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Effect of Structure on Phenothiazine Cation Radical Reactions in Aqueous Buffers

Patricia Holt Sackett and Richard L. McCreery*

Department of Chemistry, Ohio State University, Columbus, Ohio 43210. *Received May 9, 1979*

The reactions of the cation radicals of 11 phenothiazine tranquilizers were examined in mildly acidic aqueous buffers. Of the 11, those having an aminopropyl side chain in the 10 position reacted to form 0.5 mol of sulfoxide and 0.5 mol of parent drug per mole of initial radical. Cation radicals with different side chains react to form additional products, which remain to be identified but probably result from hydroxylation of the phenothiazine ring. The decay kinetics of three of the cation radicals undergoing reactions with known stoichiometry, namely, chloropromazine, promazine, and trifluprομazine, were studied in detail, and it was concluded that they all react via the same mechanism. The mechanism involves attack of the cation radical by a nucleophile, and radicals with electron-withdrawing groups in the 2 position react more quickly. Since the cation radicals with faster reaction rates with nucleophiles are more pharmacologically active, it is hypothesized that the cation radical–nucleophile interaction may be responsible for binding of phenothiazines to receptor proteins.

The mechanism of action of the phenothiazine class of major tranquilizers has remained a subject of intense study since the beginning of their use in the early 1950's.[1,2] Significant evidence has been presented that the drugs interact with dopamine receptors, and a good correlation exists between drug potency and the strength of this interaction.[2-4] In addition, several investigators have proposed that the cation radical formed by oxidation of a phenothiazine, such as chlorpromazine, is an important intermediate in the metabolism of the drug[1,5,6] and may be the active pharmacological entity.[1,5,7-9] The reasons for these hypotheses have been summarized previously and will not be repeated here.[10] The work presented herein is related to the question of radical involvement in metabolism and activity of phenothiazine major tranquilizers.

The influence of the 2-position substituent and the structure of the 10-position side chain on the antipsychotic activity of phenothiazines has been studied in detail, and it has been concluded that changes in drug structure at these positions cause variations in antipsychotic activity covering at least three orders of magnitude.[11,12] However, information about substituent effects on cation radical behavior is less prevalent due to the difficulty in studying the reactive radical cations. Given the likely involvement of the radical in the metabolism of the drugs and possibly in their activity, it is deemed useful to more fully investigate the behavior of the radicals as a function of structure.

The phenothiazine cation radicals have been studied by a variety of approaches by previous workers.[1,13-15] Due to rapid reactions of the radicals at physiological pH, all but a few of the previous studies have been carried out in strong mineral acids or in concentrated acetic acid. In one paper, an attempt was made to correlate cation radical stability in 1–9 N sulfuric acid solutions with clinical potency, but no correlation was found.[13] Until recently, the second-order kinetics of the radical decay in these solvent systems led workers to propose a disproportionation mechanism for the radical decay, leading to reduced phenothiazine and a sulfoxide.[1,5,13,14] Recent work in aqueous buffers in the pH range from 2 to 5 demonstrated that the cation radical of chlorpromazine (1) does not disproportionate but is attacked by a weak nucleophile.[15] The resulting adduct adduct is oxidized by a second molecule of the cation radical, and then decomposes to form reduced chlorpromazine and chlorpromazine sulfoxide as shown in Scheme where where $A^{+\cdot}$ represents the chlorpromazine cation radical, A represents chlorpromazine, AO its sulfoxide, and B^- a buffer anion. This mechanism is similar to that observed for thianthrene and 10-phenylphenothiazine cation radical reactions with pyridine in acetonitrile.[16,17]

In subsequent work, it was shown that the products and the rate of the reaction are highly dependent on the identity of the nucleophile, with some nucleophiles yielding hydroxylated products rather than sulfoxide.[10] Given this further knowledge about the importance of solution con-

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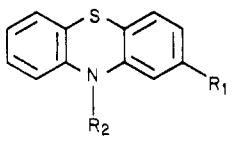
Table I. Products of Phenothiazine Radical Reactions in pH 4.0 Phosphate Buffer



compound	designation	R_1	R_2	LC results: % sulfoxide	LC results: % parent	coulometric results, % parent
chlorpromazine	1	-Cl	$-CH_2CH_2CH_2N(CH_3)_2$	51.4	52.5	51.2
promazine	2	-H	$-CH_2CH_2CH_2N(CH_3)_2$	50.8	48.0	49.6
triflupromazine	3	$-CF_3$	$-CH_2CH_2CH_2N(CH_3)_2$	52.4	48.9	47.1
acepromazine	4	$-COCH_3$	$-CH_2CH_2CH_2N(CH_3)_2$	50.5	49.5	48.7
methoxypromazine	5	$-OCH_3$	$-CH_2CH_2CH_2N(CH_3)_2$	47.7	42.2	50.5
norchlorpromazine	6	-Cl	$-CH_2CH_2CH_2NHCH_3$	48.5	52.0	
dinorchlorpromazine	7	-Cl	$-CH_2CH_2CH_2NH_2$	49.6	45.6	
promethazine	8	-H	$-CH_2C(CH_3)HN(CH_3)_2$	0	71.3	
fluphenazine	9	$-CF_3$	$-CH_2CH_2CH_2N\square N\square CH_2CH_2OH$	<5	81	
perphenazine	10	-Cl	$-CH_2CH_2CH_2N\square N\square CH_2CH_2OH$	33.4	47.0	
chlorphenethazine	11	-Cl	$-CH_2CH_2N(CH_3)_2$	7.0	82.7	

