ice bath until a precipitate developed. This was dissolved by addition of methanol and the solution was treated by a dropwise addition of a solution of 4-chlorophenacyl bromide (0.2 mole) in methanol (20 ml) while maintaining the pH at 7–7.5 by 1 *N* methanolic potassium hydroxide. The final mixture was stirred for 1 hr while cooling in ice and for an additional hour at room temperature. Dilution with cold water (60 ml) caused complete separation of the product, which was found to be the cyclized product (XXIX) as evidenced by mixed melting point determination and superimposability of IR. Repeating the reaction for a shorter period of time (15 min) in aqueous methanol again gave the cyclized product (XXIX).

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Influence of Environment and Substituents on the Stability of the Radical Cations of Several Phenothiazine Derivatives

A. ORTIZ[x], I. POYATO, and J. I. FERNANDEZ-ALONSO

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Abstract □ The UV decay spectra of the radical cations of several phenothiazine derivatives in different environments was studied. The influence of the substituents on the reference spectrum could be seen, as well as a relationship between the stability of such radicals and the acidity of the environment. There is also an influence of the substituents on the stability of the radicals in the different environments studied. The instability of the radicals in solution has been studied to relate to the pharmacological activity of neuroleptics.

Keyphrases □ Phenothiazine—derivatives, influence of environment and substituents on the stability of the radical cation □ Neuroleptics—phenothiazine derivatives, influence of environment and substituents on the stability of the radical cations □ Radical cations—influence of environment and substituents on stability, phenothiazine derivatives

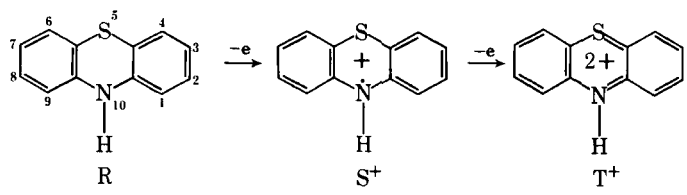
The physicochemical study of phenothiazines has increased in recent years. One of the most common properties of phenothiazine and its derivatives is that they are oxidized easily (1–3). The fact that various oxidized compounds were found (4) among metabolic degradation products suggested that the phenothiazines could act in humans in their oxidized form or produce a redox reaction. Based on this, it was proposed (5) that certain products could act in humans by means of an energy or electron transfer. The phenothiazines, then, can act as electron donors if there are adequate acceptors. Evidence has been presented that the drugs interact with dopamine receptors, and a good correlation has been found between drug potency and the strength of this interaction (6, 7). Several investigators have proposed that the cation radical formed by the oxidation of a phenothiazine derivative (8) such as chlorpromazine, could be an intermediate of the metabolism of the drug and may be the active pharmacological entity (8, 9).

In the present report a study of the kinetics of decay of the first oxidation product of several phenothiazine derivatives in different environments is described, and their instability is related to the substituents and their pharmacological activity.

BACKGROUND

The influence of the R_2 substituents and the structure of the R_{10} side chain on the antipsychotic activity of phenothiazines has been studied (10). However, information about substituent effects on the radical cation behavior is less prevalent due to the difficulty in studying the reactive cation radicals; therefore, it is useful to investigate the behavior of the radicals as a function of structure, given the possible involvement of the radicals in the metabolism of the drugs and in their activity.

The cation radicals of phenothiazine were first obtained in 1913 (11). It was stated that the phenothiazine oxidation proceeds by two steps (12):

