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Stability of Some Phenothiazine Free Radicals†

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The stability of 57 10-alkylphenothiazine free radicals has been shown to depend on both the nature of the substituent at position 2 of the phenothiazine nucleus, and the nature of the 10-alkyl substituent. The influence on radical stability of the substituent at position 2 could be predicted by the Hammett meta-substituent constant. A branched-chain aliphatic moiety at position 10 yielded a more unstable radical than did a straight-chain moiety. And the greater the number of carbon atoms between the nitrogen atom at the 10 position of the phenothiazine nucleus and that in the 10-alkyl substituent, the more stable the radical. Stability was measured in terms of the rate of decay of the radicals in H_2SO_4 solutions. Although the stability of the radicals does not appear to correlate with the usual antipsychotic doses of these compounds, the possibility that phenothiazine tranquilizers act by a mechanism involving a free radical requires further study.

In attempts to elucidate the mechanism of action of phenothiazine tranquilizers, many investigators have studied the effects of these compounds on a variety of enzyme systems *in vitro*. Chlorpromazine, the most intensively studied of these compounds, has appeared to require some transformation before it was active as an enzyme inhibitor. The time occupied by preincubation, temperature equilibration, and the assay of enzyme activity afforded opportunities for transformation by the enzyme preparations, and many studies demonstrated that preincubation was necessary for inhibition, or that the degree of inhibition increased during the assay.[2] Studies in this laboratory demonstrated that a free radical formed from chlorpromazine was a potent inhibitor of the enzyme uridine diphosphate glucose: NAD^+ oxidoreductase (1.1.1.22), and that chloropromazine itself was not inhibitory unless it was first incubated with the enzyme preparation in daylight.[2] Thus, transformation of chlorpromazine to a free radical may be required for activity *in vitro*.

Studies of the mechanism by which the phenothiazine free radicals are generated and of the mechanism by which these radicals inhibit enzyme activity were planned. There was no *a priori* reason to believe that the chlorpromazine free radical was the ideal radical to be employed in a study of enzyme inhibition; another phenothiazine free radical—either much more or much less stable—might be a more effective inhibitor. A detailed study of a large number of phenothiazine free radicals has therefore been undertaken. Fifty-seven different 10-alkylphenothiazine free radicals have been generated and characterized, and the stability of these radicals has been studied.

Experimental Section‡

Preparation of the Phenothiazine Free Radicals. The free radicals generated from 55 different 10-alkylphenothiazines were studied. The phenothiazine derivatives, their structures, names, and sources are listed in Table I. With but a few exceptions, the radicals were prepared as perchlorate salts, by a modification of the method of Merkle and his coworkers.[3]

Approximately 0.14 mmole of each phenothiazine was dissolved in 0.5 ml of 70% $HClO_4$, after which 8 μl of 30% H_2O_2 was added. The resulting intensely colored solution was diluted with an equal volume of acetone and chilled. The addition of two or three volumes of ether resulted in a heavy precipitate which was usually amorphous. After standing at −20° for an hour, the suspension was filtered. The precipitate was then washed repeatedly with small volumes of ether until it was no longer tacky, after which it was dried and stored *in vacuo*. These solid samples exhibited no change by absorption spectroscopy, and the crystals appeared grossly unchanged after storage for months in a vacuum desiccator in the dark at room temperature.

†A preliminary report of this work has appeared.[1]

‡Melting points, determined on a Thomas-Hoover melting point apparatus, were corrected. The C, H, and N analyses were performed by Clark Microanalytical Laboratory, Urbana, Ill. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Table I. Phenothiazines Studied