Scheme I

$$A^{+}\cdot + B^{-} + H_2O \underset{}{\overset{K_1}{\rightleftharpoons}} [AB(OH)]^{-}\cdot + H^{+}$$

$$[AB(OH)]\cdot + A^{+}\cdot \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [ABOH]^{0} + A$$

$$[ABOH]^{0} \xrightarrow{k_3} HB + AO$$

ditions on reaction products, rates, and mechanisms, previous discussions relating cation radical behavior in solution to clinical activity are weakened. Before one can compare cation radical reactivity and clinical activity, one must observe that the same mechanism of decay applies to the systems being compared. It is not meaningful to compare kinetic parameters of different drugs unless those parameters represent the same reactions.

The objective of the present work is to examine the influence of cation radical structure on the products and kinetics of radical decay in aqueous buffers in the pH range 2–7. Because of the importance of the buffer (and therefore nucleophile) to the reaction,[10] the solutions will be limited to phosphate and acetate buffers. Of particular interest will be the influence of the electron-withdrawing ability of the 2-position substituent and the structure of the 10-position side chain. The relationship of this information to the clinical activity and metabolism of promazine (**2**), chlorpromazine (**1**), and triflupromazine (**3**) will be discussed.

Experimental Section

Electrochemistry. Cyclic voltammetry using a graphite paste electrode and coulometry using a carbon cloth electrode were performed as described previously.[15] Oxidation potentials were determined from anodic peak potentials and are expressed as E_p vs. SCE.

Materials. Triflupromazine hydrochloride and fluphenazine dihydrochloride were gifts from E. R. Squibb and Sons; promazine hydrochloride and promethazine hydrochloride were donated by Wyeth Laboratories, Inc. Acepromazine maleate was given by Ayerst Laboratories, methoxypromazine maleate by Lederle Laboratories, and perphenazine dihydrochloride by Schering Corp. Chlorphenethazine hydrochloride, norchlorpromazine hydrochloride and dinorchlorpromazine hydrochloride were gifts from Dr. A. A. Manian of the Psychopharmacology Research Branch at NIMH. All phenothiazines were used without further purification. Cation radical perchlorate salts were synthesized electrochemically in perchloric acid as described previously.[15] Anal. Calcd for triflupromazine radical perchlorate, $C_{18}H_{20}F_3N_2S\cdot 2(ClO_4)\cdot H_2O$: C, 37.91; H, 3.89; F, 9.99; N, 4.91; S, 5.62; Cl, 12.43. Found: C, 37.79; H, 3.96; N, 4.92; S, 5.50; Cl, 12.66. The melting point of the solid was 196.5–197 °C (oil bath); the molar absorptivity of the radical in 6 M H_2SO_4 was 7209 M^{-1} cm^{-1} at 500 nm. Anal. Calcd for promazine radical perchlorate, $C_{17}H_{21}N_2S\cdot 2(ClO_4)\cdot 0.5H_2O$: C, 41.39; H, 4.50; N, 5.68; S, 6.50; Cl, 14.37. Found: C, 41.31; H, 4.34; N, 6.00; S, 6.42; Cl, 14.94. The melting point of the solid was 225–226 °C (microblock); the molar absorbtivity of the cation radical in 6 M H_2SO_4 was 6507 M^{-1} cm^{-1} at 518 nm. The characteristics of chlorpromazine cation radical perchlorate have been reported earlier.[15]

Product Analysis. A high-pressure liquid chromatograph composed of modular components was used for product analysis. An octadecylsilane reverse-phase column (2 × 250 mm) was used for separation of triflupromazine and promazine products. Eluent was composed of 50:50:1 H_2O–EtOH–HOAc (v/v) and 0.01 M 1-heptanesulfonic acid sodium salt ion-pairing reagent (Eastman Chemicals) adjusted to a pH of 4.5 with base. The separation conditions for chlorpromazine mixtures have been described elsewhere.[15] In all cases, quantitation was achieved by the use of calibration curves based on peak heights, which were linear throughout the required range. Coulometry was also used to quantitate the amount of precursor regenerated by adding strong sulfuric acid to an aliquot of the reaction product mixture. In such an acidic medium, oxidation of the phenothiazine to its sulfoxide is quantitative, and the oxidation could be used to analyze for reduced phenothiazine.[18]

Kinetics. Kinetic runs were conducted by monitoring cation radical decay at 500, 525, and 518 nm for triflupromazine, chlorpromazine, and promazine cation radicals, respectively. The reaction was initiated by introduction of radical salt dissolved in double-distilled demineralized water to an equal volume of buffer. NaCl was used to adjust the ionic strength of the buffer to a uniform value of 0.5 M. A double-beam spectrophotometer and strip-chart recorder were used to record the radical decay. Absorbance vs. time curves were converted to x and y coordinates by a digitizer and analyzed by the university computer. All solutions were thermostated at 25 ± 0.5 °C during kinetic runs.