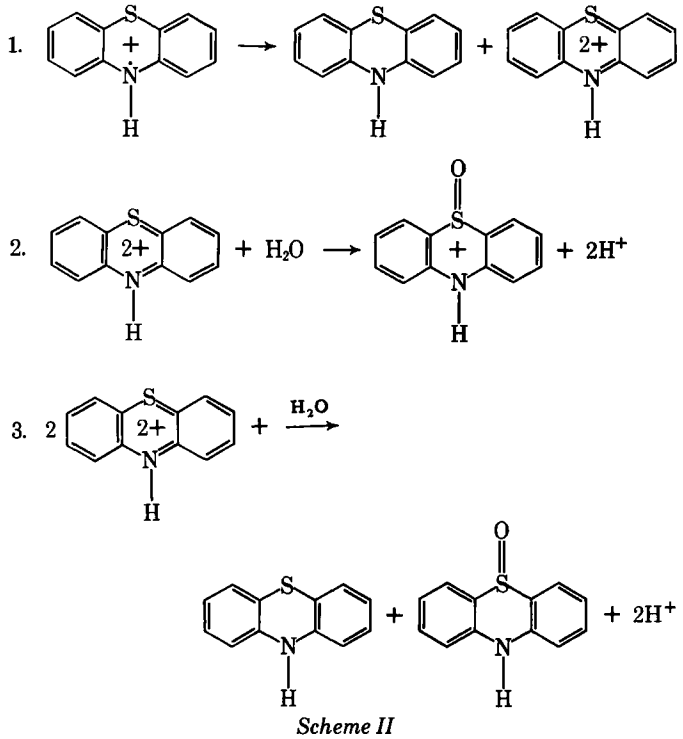


Scheme I

0022-3549/83/0100-0050$01.00/0

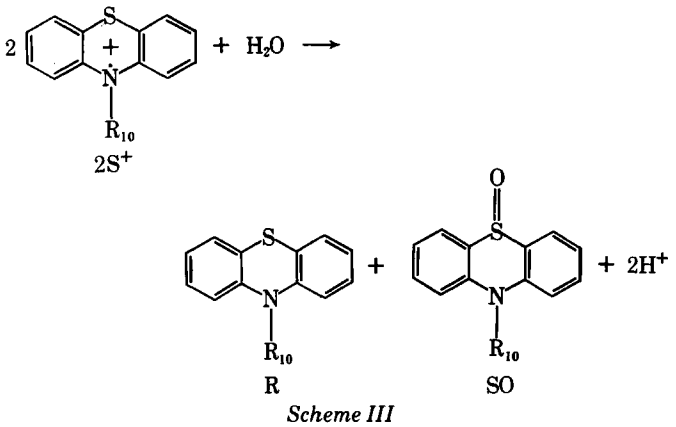
where R produces an uncolored solution, S^+ produces a colored solution, and T^+ an intense red solution.

The radical in solution is stable only in a concentrated acid environment, otherwise disproportionation occurs in three steps:



Scheme II

The first and second steps can be eliminated by acidifying the environment. The third step does not occur in the case of substituted R_{10} derivatives. Therefore, the reaction ends at step 2 and it can be expressed (13):



Scheme III

While the S^+ produces a colored solution, the R and sulfoxide solution are decolorized.

EXPERIMENTAL

Materials—Phenothiazine derivatives with R_2 and R_{10} substituents were used (Table I). The products were pharmacologically pure and were used without further purification. Perchloric acid (70%) was the oxidizing agent.

The solvents were bidistilled water, sulfuric acid (9 *N* and 2 *N*), and solutions with different pH values (pH 1, 2.2, and 3.5). UV and visible spectrophotometers were used to obtain the spectra to which the kinetic decay of the radicals in solution adjust.

Method—A method reported previously was used to obtain the cation radicals in the solid state (14). Analysis for phenothiazine derivative radical perchlorates were calculated.

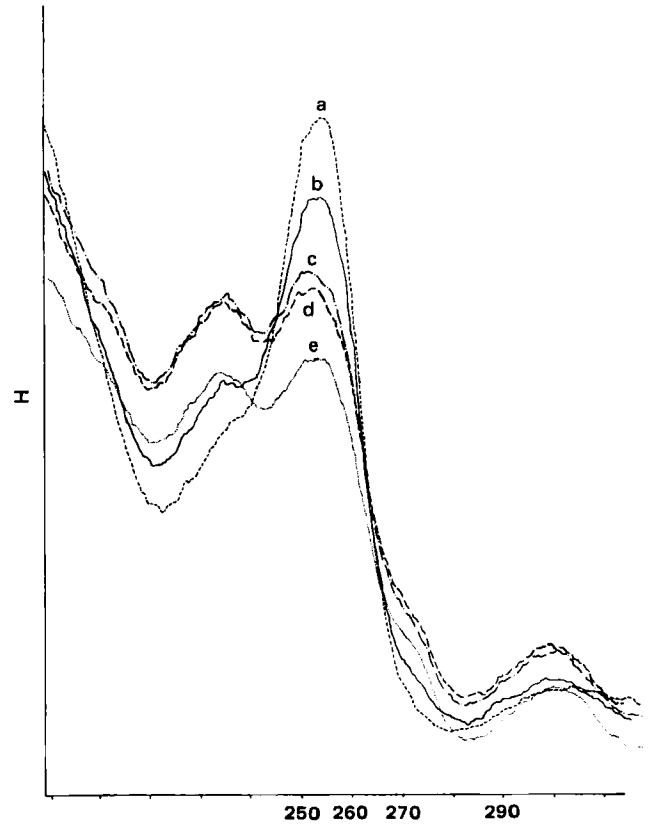


Figure 1—*Decay of the trifluoperazine radical cation in the following solutions (350–200 nm* versus *absorption) (a) water; (b) sulfuric acid pH 3.5; (c) sulfuric acid, pH 2.2; (d) sulfuric acid, pH 1; (e) 2 N sulfuric acid.*

Perazine—Anal.—Calc.: C, 43.80; H, 4.96; S, 5.84; N, 7.66; Cl, 12.93. Found: C, 43.14; H, 4.91; S, 5.92; N, 7.66; Cl, 12.91.