No.	Substituent	Names	Source
	A. Ring-Substituted Derivatives of 10-(3-Dimethylaminopropyl)phenothiazine		
I		Promazine, Sparine	*a*
II	2-Acetyl	Acepromazine	
III	2-Acetoxy	RP 7,306	*b*
IV	2-*tert*-Butyl	RP 9,960	*b*
V	2-Butyryl	WY 1321	*a*
VI	2-Hexanoyl	WY 1479	*a*
VII	2-Chloro	Chlorpromazine, Thorazine	*c*
VIII	2-Cinnamoyl	WY 2014	*a*
IX	2-Cyano	RP 7,210	*b*
X	2-Diethylaminopropionyl	WY 1358	*a*
XI	2-Formyl	RP 11,718	*b*
XII	2-Hydroxymethyl	WY 1417	*a*
XIII	2-Methoxy	Methoxypromazine, Tentone	*d*
XIV	2-Methyl	RP 4,627	*b*
XV	2-Propionyl	WY 1310	*a*
XVI	2-Trifluoromethyl	Triflupromazine, Vesprin	*e*
	B. 10-Alkyl Derivatives of Phenothiazine		
XVII	10-Diethylaminoethyl	Diethazine, Diparcol	*b*
XVIII	10-(2-Diethylaminopropyl)	Ethopropazine, Parsidol	*c*
XIX	10-(3-Diethylaminopropyl)	RP 3,012, WY 1107	*a, b*
XX	10-(4-Dimethylaminobutyl)	WY 1197	*a*
XXI	10-[3-(Dimethylamino)-2-methylpropyl]	Trimeprazine, Temaril	*a, c*
XXII	10-(2-Dimethylaminopropyl)	Promethazine, Phenergan	*a*
XXIII	10-{3-[1-(2-Hydroxyethyl)-4-piperazinyl]-2-methylpropyl}	AHR 0791	*f*
XXIV	10-(3-Methylaminopropyl)	RP 17,461	*b*
XXV	10-[3-(4-Methyl-1-homopiperazinyl)propyl]	WY 2805	*a*
XXVI	10-[(1-Methyl-3-piperidyl)methyl]	Mepazine, Pacatal	*g*
XXVII	10-[(1-Methyl-3-pyrrolidinyl)methyl]	Methdilazine, Tacaryl	*h*
XXVIII	10-[2-(1-Pyrrolidinyl)ethyl]	Pyrathiazine, pyrolazote	*i*
XXIX	10-[3-(1-Pyrrolidinyl)propyl]	RP 4,695	*b*
	C. 10-Alkyl Derivatives of 2-Chlorophenothiazine		
XXX	10-{3-[1-(2-Acetoxyethyl)-4-piperazinyl]propyl}	Thiopropazate, Dartal	*j*
XXXI	10-(3-Aminopropyl)	RP 4,728	*b*
XXXII	10-[3-(4-Carbamoylpiperidin-1-yl)propyl]	Pipamazine, Moridine	*j*
XXXIII	10-(3-Diethylaminopropyl)	Chlorproethazine, RP 4,909	*b*
XXXIV	10-{3-[1-(2-Hydroxyethyl)-4-homopiperazinyl)propyl}		*k*
XXXV	10-{3-[1-(2-Hydroxyethyl)-4-piperazinyl]propyl}	Perphenazine, Trilafon	*k*
XXXVI	10-(3-Methylaminopropyl)	RP 5,815	*b, c*
XXXVII	10-[3-(1-Methyl-4-homopiperazinyl)propyl]	WY 2830	*a*
XXXVIII	10-[3-(1-Methyl-4-piperazinyl)propyl]	Prochlorperazine, Compazine	*c*
XXXIX	10-[3-(1-Pyrrolidinyl)propyl]	RP 4,670	*b*
	D. Ring-Substituted Derivatives of 10-(2-Dimethylaminopropyl)phenothiazine		
XL	2-Bromo	RP 4,692	*b*
XLI	2-Formyl	RP 11,629	*b*
XLII	2-[2-(4-Methyl)thiazolyl]	RP 12,523	*b*
XLIII	2-Proplonyl	Propiomazine, Largon	*a*
	E. Ring-Substituted Derivatives of 10-[3-(Dimethylamino)-2-methylpropyl]phenothiazine		
XLIV	2-Methoxy	Methotrimeprazine, Levoprome	*d*
XLV	2-Trifluoromethyl	Trifluomeprazine	*c*
	F. 10-Alkyl Derivatives of 2-Trifluoromethylphenothiazine		
XLVI	10-{3-[1-(2-Hydroxyethyl)-4-piperazinyl]propyl}	Fluphenazine, Prolixin	*e*
XLVII	10-[3-(1-Methyl-4-piperazinyl)propyl]	Trifluoperazine	*c*
	G. 2-Sulfur-Containing 10-Alkyl Derivatives of Phenothiazine		
XLVIII	2-Dimethylaminosulfonyl-10-[3-(-methyl-4-piperazinyl)propyl]	Thioproperazine, Majeptil	*b*
XLIX	2-Ethylthio-10-[3-(4-methyl-1-piperazinyl)propyl]	Thiethylperazine, Torecan	*j*
L	2-Methylsulfinyl-10-[2-(1-methyl-2-piperidyl)ethyl]	Thioridazine sulfoxide, Serentil	*l*
LI	2-Methylthio-10-[2-(1-methyl-2-piperidyl)ethyl]	Thioridazine, Mellaril	*l*
	H. 2-Acyl-10-Alkyl Derivatives of Phenothiazine		
LII	2-Acetyl-10-{3-[4-(2-hydroxyethyl)piperidino]propyl}	Piperacetazine, Quide	*m*
LIII	2-Acetyl-10-{3-[1-(2-hydroxyethyl)-4-piperazinyl]propyl}	Acetophenazine, Tindal	*k*
LIV	2-Propionyl-10-{3-[1-(2-hydroxyethyl)-4-piperazinyl]propyl}	Carphenazine, Proketazine	*a*
LV	2-Butyryl-10-[3-(1-methyl-4-piperazinyl)propyl]	AHR 3000	*f*