Results

Product Analysis. The results of electrochemical and liquid chromatographic analysis for several reactions of phenothiazine cation radical perchlorates in pH 4 phosphate buffer are shown in Table I. The yields are based on the original quantity of radical salt dissolved in the buffer, with percent parent representing the quantity of regenerated reduced phenothiazine measured both by LC and by coulometry. For the first seven radicals, which are 2-substituted promazines or N-demethylated chlorpromazines, the yields are within a few percent of 50% sulfoxide and 50% parent drug; similar values result for

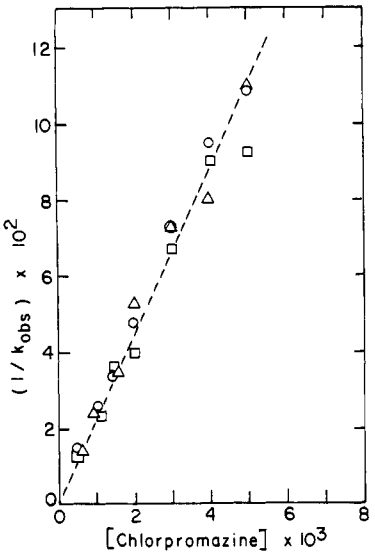


Figure 1. Reciprocal of observed second-order rate constant for chlorpromazine radical decay plotted vs. reduced chlorpromazine concentration: △, pH 3.2; ○, pH 3.7; □, pH 4.2. Acetate buffer, acetate anion concentration kept constant at 0.05 M.

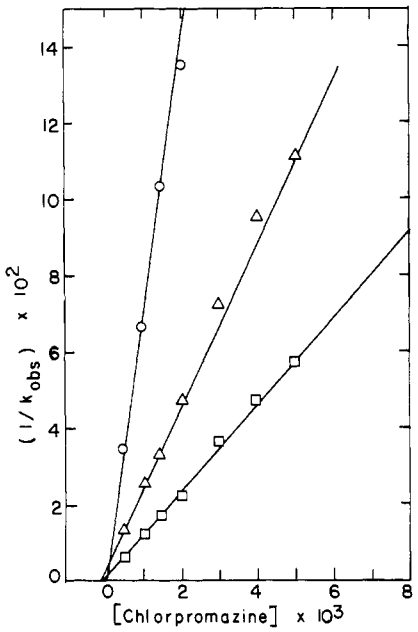


Figure 2. $1/k_{obs}$ for chlorpromazine radical decay vs. reduced chlorpromazine concentration as a function of acetate concentration, pH 3.7: ○, 0.027 M acetate; △, 0.054 M acetate; □, 0.078 M acetate.

reactions of these species in acetate buffer. Thus, for these seven systems, the overall stoichiometry of the radical decay is represented by eq 1, where $A^{+}\cdot$ is a phenothiazine

$$2A^{+}\cdot + H_2O \rightarrow A + AO + 2H^{+} \quad (1)$$

radical and AO is its sulfoxide.

For entries 8–11 in Table I, the product distribution is obviously much different, with much lower yields of sulfoxide. For these compounds, the sulfoxide plus parent do not total 100% because of the presence of unidentified products, which appear in the chromatograms. The early peaks in the promethazine chromatogram have spectral, chromatographic, and electrochemical characteristics identical with 2,3-dioxopromazine, so they are likely to represent products of hydroxylation rather than sulfoxidation of the radical.[10] Further work is under way to prove this hypothesis. It is apparent that only the first seven compounds of Table I follow the stoichiometry of eq 1; therefore, these compounds were chosen for detailed kinetic investigation.

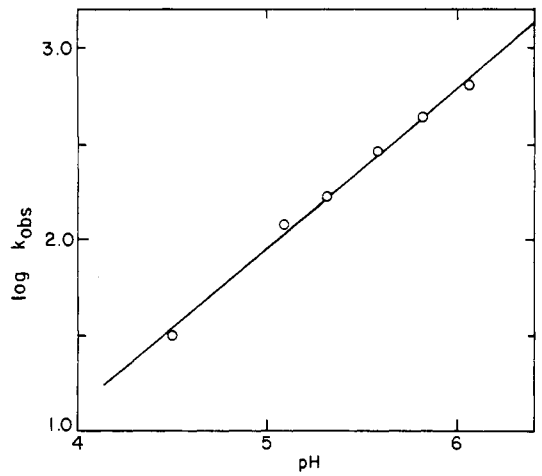


Figure 3. Log k_{obs} vs. pH for promazine radical decay in 0.1 M phosphate buffer. Initial radical concentration = 2×10^{-4} M; promazine concentration = 1×10^{-3} M; slope = 0.85.