Trifluoperazine—Anal.—Calc.: C, 40.88; H, 4.25; N, 6.81; S, 5.20; Cl, 11.50; F, 9.24. Found: C, 40.28; H, 4.21; N, 7.05; S, 5.29; Cl, 11.6; F, 9.38.

Prochlorperazine—Anal.—Calc.: C, 41.21; H, 4.49; N, 7.20; S, 5.50; Cl, 18.25. Found: C, 41.21; H, 4.43; N, 7.35; S, 5.18; Cl, 17.84.

Thiethylperazine—Anal.—Calc.: C, 43.42; H, 5.05; N, 6.90; S, 10.53; Cl, 11.65. Found: C, 43.7; H, 5.20; N, 7.01; S, 11.0; Cl, 10.92.

Promazine—Anal.—Calc.: C, 41.38; H, 4.49; S, 6.49; N, 5.67; Cl, 14.37. Found: C, 41.15; H, 4.35; S, 6.53; N, 5.61; Cl, 14.52.

These values agreed with the proposed formula given in an earlier report for chlorpromazine perchlorate (14): (phenothiazine derivative) + $ClO_4^-.ClO_4H.\frac{1}{2}H_2O$. The melting points of these compounds ranged from 170 to 224°.

RESULTS AND DISCUSSION

The radicals dissolved in all solvents used. An intense coloration appeared which corresponded to the oxidized form. The radicals remained indefinitely stable only in 9 *N* sulfuric acid solution because of its high acidity. Disproportionation of the cation radicals occurred in all other solvents employed, with the characteristic coloration of the cation radical disappearing.

To study the kinetics of these compounds, the cation radical perchlorates were used as the oxidized form in the solid state. For each radical a control in 9 *N* sulfuric acid solution, the acid-stabilized solution, was used as a comparison standard of fixed concentration.

To determine the concentration of the radical, electron-spin resonance (ESR) spectroscopy was employed. As a reference, a benzene solution of 1,1-diphenyl-2-picrylhydrazyl, as free radical standard was used. An acid-stabilized control sample of each compound with *concentrations* of 10^{-2}–10^{-3} *M* served as the reference.

The characteristic superfine structure of the molecular structure of phenothiazine was suppressed; consequently, the ESR spectra of the radical cations consisted of a single line. The proportion of the ESR signal height with respect to the radical concentration gave a concentration measurement accurate to 3%. The comparison with the free radical

Table I—UV Spectra Data for the Radical Cations of the Phenothiazine Derivatives in 9 *N* Sulfuric Acid

Compounds[a]	R_2	Concentration	λ_{max}, nm (log ϵ)	λ_{max}, nm (log ϵ)	λ_{max}, nm (log ϵ)
I Perazine[b]	—H	4×10^{-4} mole/liter	271(3.50)	265(3.48)	212(3.25)
II Promazine[c]	—H	4×10^{-4} mole/liter	271(3.51)	265(3.48)	212(3.25)
III Thiethylperazine[d]	$-SC_2H_5$	6×10^{-4} mole/liter	296(3.29)	267(3.12) 245–236(2.98)	205(2.95)
IV Prochlorperazine[e]	—Cl	5.14×10^{-4} mole/liter	275(3.39)	268(3.37)	216(3.20)
V Trifluoperazine[f]	$-CF_3$	4×10^{-4} mole/liter	271(3.60)	—	213(3.24)

[a] For I, III–V R_{10} = $-CH_2-CH_2-CH_2-N\langle(H)\rangle N-CH_3$; for II R_{10} = $-CH_2-CH_2-CH_2-N-(CH_3)_2$. [b] Rhodia. [c] Promonta. [d] Upjohn. [e] Sandoz. [f] Squibb.

standard showed that, within the accuracy of the method, the acid-stabilized control was entirely in the free radical cation form.