[a] Wyeth Laboratories Inc., Philadelphia, Pa. [b] Society des Usines Chimiques Rhône-Poulenc, Paris. [c] Smith Kline & French Laboratories, Philadelphia, Pa. [d] Lederle Laboratories, Pearl River, N. Y. [e] The Squibb Institute for Medical Research, New Brunswick, N. J. [f] A. H. Robins Company, Inc., Richmond, Va. [g] Warner Lambert Research Institute, Morris Plains, N. J. [h] Mead Johnson Research Center, Evansville, Ind. [i] The Upjohn Company, Kalamazoo, Mich. [j] G. D. Searle & Co., Chicago, Ill. [k] Schering Corporation, Bloomfield, N. J. [l] Sandoz Pharmaceuticals Division of Sandoz, Inc., Medical Department–West Coast, San Francisco, Calif. [m] Pitman-Moore Division of the Dow Chemical Company, Zionsville, Ind.

The radicals of three promethazine derivatives—the 2-propionyl, 2-formyl, and 2-dimethylsulfonamido—were too unstable to be isolated as their solid perchlorate salts. These were prepared by oxidation of the phenothiazine in solution in 50% H_2SO_4 with H_2O_2 and studied immediately.

Analysis of Radical Salts. Because elemental analysis and other studies (*e.g.*, esr, energy of activation) of all of the large number of compounds was prohibitively expensive, both in terms of money and of time, seven representative free radicals were selected for these studies; the radicals chosen provided a wide range of stability. Melting points and elemental analyses of the representative free radical diperchlorate salts were as follows: I, mp 227–228° [*Anal.* ($C_{17}H_{21}Cl_2N_2O_8S$) C, N; H: calcd, 4.34; found, 3.27]; VII, mp 199–200° [*Anal.* ($C_{17}H_{20}Cl_3N_2O_8S$) H, N; C: calcd, 39.34; found, 38.21]; XVI, mp 214–215° [*Anal.* ($C_{18}H_{20}Cl_2F_3N_2O_8S$) C, N; H: calcd, 3.62; found, 3.04]; XX, mp 209–210° [*Anal.* ($C_{18}H_{23}Cl_2N_2O_8S$) H, N; C: calcd, 43.37; found 42.75]; XXI, mp 220–221° [*Anal.* ($C_{18}H_{23}Cl_2N_2O_8S$) C, N; H: calcd, 4.62; found, 3.88]; XXII, mp 188–189° [*Anal.* ($C_{17}H_{21}Cl_2N_2O_8S$) C, N; H: calcd, 4.34; found, 3.63]; XL, mp 162–163° [*Anal.* ($C_{17}H_{20}BrCl_2N_2O_8S$) C, N; H: calcd, 3.55; found, 2.95]. The reason for the large deviations in some of the analytical results is not evident at this time. Merkle, *et al.*,[3] have proposed that the free radical salt of VII exists as a hemihydrate; neither their analysis nor that presented here enables a choice between a hemihydrate and the anhydrous salt. The formulas given are for the anhydrous salts. It must be admitted that there is uncertainty regarding the structures of these salts which is not clarified by the elemental analyses.

Spectra. Absorption spectra of the free radicals in solution in 50% H_2SO_4 were recorded by means of a Beckman DK2A ratio-recording spectrophotometer. Esr spectra were obtained on seven selected free radicals, employing the 9.3-GHz esr spectrometer described by Tozer and Tuck.[4] Spectra were recorded both from solutions of the radicals in 50% H_2SO_4 and from solid samples of the free radical salts. These seven radicals were also characterized by elemental analysis. The concentration of free radical was determined by double integration of the esr spectrum, correcting for instrumental drift by the method recently described by Loveland and Tozer.[5]