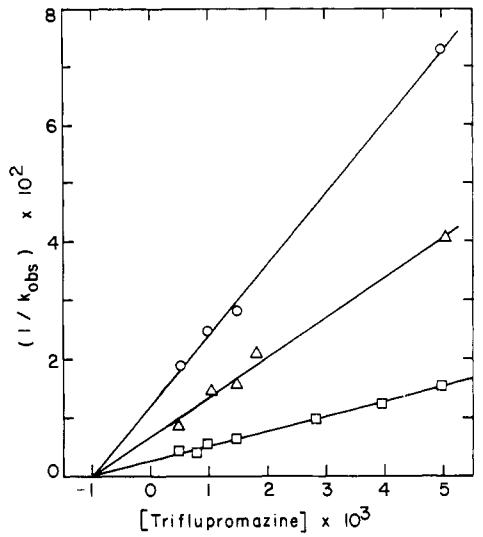


Figure 4. $1/k_{obs}$ for triflupromazine radical decay vs. reduced triflupromazine concentration as a function of acetate concentration, pH 3.7: ○, 0.0068 M acetate; △, 0.0135 M acetate; □, 0.027 M acetate.

Table II. Observed Rates of Reaction of Phenothiazine Radicals with Acetate and Phosphate Nucleophiles

no.	k_{obs} in acetate buffer,[a] M^{-1} s^{-1}	k_{obs} in phosphate buffer,[b] M^{-1} s^{-1}
1	118	22.9
2	5.3	0.89
3	690	70.5
4	925	57.4
5	22.4	14.7
6	174	6.7
7	212	9.0

[a] Conditions for acetate reactions: $[CH_3COO^{-}] = 0.08$ M, pH 3.7; concentration of reduced precursor = 0.5 mM; initial radical concentration = 1×10^{-4} M. [b] Conditions for phosphate reactions: $[H_2PO_4^{-}] = 0.08$ M, pH 2.7; concentration of reduced precursor = 0.5 mM; initial radical concentration = 1×10^{-4} M.

Kinetics. Since promazine, chlorpromazine, and triflupromazine are the most clinically important of compounds 1–7, they were studied in greatest detail. Previous work with chlorpromazine cation radical in phosphate and citrate buffers indicated that the reaction rate was second

order in radical and depended on the pH, the identity, and the concentration of the nucleophile (buffer) and the concentration of reduced chlorpromazine in the solution.[15] Detailed kinetic experiments examining each of these variables were performed with cation radicals from compounds 1–3 in acetate and phosphate buffers. The reactions were second order in radical in all cases, and the observed second-order rate constant will be abbreviated k_{obs}.

Chlorpromazine Radical Decay. The reactions of chlorpromazine radical in phosphate have been discussed previously,[15] and the mechanism of Scheme I was concluded. The reaction of chlorpromazine radical in acetate buffer was second order, but its rate was pH independent over a range of at least 3 pH units. The data for 3 pH values and several reduced chlorpromazine concentrations are summarized in Figure 1. Note that a plot of $1/k_{obs}$ vs. chlorpromazine concentration is linear, pH independent, and intercepts the origin. The reaction rate is first order in acetate concentration but has a dependence on reduced chlorpromazine concentration shown in Figure 2. Again $1/k_{obs}$ vs. chlorpromazine concentration is linear with an intercept at the origin.

Promazine Radical Decay. Although much slower, the reactions of promazine radical with both acetate and phosphate nucleophiles were qualitatively identical with those of chlorpromazine. In phosphate buffer, a plot of log k_{obs} vs. pH was linear with a slope close to 1, as shown in Figure 3. Plots of $1/k_{obs}$ vs. promazine concentrations were linear, with an intercept at the origin in phosphate buffer. In acetate buffer, the rate was pH independent, and plots of $1/k_{obs}$ vs. reduced promazine concentration were linear with intercepts at the origin.

Triflupromazine Radical Decay. Triflupromazine reacted faster than chlorpromazine under comparable conditions (see Table II). As with the other two radicals, the radical decay was second order in radical and first order in nucleophile. Also consistent with the other systems was a pH-independent rate in acetate buffer and a rate which increased with increasing pH in phosphate. Plots of $1/k_{obs}$ vs. reduced triflupromazine concentration were linear in both buffers but did not intercept the origin, as shown in Figure 4 for acetate. The abscissa intercept was about -1×10^{-3} M for a variety of acetate concentrations and pH's, and -2.2×10^{-3} M for phosphate buffer.

Methoxypromazine and Acepromazine. While the cation radicals of compounds 4 and 5 did yield 50:50 product mixtures, the kinetics of their decay were qualitatively different from any of the three cation radicals discussed above. For methoxypromazine, the reaction actually slowed down with increasing pH in phosphate buffer and acepromazine was pH independent in phosphate. Since these compounds appeared to react via a different mechanism, a comparison of reaction rates was inappropriate and their kinetic behavior was not pursued further.