The promazine and perazine cation radicals decayed slowly enough so that successive passes through the UV region (350–200 nm) could be made, and it was possible to study the entire spectrum with respect to time. For the other products, only the λ_{max}, which refers to the oxidized form, were taken, and the time dependence of the absorbance was recorded.

Influence of the Substituents of the Radical Cations in 9 *N* Sulfuric Acid Solution—Table I presents the transitions corresponding to the cation radicals in 9 *N* sulfuric acid solution (15). The reference used to study the substituent effects was perazine.

Perazine (I) and promazine (II) generate no change from the reference spectrum with R_2 = H and different substituents at R_{10}.

The radical cations with different substituents at R_2 and the same at R_{10} give different changes. Thus, the —Cl and $-SCH_2CH_3$ substituents show a resonance effect upon the π structure of the nucleus of the phenothiazine derivative which manifests itself in bathochromic shifts of the peaks (16). However, R_2= $-CF_3$, with a strong electron-withdrawing effect, seems not to affect the transitions corresponding to the radical cation.

In the perazine spectrum, it can be seen that the —Cl substituent shows a bathochromic shift in all bands.

The $-SCH_2CH_3$ caused an intense bathochromic shift in the first band, the second remained at 265 nm, and a transition peak appeared at 245–228 nm, which represents a vibratory structure. This transition peak could be caused by the sulfur atom in the R_2 substituent [the same transitions also appeared at the same wavelength in the spectra of an identical neutral compound (17)].

The $-CF_3$ derivative produces no shift in the bands; however, the second maximum at 265 nm disappeared.

Through log ϵ, a relationship can also be established between the intensity of the bands of each radical cation and the substituents. Thus, the R_{10} substituent does not exert any influence on the intensity of the bands; with perazine, however, derivatives with R_2 substituents such as —Cl or $-SC_2H_5$ seem to exert a negative influence on such bands showing a hypochromic effect. On the contrary, the $-CF_3$ substituent affects the π electronic structure of the tricyclic nucleus producing hyperchromic shifts on its maxima (16).

Decay of the Radicals—Figures 1 and 2 show the final spectra of the decay of the radical cation in the different solutions used. The corresponding λ_{max} and log ϵ values are given in Table II. The values of λ for the sulfoxide are on the right side of the table and the values for λ corresponding to neutral phenothiazine derivatives are on the left. The neutral form and sulfoxide of each radical cation were identified from earlier reports (18–20).

From these spectra the following can be seen. (*a*) The radical cations stay stable only in 9 *N* sulfuric acid; (*b*) The radicals are not stable in 2

Table II—UV Spectra of the Phenothiazine Derivatives in Sulfuric Acid Solutions of Different Acidity

Product (concentration)	Solvent	Neutral form λ_{max} (log ϵ)	Sulfoxide λ_{max} (log ϵ)
Promazine (4×10^{-4} mole/liter)	2 *N* H_2SO_4	250 (3.21) 298s (2.72)	270 (2.91) 235s (3.14) 222s (3.09)
	H_2SO_4 pH 1	251 (3.32) 298s (2.79)	270.5s (2.88) 238 (3.24) 224 (3.18)
	H_2SO_4 pH 3.5	250.5 (3.21) 298s (2.71)	271 (2.78) 236 (3.14) 225 (3.10)
	distilled water	250.5 (3.18) 298s (2.54)	270 (2.48) 235 (3.06) 224 (3.03)
Perazine (4×10^{-4} mole/liter)	2 *N* H_2SO_4	251s (3.04) 295s (2.48)	270 (2.95) 234 (3.15)
	H_2SO_4 pH = 1	251s (3.09) 297s (2.62)	271s (2.92) 234 (3.16) 243 (3.13)
	H_2SO_4 pH = 3.5	251 (3.06) 297 (2.48)	271 (2.88) 234 (3.05)
	distilled water	251 (3.12) 297 (2.52)	271s (2.85) 235 (3.03)
Thiethylperazine[a] (6×10^{-4} mole/liter)	2 *N* H_2SO_4	267 (3.25)	260 (3.25) 222–225s (3.07)
	distilled water	267 (3.25)	260 (3.23) 221–225 (3.06)
Trifluoperazine (4×10^{-4} mole/liter)	2 *N* H_2SO_4	251.5 (3.16) 300s (2.54)	271s (2.70) 234.5 (3.15)
	H_2SO_4 pH 1	252.5 (3.22) 301s (2.68)	270s (2.76) 234 (3.21)
	H_2SO_4 pH 2.2	251.5 (3.22) 301s (2.65)	270s (2.72) 235 (3.21)
	H_2SO_4 pH 3.5	251.5 (3.21) 300 (2.62)	271s (2.72) 235.5 (3.17)
	distilled water	254 (3.36) 302s (2.54)	235s (3.10)
Prochlorperazine (5.35×10^{-4} mole/liter)	2 *N* H_2SO_4	250 (3.17) 300 (2.53)	275s (2.60) 240s (3.13)
	distilled water	250 (3.15) 300s (2.54)	240 (3.05)

[a] There is an intense overlap of the bands corresponding to the sulfoxide and to the neutral derivative.