Study of Free Radical Stability. Stability of the free radicals was measured in terms of the decay of the free radicals with respect to time. A stock solution of the free radical was prepared by dissolving the free radical perchlorate in 50% H_2SO_4. A 4-μl aliquot of the stock solution was transferred to a 10-ml erlenmeyer flask, and 4 ml of one of a number of H_2SO_4 solutions was added to a flask as a stopwatch was activated. The flask was swirled, and its contents poured into a quartz cuvette; the cuvette was immediately placed in the temperature-controlled cell holder of the Beckman DK2A spectrophotometer, and the change in absorbance at the wavelength in the visible range characteristic of the absorption maximum of the free radical solution was recorded as a function of time. This absorption maximum was unchanged for each radical over the range of $[H^+]$ studied. The temperature was maintained at 25 ± 0.5°. Dilutions of the stock free radical solution in 50% H_2SO_4 were employed to calibrate the spectrophotometer, and for recording the absorption spectrum. Decay of the free radical was measured in 1.8, 3.6, 5.4, 7.2, and 9.0 N H_2SO_4; in a few instances, the decay was also measured in 0.9 or 10.8 N H_2SO_4 solutions. The Hammett acidity function, H_0, was measured directly for each of the acid solutions used in the stability studies, and was found to be virtually identical with the values given by Paul and Long.[6]

The rate constant of decay of the free radical was determined from a plot of the reciprocal of the concentration of the free radical remaining at each time interval as a function of time. A straight line was fitted to the measurements made at intervals of 1 min between 5 and 18 min by the method of least squares; the slope of the line represented the apparent second-order decay constant, k'. For each radical, the common logarithm of k' was plotted as a function of H_0, again fitting the best straight line by means of the method of least squares; five to seven points were used in each least-squares computation.

Table II. Absorption Maxima Characteristic of 10-Alkylphenothiazine Free Radicals

Spectrum	λ_{max}, nm
A	266, 273, 315, 509–516
B	268, 276, 325, 523–529
C	251, 287, 508–516
D	248, 285, 555 or 565
E	273, 498–500

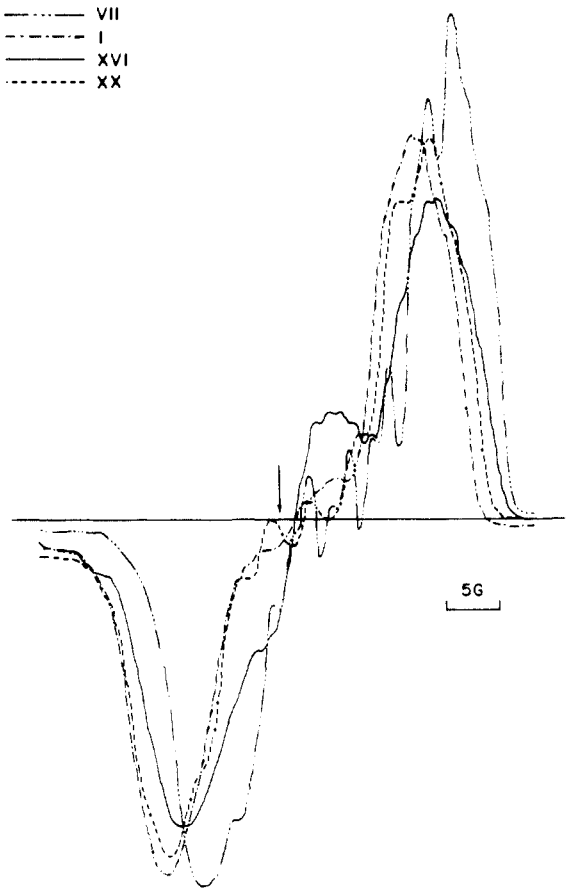

Figure 1. Esr spectra of 10^{-2} M solutions of phenothiazine free radicals (VII, I, XVI, XX) in 50% H_2SO_4. The arrow indicates a g value of 2.0023.

Results

Absorption Spectra. Solutions of the free radicals of each of 46 compounds in 50% H_2SO_4 yielded one of five absorption spectra (Table II). With only minor variations, all radicals not substituted in the ring yielded spectrum A. All radicals with a 2-chloro substituent yielded spectrum B; the spectrum of the radical XL differed from spectrum B only in having its visible absorption maximum at 535 nm, and that of radical XII also showed a similar spectrum with a maximum at 530 nm. Spectrum C was associated with the free radicals of the 2-acyl derivatives. The radicals of the two 2-methoxy-substituted compounds yielded spectrum D, and those with 2-trifluoromethyl substituents yielded spectrum E. The existence of more than one radical species in the solutions in which these studies were carried out cannot be excluded, although the conditions of preparation and study make this unlikely.