Demethylated Chlorpromazines. The kinetics of decay of radicals from compounds 6 and 7 were qualitatively identical with those of chlorpromazine. In acetate buffer, the demethylated compounds reacted slightly faster than 1, while in phosphate they reacted slightly more slowly. A comparison of observed rates for all seven compounds studied is shown in Table II for both acetate and phosphate buffers. Table II serves to indicate the effect of structure on observed rates; however, the phosphate results will be pH dependent. For comparisons of buffers at various values of pH, previous work should be consulted.[10,15]

Discussion

Product Distribution. Table I and Figure 1 clearly indicate that for the compounds studied an unbranched aminopropyl side chain is necessary to produce a 50:50 mixture of neutral drug and sulfoxide. A shorter carbon chain or piperazine ring contained in the side chain will lead to different products. While there is not enough information to deduce the structural basis for this observation, it is apparent that structural changes several carbons away from the cation radical ring system drastically affect the course of the reaction. In any case, in order to relate reaction rate to structure, one must deal with the same reaction, so the radicals leading to a product distribution other than 50:50 precursor/sulfoxide will not be compared kinetically. The next step in relating structure to reactivity is to demonstrate that the reactions being compared have the same mechanism.

Kinetics. The rate law for the mechanism depicted in Scheme I, assuming steady-state conditions for intermediates, is represented by eq 2, where $A^{+}\cdot$ is the phenothiazine cation radical, A is its reduced form, and the term in brackets is k_{obs}, the observed second-order rate constant. In earlier work, it was concluded that this rate law applied to the chlorpromazine radical reaction with phosphate and citrate, and it was noted that the reaction was faster for diprotic nucleophiles such as phosphate and citrate than for monoprotic nucleophiles such as acetate under the conditions used. In addition, it was shown that the first step was an equilibrium, while the second two steps were of comparable rate.[15]

$$\frac{d[A^{+}\cdot]}{dt} = -\left[\frac{2K_1(k_2/k_{-2})k_3[H_2O][B^-]}{([A] + k_3/k_{-2})[H^+]}\right][A^{+}\cdot]^2 \quad (2)$$

Since the k_{obs} for the reaction of the chlorpromazine cation radical with acetate is pH independent, eq 2 must not apply, and therefore Scheme I must not be valid for the chlorpromazine/acetate reaction. Scheme II and eq 3 are an alternative scheme and rate law which account for the observations.

Scheme II

$$A^{+}\cdot + RCO_2^- \underset{}{\overset{K_1}{\rightleftharpoons}} [A(RCO_2)]\cdot$$

$$[A(RCO_2)]\cdot + A^{+}\cdot \overset{K_2}{\rightleftharpoons} [A(RCO_2)]^+ + A$$

$$[A(RCO_2)]^+ + H_2O \xrightarrow{k_3} AO + RCO_2H + H^+$$

$$\frac{d[A^{+}\cdot]}{dt} = -\left[\frac{2K_1K_2k_3[H_2O][RCO_2^-]}{[A]}\right][A^{+}\cdot]^2 \quad (3)$$

Note that this mechanism is first order in nucleophile, k_{obs} (the term in brackets) is pH independent, and a plot of $1/k_{obs}$ vs. [A] will have an intercept at the origin and a slope which depends inversely on $[RCO_2^-]$ but not on pH, consistent with Figures 1 and 2. The last reaction may take several steps, but the data do not permit such a conclusion.

A slight modification to the mechanism for the reaction between dihydrogen phosphate and chlorpromazine cation radical should be made to allow the reactions to be consistent in terms of mechanism. Scheme III and eq 4 ac-

Scheme III

$$A^{+}\cdot + H_2PO_4^- \underset{}{\overset{K_1}{\rightleftharpoons}} [A(HPO_4)]^{-}\cdot + H^+$$

$$A^{+}\cdot + [A(HPO_4)]^{-}\cdot \overset{k_2}{\rightleftharpoons} [A(HPO_4)]^0 + A$$

$$[A(HPO_4)] + H_2O \xrightarrow{k_3} AO + H_2PO_4^- + H^+$$

Table III

$$\frac{d[A^+]}{dt} = -k_{obs}[A^+]^2$$

radical nucleophile	mechanism	k_{obs}	abscissa intercept for $1/k_{obs}$ vs. [A]
chlorpromazine/acetate promazine/acetate	Scheme II	$\frac{2K_1K_2k_3[H_2O][RCO_2^-]}{[A]}$	0
triflupromazine/acetate	Scheme II, 2nd step nonequilibrium	$\frac{2K_1(k_2/k_{-2})k_3[H_2O][RCO_2^-]}{[A] + k_3[H_2O]/k_{-2}}$	$-k_3[H_2O]/k_{-2}$
chlorpromazine/$H_2PO_4^-$ triflupromazine/$H_2PO_4^-$	Scheme III	$\frac{2K_1(k_2/k_{-2})k_3[H_2O][H_2PO_4^-]}{([A] + k_3[H_2O]/k_{-2})[H^+]}$	$-k_3[H_2O]/k_{-2}$
promazine/$H_2PO_4^-$	Scheme III, 2nd step in equilibrium	$\frac{2K_1K_2k_3[H_2O][H_2PO_4^-]}{[A][H^+]}$	0