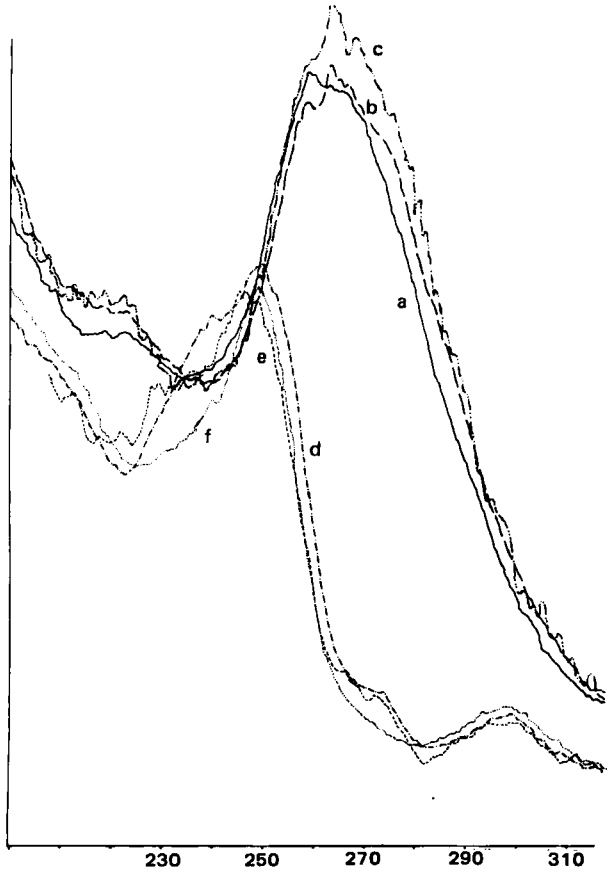


Figure 2—*Final decay spectra of prochlorperazine and thiethylperazine radical cations in the following solutions: 350–200 nm* versus *absorption) thiethylperazine in (a) 2* N *sulfuric acid; (b) water; (c) sulfuric acid, pH 1. Prochlorperazine in (d) 2* N *sulfuric acid; (e) pH 1 sulfuric acid; (f) water.*

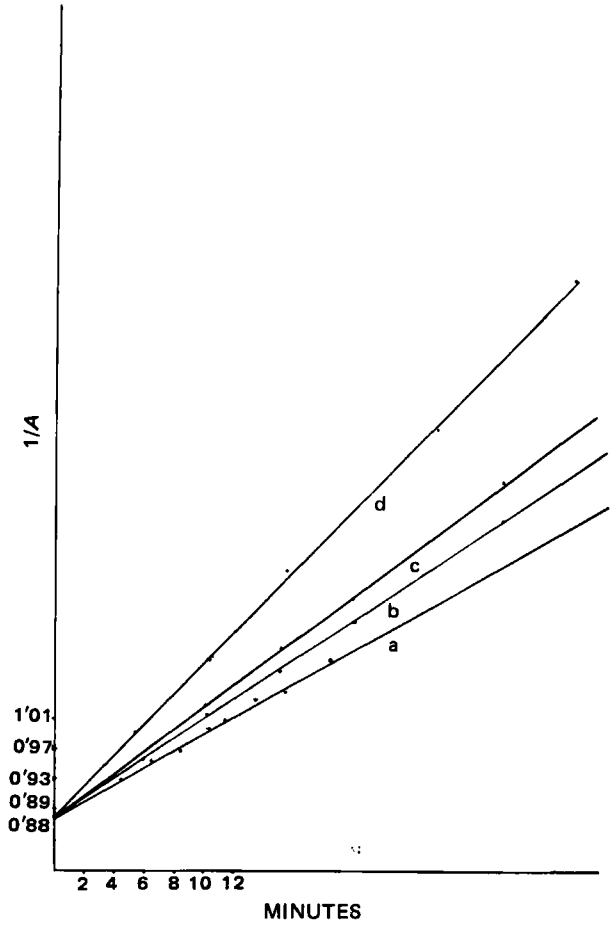


Figure 3—*1/*A versus *time (minutes) for promazine acid; in (a) 2* N *sulfuric acid; (b) pH 1 sulfuric acid; (c) pH 3.5 sulfuric acid; (d) water.*

