and other drugs with an *n*-propyl side chain terminated by a basic amine (i.e., **2** and **3**), the terminal amine will not be involved in radical decay, except for the case where no other nucleophiles are present. When the length of the side chain is reduced to two carbons as in promethazine, the terminal amine is sufficiently acidic in the radical to act as a nucleophile and form a dimer. There is clearly an intermolecular interaction between two promethazine radicals which involves deprotonation of a terminal amine. Therefore, hydroxylation results from an intermolecular attack by a deprotonated amine, as with chlorpromazine and Mes. At pH 7, the promethazine will both hydroxylate directly and disporportionate, but in either case the terminal amine must deprotonate.

For radicals with piperazine side chains, e.g., perphenazine and fluphenazine, the pK_a values of the side-chain amines will be about 3.9 and 8.0.[12] Thus, one of the two amines will be deprotonated at pH 7, creating the possibility for the side chain to act as a nucleophile. Even in the presence of a fairly active oxygen nucleophile like phosphate, the deprotonated piperazine amine is sufficiently reactive to promote hydroxylation. The results indicate that prochlorperazine and trifluoperazine are less prone to hydroxylate than fluphenazine or perphenazine. The reasons for this difference are not clear, since the side chains differ by only a CH_2OH group. It is possible that the nucleophilcity of the amine may change with the addition of a CH_2OH group, but there is no evidence to support or refute this hypothesis.

Given that a deprotonated side-chain nitrogen is the important nucleophile for the piperazine drugs, the question arises of whether nucleophilic attack is intermolecular (as with promethazine) or intramolecular. Molecular models indicate that an intramolecular attack is possible at several sites on the ring system for the piperazine derivatives, so this possibility cannot be ruled out on steric grounds. The two mechanisms cannot easily be resolved on kinetic grounds, because nucleophile attack is not rate limiting, and both inter- and intramolecular mechanisms will appear second order, since a second radical is required to carry out the overall reaction in both cases. While the side-chain amine is clearly involved in

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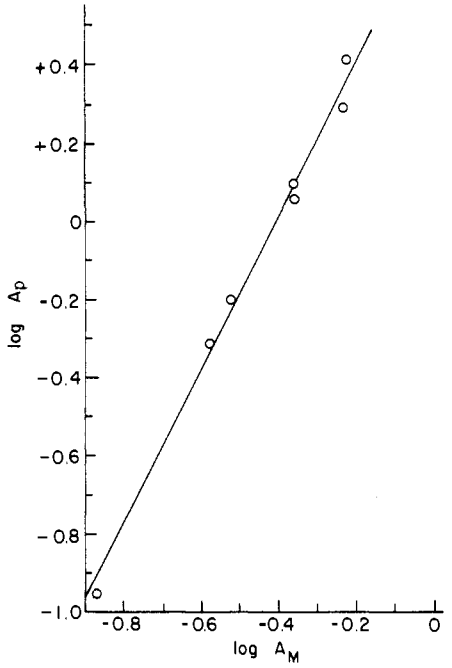

Figure 5. Variation in absorbance of the bands in Figure 4 as a function of total promethazine radical concentration with pH 2.5. A_p is the absorbance at 422 nm; A_M is absorbance at 519 nm. The slope is 2.01, the range of total radical concentration is 0.09 to 0.7 mM, and the buffer is monochloroacetate.

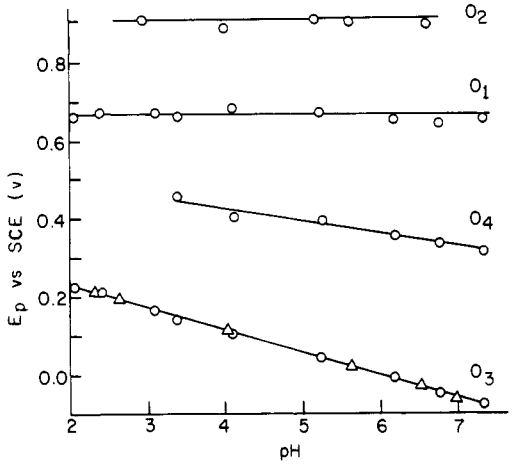

Figure 6. Anodic peak potential of perphenazine oxidation as a function of pH; conditions are as in Figure 2. Triangles on O_3 curve indicate potentials for authentic 2,3-dihydroxyperphenazine; circles are for perphenazine.

reactions of piperazine radicals, it is presently an unanswered question of whether the amine attacks from the radical's own side chain or a different molecule or radical, or parent, drug.

The mechanisn of the amine-induced hydroxylation was not examined in detail, because a mixture of products was obtained and a quantitative stoichiometric reaction could not be deduced. The process was further complicated by the fast oxidation of hydroxylated products by unreacted phenothiazine radical. Although the kinetics of the amine reactions are easily monitored, they are difficult to intepret given the variety of reactions occurring that lead to hydroxylated products. By analogy to 10-phenylphenothiazine reactions with pyridine in acetonitrile,[15] the amine can attack at the sulfur or at the 7 position, para to the thiazine nitrogen. Attack at the sulfur could induce hydroxylation on the ring and eventual loss of the amine from the sulfur. Alternatively, amine attack at the 7 position could lead to an intermediate which is hydrolyzed to form a hydroxylated product. In either case, the amine is catalytic, but it leads to a ring substitution rather than sulfoxide formation.

The biological importance of these results lies mainly in the area of metabolism. Promethazine and the piperazine antipsychotics have a greater tendency to hydroxylate, since the amine nucleophile is present in the molecule and shows a strong tendency to interact with the radical. For chlorpromazine, hydroxylation is less likely unless a nucleophilic amine is available from an external source. Otherwise, carboxylates or phosphates will attack the radical faster than its own side chain to produce sulfoxide. These results also support the hypothesis that the cation radical is an important intermediate in vivo, since two major classes of metabolites result from reactions of the radical with physiologically occurring materials. It is important to note that the doubly oxidized form of the parent drug, the dication, is not likely to be an important intermediate in vivo as has been proposed.[1,2] Dication formation requires a thermodynamically more difficult oxidation potential than the cation radical (except for promethazine), and the dication will be present in much lower concentration than the radical. Since the common sulfoxide and hydroxylated metabolites can be made from the radical, it is not necessary to require the dication as an intermediate. Furthermore, disproportionation of cation radical to form dication has been ruled out previously.[6] Promethazine is a possible exception to this statement, since the doubly oxidized species is favored above pH 3 and, therefore, may be involved in vivo.

With regard to the fate of the cation radical in vivo, it is clear that deprotonated amines are the most reactive of the nucleophiles studied, including carboxylates, phosphates, and a variety of amines. Earlier work indicates that gluthathione and similar mercaptan nucleophiles are more reactive than amines, but their reactions have not been studied in detail.[14] It has been reported[17] that ascorbic acid is a very rapid reducing agent for chlorpromazine radical, but this reaction will lead to parent drug and not metabolic products. It is difficult to correlate the product distributions observed here with metabolic profiles from animals or humans. As mentioned earlier, the relative importance of sulfoxides and hydroxylated metabolites varies widely with species,[5] implying that many factors besides radical structure affect metabolite distribution. Nevertheless, the results presented here indicate the likely modes of decay of radical to form two of the three major metabolite groups.

In conclusion, a phenothiazine cation radical untouched by an amine will react with even very weak nucleophiles to produce sulfoxide and parent by an established route. However, if a deprotonated amine is present, even on the molecule's own side chain, significant hydroxylation will occur, with the degree of hydroxylation being determined by structural factors on the drug molecule.

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