Table IV

	1	2	3	4	5
k_{obs},[a] M^{-1} s^{-1}	118	5.3	690	925	22.4
k_{obs},[b] M^{-1} s^{-1}	22.9	0.89	70.5	57.4	14.7
σ_p^{20}	+0.227	0.00	+0.54	+0.502	−0.268
σ_m^{20}	+0.373	0.00	+0.43	+0.376	+0.115
E_p vs. SCE, V	0.68	0.58	0.75	0.67	0.55
av clinical dose,[c] μmol kg^{-1}	12.0	33.0	4.6	5.0	30.1

[a] 0.08 M acetate, pH 3.70. [b] 0.08 M $H_2PO_4^-$, pH 2.70. [c] Reference 3 and 11.

$$\frac{d[A^+\cdot]}{dt} = -\left[\frac{2K_1(k_2/k_{-2})k_3[H_2O][H_2PO_4^-]}{([A] + k_3[H_2O]/k_{-2})[H^+]}\right][A^+\cdot]^2 \quad (4)$$

count for all observations. This mechanism differs from the original (Scheme I) only in the source of the proton causing the pH dependence. The fact that a proton may be produced from the nucleophile with phosphate and citrate nucleophiles makes the rates of cation radical decay in these buffers highly pH dependent, while nucleophiles derived from monoprotic acids do not show such a dependence. The major conclusion of the original mechanism, namely, that the buffer anion acts as a nucleophile attacking the radical, without an intervening disproportionation, is unchanged and applies to all systems examined here.

Equation 3 was derived assuming that the first two reactions were fast relative to the third and were in equilibrium. This situation was observed for both chlorpromazine and promazine reacting with acetate ion. The equilibrium nature of the first two steps can be deduced from the intercept at the origin in Figures 1 and 2. In the case of triflupromazine, the abscissa intercept of Figure 4 is negative and independent of buffer or hydrogen ion concentration. This observation is consistent with the second step of Scheme II being comparable in rate to the third step, leading to the rate law represented by eq 5. Note that

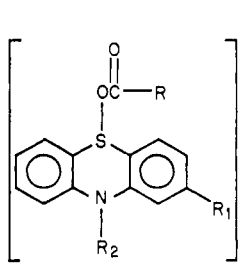
$$\frac{d[A^+\cdot]}{dt} = -\left[\frac{2K_1(k_2/k_{-2})k_3[H_2O][RCO_2^-]}{([A] + k_3[H_2O]/k_{-2})}\right][A^+\cdot]^2 \quad (5)$$

a plot of $1/k_{obs}$ vs. [A] will have an abscissa intercept of $-k_3[H_2O]/k_{-2}$, independent of buffer concentration and pH, as observed experimentally (Figure 4).

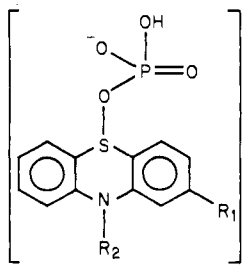
The qualitatively identical kinetic behavior of triflupromazine and chlorpromazine in phosphate buffer allows the conclusion that the reaction of **3** with $H_2PO_4^-$ follows the mechanism of Scheme III, with the second and third steps of comparable rate. Values for $k_3[H_2O]/k_{-2}$ and K_1k_2 may be deduced from the plot of $1/k_{obs}$ vs. [A] for triflupromazine and have the values 2.18×10^{-3} and 2.09×10^{-5}, respectively. The corresponding values for chlorpromazine are 1.25×10^{-3} and 1.92×10^{-6}. The larger value of K_1k_2 for triflupromazine compared to that for chlorpromazine implies that the first two steps of Scheme III are faster for triflupromazine, consistent with the faster overall rate for **3**. The intercept of $1/k_{obs}$ vs. [A] for promazine in phosphate is at the origin, implying that steps 1 and 2 of Scheme III are in equilibrium. As a consequence, the values for k_3/k_{-2} and K_1k_2 cannot be calculated.

A summary of the kinetic data for the reactions of compounds **1** to **3** with acetate and phosphate is presented in Table III. The main conclusion available from these data is that the three systems follow the same mechanism, with different radical/nucleophile combinations resulting in variations in microscopic rate constants which cause different overall observed kinetic behavior. Methoxypromazine **5** and acepromazine **4** differed greatly in kinetic behavior and, therefore, probably decay by different mechanisms. The small differences in rate between **1** and **6** or **7** lead to the conclusion that demethylation of the side-chain nitrogen has only a small effect on the rate.