N sulfuric acid, decoloration occurs, and new bands appear, which are different from those of the radical cation. The sulfoxide coexists with the neutral form, with the sulfoxide being more important. (*c*) In solutions of pH 1 and 3.5 decoloration goes fast, and it can be seen that the band corresponding to the neutral phenothiazine derivative is of a stronger intensity than the one corresponding to the sulfoxide. (*d*) In solutions in distilled water, the decay is almost simultaneous and decoloration is practically immediate. There is a strong prevalence of the band corresponding to the neutral phenothiazine derivative over the one corresponding to the sulfoxide. However, upon observing the spectra, an overlapping of some bands can be noticed (Fig. 2). Nevertheless, the decay of the radical cations depends upon the acidity concentration of the environment and substituents R_2 and R_{10}.

There is a correlation between the bands characteristic of each compound with the environment. Thus, in solutions with higher acidity, the sulfoxide dominates; in solutions with lower acidity, the neutral derivative dominates; and in solutions at pH 2.2 and 3.5, the proportion between these two forms is similar.

Kinetics—The decay velocity of the radicals in each solution studied was obtained from spectral data on the assumption that the absorbance was linearly related to the free radical concentration, representing $1/A$ *versus* time. In this way the initial absorbance was computed from the intercept of zero time with $1/A$. The velocity constant was obtained from:

$$K = (1/t)\,(1/A - 1/A_0) \qquad \text{(Eq. 1)}$$

where A_0 is the initial, extrapolated absorbance, A is the absorbance at time t, and t is time in minutes.

In all cases the data produced a straight line (Figs. 3 and 4) indicating a second-order decay of the semiquinone or oxidated form S^+, which agrees with 2 S+ - - - - R + SO, as proposed in other reports (13, 21).

Since the oxidized form is unstable in insufficiently acidic solutions, the initial concentration is no longer 100% but is lower from the beginning. This makes it necessary to extrapolate at time 0 to calculate A_0, which can be compared with the absorbance of the coresponding radical in 9 *N* sulfuric acid, taken as the reference, in which the concentration is 100%, because the radical stays stable indefinitely. Thus, in 2 *N* sulfuric acid the calculated initial concentration of the radicals studied are between 85 and 100% of the same compound in 9 *N* sulfuric acid.

In the less acidic solutions, the measurements are even less accurate because of the speed of the radical decay, and at lower pHs, the extrapolated initial concentrations were still lower than for 2 *N* sulfuric acid. However, it can be considered sufficient for obtaining relative reaction rates on the disproportionation of the radicals.

Effect of the Environment pH on K Decay—Least-square fits were made to obtain constants for K, experimental errors, ϵ, coefficients of regression, R^2, and $1/A_0$ data for every compound in the different media employed (Table III).

In Fig. 3 it can be seen that, as for any of the compounds studied, K increases as the acidity of the solvent decreases.

Compounds I and II with R_2 = H and a Side Chain at R_{10}—Compounds I and II differ by their R_{10} substituents and from the values obtained it could be pointed out that the chain of I, which is longer than that of II, is capable of producing a more stable radical.

It can be seen from Table III that for compound I the K decay values are very similar, in spite of the different solutions employed. This appears to indicate that acidity has little influence on the stability of this radical in solution.

However, for II, K-values depend more on the solutions used, and the relation of the K decay of these two compounds in water is double for II than for I, that is, II decays two times faster than I in water.

Effects of R_2 Substituents—A clear influence of R_2 on K is observed; thus, it can be seen that the K-values for the same solution, 2 *N* sulfuric acid, (Fig. 4) or distilled water, follow the sequence: —H < —SCH_2CH_3 < —Cl < —CF_3. Similar to the first case with R_2 = H, the K-values increase as the acidity of the solution decreases; however, this K increase is stronger for compounds with $R_2 \neq$ H.

The strongest increase of the decay constant of the radicals can be seen in the product with the electron-withdrawing substituent, R_2 = —CF_3.