The kinetic data indicate the composition of the intermediates $[ARCO_2]\cdot$ and $[AHPO_4]^{-}\cdot$, but their structures cannot be rigorously concluded. Given the localization of positive charge on the sulfur atom in the cation radicals, reasonable structures for these intermediates are **12** and



12



13

13. The reasons for the changes in observed kinetic behavior with changes in R_1 presumably result from inductive effects of R_1 on the stability of 12 and 13 and also on the rate of oxidation of these adducts by another cation radical. For example, the higher oxidation potential for 3 might make k_2 large relative to k_{-2}, resulting in a nonequilibrium situation for the second step, causing a nonzero abscissa intercept for $1/k_{obs}$ vs. [A]. In any case, the substituent changes do not alter the mechanism for 1, 2, and 3 but merely the microconstants in the rate expressions.

Since promazine, chlorpromazine, and trifluprom azine radicals react via the same mechanism, their comparative reaction rates legitimately indicate the influence of ring substituents at the 2 position on radical reactivity. While the most precise comparison would be between the value of K_1 and a substituent parameter for the 2 position, the kinetic results do not allow individual values for K_1 to be obtained. However, the overall rates do parallel the K_1k_2 values for the systems where the data allow comparison, so overall rate constants will be used to compare substituent effects. Table IV summarizes kinetic data for compounds 1–5, as well as oxidized potentials, Hammett σ values, and clinical doses. As one would expect, the rates of reaction for compounds 1–3 increase with the electron-withdrawing ability of the 2-position substituent, as indicated by σ_p values. Correlations of radical decay rates in strong sulfuric or acetic acid with σ_m values have been pointed out before,[13,14] but these are inappropriate given that the sulfur atom is the reacting center and is not meta to the 2 position. The exception of methoxypromazine from the trend presumably results from the fact that the decay of its radical follows a different mechanism. Given the multistep nature of the radical decay, it is not surprising that the Hammett correlation is not linear, but the trend is clear.

The oxidation potentials also follow a trend with Hammett σ_p values, with electron-withdrawing groups increasing the potential, again as one would predict. In addition, the resulting cation radical is more electron deficient and, therefore, more subject to nucleophilic attack, reflected in faster reaction rates.

The significance of these results to the biological activity of the compounds stems from several points. First, this work extends earlier investigations in strong acid media into the much milder conditions of the pH region 2–5. While the reactions were too fast to study rigorously at pH 7, the conditions used certainly more closely approximated a physiological environment than those used in earlier work. Second, the products of radical decay are highly dependent on radical structure, with changes to the side chain leading to hydroxylated products rather than (or as well as) sulfoxides. Numerous reports have indicated that the cation radical is an intermediate in phenothiazine metabolism;[1,5,9,19] the present work provides insights into structural factors influencing the formation of metabolites from the radical. Third, the lack of a correlation between radical stability and clinical activity reported by others[13] is not conclusive, since different radicals react to form different product distributions or their decays proceed via different mechanisms. Furthermore, the mechanisms and rates of reaction also depend strongly on the identity of the nucleophile; it is indeed difficult to predict the environment of a radical formed in vivo. Fourth, it should be noted that for those radicals having a common mechanism, those derived from 1 to 3, the more reactive radicals have lower clinical doses. Based on the relationship between dopamine receptor binding and clinical activity, it was proposed that a cation radical–nucleophile interaction could be the molecular form of this receptor binding.[10] The radical–receptor binding would be relatively stable and would alter the receptor's characteristics until the radical were oxidized and the reaction proceeded to regenerate the nucleophile and the phenothiazine sulfoxide. Given the low concentration of radicals in vivo, the oxidation of the radical–receptor adduct would be much slower than the rates observed in the solutions used here. In the present work, the most reactive cation radicals, and therefore those with the fastest radical–nucleophile interaction, are the most pharmacologically active. A series of three compounds can hardly be considered a correlation, but the relationship is valid for the three systems which have the same mechanism and therefore can be compared on a basis of reactivity. It should be emphasized that the comparison of reactivity and clinical potency can only be made after the variables of product distribution and mechanism have been removed. Once the radicals with the same decay mechanism are compared, the present work suggests that the rate of radical reactivity with nucleophiles is important to clinical activity rather than overall radical stability.

Conclusion

Of the 11 phenothiazine cation radicals examined, 7 react in pH 2–5 acetate and phosphate buffers to yield 50% sulfoxide and 50% reduced phenothiazine. The remainder form additional products which are likely to result from ring hydroxylation. Of the seven compounds, promazine, chlorpromazine, and trifluprom azine react with the same mechanism (Schemes II and III) involving nucleophilic attack of the radical by acetate or dihydrogen phosphate, although the observed kinetics differ somewhat due to variations in rate constants. An electron-withdrawing group para to the sulfur increases the rate of reaction of the cation radical, and radicals with high reactivity have higher clinical activity. It is hypothesized that the interaction of phenothiazine cation radicals with nucleophiles associated with receptor sites may be related to receptor binding, and therefore more reactive radicals are clinically more active. In addition, the distribution of metabolites from phenothiazine drugs may result from different modes of radical decay caused both by radical structure and the identity of an attacking nucleophile. It is apparent that the fate of metabolically generated phenothiazine cation radicals is a question of significant complexity and that simple disproportionation mechanisms are not significant in weakly acidic buffers or near the physiological pH range.

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Potential Antitumor Agents. 32. Role of Agent Base Strength in the Quantitative Structure–Antitumor Relationships for 4'-(9-Acridinylamino)methanesulfonanilide Analogues

William A. Denny, Graham J. Atwell, and Bruce F. Cain*

Cancer Chemotherapy Research Laboratory, University of Auckland School of Medicine, Auckland, New Zealand.
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Several homologous series of 9-anilinoacridines, each bearing a different pK_a modulating acridine substituent, have been synthesized and screened in the L1210 leukemia system, to examine if measures of agent lipophilic–hydrophilic balance utilized in regression analysis should be corrected for the effects of changing base strength. The measure of tumor selectivity modeled was the maximum increase in life span in L1210 tests (ILS_{max}), and dose potency was gauged as D_{40}, the molar drug dose necessary to provide 40% extension in life span. Agent lipophilic–hydrophilic balance was measured as chromatographic R_m values, and the pK_a modulating factors examined were log $(1 - \alpha)$ and log α, where α is the fraction of drug ionized at physiological pH. Regression analyses of data from 78 L1210-active compounds show that the measured R_m values, unmodified by pK_a correction factors, furnish superior correlation equations. An equation in R_m^2, with indicator variables denoting the presence of acridine 3-NO_2 or 4-CONRR' substituents, successfully models ILS_{max}. To develop successful regression equations for D_{40} it was necessary to restrict attention to close structural congeners which are likely to be metabolized by similar routes. Results for a series of 3-nitroacridine derivatives which may be reduced in vivo to more dose-potent and/or more hydrophilic compounds could not be incorporated. Acceptable equations developed for D_{40} contain terms in R_m, R_m^2, and pK_a or $t_{1/2}$. The latter provides a measure of the rate of agent thiolytic cleavage, a prominent contributor to drug decay in vivo.

The high, broad spectrum antitumor activity of previously examined members[1-8] of the 4'-(9-acridinylamino)-methanesulfonanilide series has lead to the clinical trial of one congener (**22**, Table I, *m*-AMSA, NSC249992).[9] To aid the discovery of more effective second-generation analogues, it was hoped to discern those drug features associated with tumor selectivity by development of quantitative structure–activity relationships (QSAR) for this series. With 12 apparently nonequivalent drug positions available for substitution and varying dependence of biologic activity on the steric and electronic contributions of substituents at each of these positions, further compounded by the less than desirable accuracy of antitumor screening data, we have been unable to develop satisfactory QSAR by conventional approaches. A possible contributor to this lack of success is the varying pK_a values of the drugs. The magnitudes of the ionization constants (see Table I for examples) are such that each substituted agent will likely provide a different percentage of cationic species at physiologic pH values. It has been shown that virtually any drug substituent, appended to either the acridine or 9-anilino ring system, will alter agent base strength.[8] Certain qualitative SAR in this drug series are difficult to rationalize without ascribing a critical role to agent base strength.[6] Additionally, in an earlier QSAR study of unrelated series of antitumor drugs we had successfully quantified the contribution of agent pK_a values to antitumor activity.[10] With the failure of conventional methods of developing QSAR, in the present series it became necessary to design an alternate method of gauging the role of base strength in antitumor activity, and such a study forms the basis of this article.

Chemistry. Agent generation involved the general procedures evolved earlier.[1-3] Thus, Jourdan–Ullmann condensation of a substituted 2-chlorobenzoic acid and the requisite aniline afforded a *N*-arylanthranilic acid.[2] Ring closure then provided the substituted 9(10*H*)-acridones, which with $SOCl_2$/DMF[2] furnished the corresponding 9-chloroacridines. Acid-catalyzed coupling of the latter with the appropriate 3'-methoxy-4'-aminoalkanesulfonanilide[3] provided the bulk of the cogeners required.

4-Hydroxy-9(10*H*)-acridone could be conveniently prepared by pyridine hydrochloride dealkylation of 4-methoxy-9(10*H*)-acridone. The 4-*O*-alkylacridones necessary for preparation of **41–43** were then prepared by alkylation (RBr/Me_2SO) of the sodium salt of the 4-hydroxy-9-(10*H*)-acridone. Reaction of the latter under similar conditions with 4-bromobutyronitrile gave a cyano ether which readily hydrolyzed to the corresponding acid. The latter was elaborated, as in earlier work,[5] to the amide variant **47**.

Selective reaction of the acid chloride function of 9-chloroacridine-4-carbonyl chloride[5] with *n*-butylamine in alkaline media at low temperatures afforded the corresponding *n*-butylamide. Acid-catalyzed coupling with the appropriate aromatic amine component then furnished **71–73**. For other 4-carboxamide variants, the previously[5] prepared 4'-[9-[4-[(4-nitrophenoxy)carbonyl]acridinyl]-amino]methanesulfon-*m*-anisidide was reacted with glycine methylamide to afford **77** or with 6-aminohexanol. In the latter case, further reaction of the resultant alcohol with limited quantities of methanesulfonyl chloride in pyridine

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