Table III—$K(10^{-3})$ [a] of the Cation Radicals in Sulfuric Acid

Parameters	Products I	II	III	IV	V
$1/A_0$	0.82	0.884	1.072	0.958	0.798
K	7.12	11.82	30.01	37.03	46.72
2 N					
ϵ	±0.046	±0.446	±1.65	±0.60	±0.22
R^2	0.9997	0.991	0.993	0.998	0.9997
$1/A_0$	0.89	0.989	1.04	1.216	1.464
K	7.88	13.85	32.45	36.55	50.55
pH 1					
ϵ	±0.060	±0.33	±1.79	±0.86	±0.71
R^2	0.998	0.998	0.987	0.997	0.9990
$1/A_0$	—	—	—	—	1.500
K	—	—	—	—	55.86
pH 2.2					
ϵ	—	—	—	—	±0.24
R^2	—	—	—	—	0.99992
$1/A_0$	0.93	1.227	—	—	0.909
K	8.58	14.36	—	—	56.73
pH 3.5					
ϵ	±0.02	±0.44	—	—	±0.51
R^2	0.9998	0.980	—	—	0.909
$1/A_0$	0.938	1.34	1.21	1.426	1.003
K	8.97	18.6	49.29	56.66	60.04
Water					
ϵ	±0.65	±0.91	±1.51	±0.70	±0.72
R^2	0.979	0.992	0.994	0.9997	0.9995
$E^{1/2}$, mV	503[c]	525[b]	540[b]	570[b]	680[b]

[a] In moles/liter minute. [b] Reference 19. [c] Reference 20.

The introduction of an R_2 substituent, activated or deactivated, is capable of increasing considerably the decay of the radicals. It could have been expected that the electron-donating substituents, such as $-SCH_2CH_3$, would tend to stabilize the cation radicals in solution (22); however, in this case this was not so.

The constant decay can be related also to the $E_{1/2}$ (23, 24). The R_2 and R_{10} substituents affect the halfwave potentials as given in Table III. It is observed that a higher $E_{1/2}$ corresponds to a higher constant decay of the compound or at higher halfwave potential, less stability of the radical.

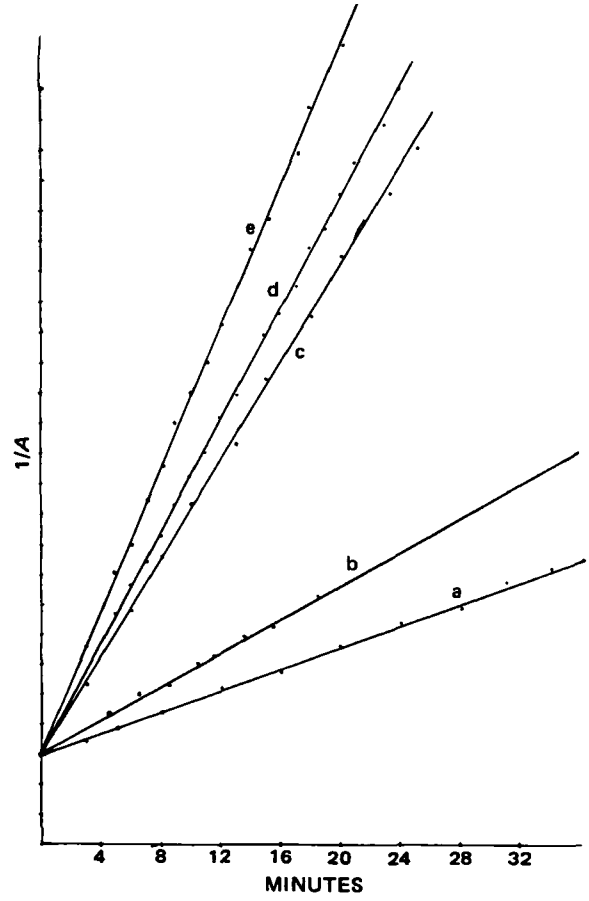


Figure 4—1/A versus *time for (a) perazine; (b) promazine; (c) thiethylperazine; (d) prochlorperazine; (e) trifluoperazine, in 2 N sulfuric acid.*

Relative Stability and Pharmacological Activity of the Radicals—It has been suggested that the phenothiazine derivative radical cation may be of some importance in the biological activity of phenothiazine (25), and in some cases it has been related to the stability of these radicals.

Since the pharmacological activity of the compounds studied, such as neuroleptics, depends on the R_2 substituents as: $-CF_3 > -Cl > -SCH_2CH_3 > -H$, and as it has been determined that the stability of their radicals varies inversely with the activity as neuroleptics, then the activity of these compounds cannot be related to the stability of the corresponding radical. However, the activity of these compounds could be related to the ability to form (CTC) charge transfer complexes whenever there are adequate receptors, with the cation radical as an intermediate in the biological action as has been suggested (26).

CONCLUSIONS

It can be concluded that the R_{10} substituents seem not to affect the transitions corresponding to the radical cation, but the R_2 substituents considerably affect such transitions in two ways: first, on the λ characteristics of the reference spectrum by bathochromic shifts (—Cl, and $-SCH_2CH_3$) and second, increasing (the $-CF_3$) or decreasing (—Cl and $-SCH_2CH_3$) the intensity of the corresponding bands.

From the final decay spectra of the radicals it can be seen that there exists a correlation between the band characteristics of the sulfoxide and the neutral form of each radical cation with the milieu, depending on the prevalence of one of the forms on the acidity of the solution employed. Thus, the neutral derivative dominates over the sulfoxide in water; however, this last compound seems to dominate the former in acidic solutions.

From the K-values obtained, it can be concluded that for each compound studied the radicals are only stable in high acidic solutions, and their stability decreases as the acidity of the environment decreases. Here it can be seen that the R_2 and R_{10} substituents' effect on the stability of radicals with longer chains are capable of producing a more stable radical. On the contrary, none of the R_2 substituents studied different than $R_2 \neq H$ are capable of generating a more stable radical. All substituents affect considerably the stability of the corresponding radicals. The more unstable the product with a strong electron-withdrawing effect ($-CF_3$), the more the other compounds (—Cl and $-SCH_2CH_3$) will also decrease the stability of their radicals but less than $-CF_3$.

This seems to indicate that the stability of the radicals can not be related to the activity of these compounds as neuroleptics, for the more

active (trifluoperazine) seems to be the less stable, and as soon as the radical is free and independent in solution, it suffers disproportionation and disappears as that radical.

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Simultaneous Stability-Indicating Determination of Phenylephrine Hydrochloride, Phenylpropanolamine Hydrochloride, and Guaifenesin in Dosage Forms by Reversed-Phase Paired-Ion High-Performance Liquid Chromatography

GARY W. SCHIEFFER ˣ and DAVID EMLYN HUGHES

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Abstract □ A method for the quantitative determination of phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and guaifenesin in commercial formulations was developed. A reversed-phase paired-ion high-performance liquid chromatographic technique resolves the active from degradation products, colorings, and flavor and was found applicable to seven commercial dosage forms.

Keyphrases □ Phenylephrine hydrochloride—simultaneous determination with phenylpropanolamine hydrochloride and guaifenesin, reversed-phase paired-ion high-performance liquid chromatography □ Phenylpropanolamine hydrochloride—simultaneous determination with phenylephrine hydrochloride and guaifenesin, reversed-phase paired-ion high-performance liquid chromatography □ Guaifenesin—simultaneous determination with phenylephrine hydrochloride and phenylpropanolamine hydrochloride, reversed-phase paired-ion high-performance liquid chromatography □ High-performance liquid chromatography—reversed-phase paired-ion, simultaneous determination of phenylephrine hydrochloride, phenylpropanolamine hydrochloride, and guaifenesin

The simultaneous determination of the active components in a specific dosage form offers advantages to separate analyses. Simultaneous GLC determinations are typically successful in assaying for phenylpropanolamine hydrochloride and other amines (1–8). At least one GLC assay for guaifenesin (glyceryl guaiacolate) is available in which guaifenesin is extracted and derivatized (9). Simple and reliable procedures for the simultaneous GLC determination of the underivatized phenylephrine hydrochloride and other amines are absent from the chemical literature. Studies are available in which 71 drugs were determined using nitrogen-selective and flame-ionization (FID) detectors (2); 50 amines of pharmaceutical interest (5) and 23 physiologically active amines (8) were determined. None of these methods were responsive to phenylephrine hydrochloride.

To overcome problems arising from the presence of phenylephrine hydrochloride in pharmaceutical formulations, high-performance liquid chromatographic (HPLC) procedures have been developed for simultaneous assay. The desired chromatographic separation involves phenylephrine hydrochloride (I), phenylpropanolamine hydrochloride (II), and guaifenesin (III). The only HPLC method reported in the literature separating I, II, and III with high resolution used a bonded phase cation exchange column (10). A reversed-phase HPLC method employing ion-pairing was preferred, since bonded-phase ion-exchange columns tend to have short lifetimes and poor reproducibility from column to column (11).

Numerous reversed-phase ion-pairing methods have been reported for various combinations of I, II, and III and other drugs. A previous report (12) used a nitrile column

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