Esr Spectra. Under low resolution, the free radicals gave esr spectra which were related to the chemical structures of the radicals. Four free radicals with a straight chain of three or four carbon atoms between the nitrogen in the phenothiazine ring and the first nitrogen in the substituent yielded similar esr spectra (Figure 1) in 50% H_2SO_4 solutions; these radicals had a variety of substituents in position 2. Three other radicals, having in common a branched chain of three or four carbon atoms between the ring and side-chain nitrogens, yielded spectra with better resolved hyperfine structure (Figure 2) than those of the radicals possessing straight carbon chains. The esr spectra recorded from solid samples of free radicals of promazine and two of its derivatives are

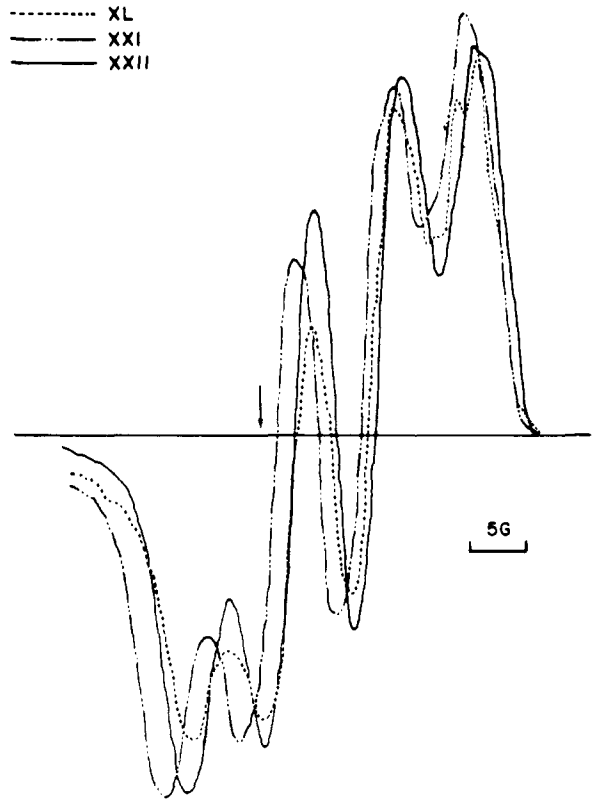

Figure 2. Esr spectra of 10^{-2} *M* solutions of phenothiazine free radicals (XL, XXI, XXII) in 50% H_2SO_4. The arrow indicates a *g* value of 2.0023.

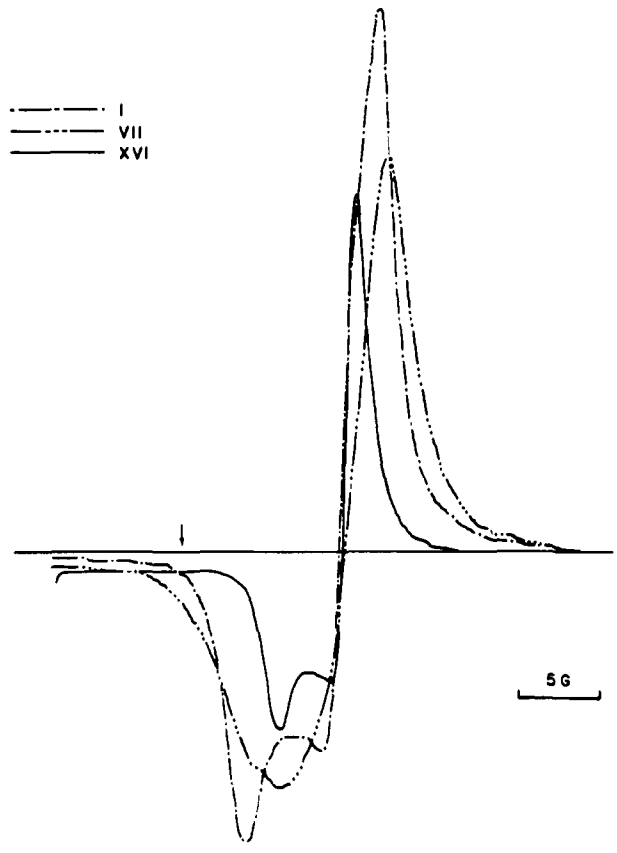

Figure 3. Esr spectra of solid samples of phenothiazine free radicals. I: 1.5 × 10^{-6} mole, amplification 400×; VII: 1.7 × 10^{-6} mole, amplification 500×; XVI: 1.2 × 10^{-6} mole, amplification 100×. The arrow indicates a *g* value of 2.0023.

shown in Figure 3. These spectra show similar but not identical *g*-tensor anisotropy.[7]

It is interesting to note that, whereas the absorption spectra appear to correlate best with the nature of the substituent on the 2 position of the phenothiazine nucleus, the esr spectra appear to vary according to the nature of the 10 substituent. Free radical stability depends upon the substituents in both positions.

Concentrations of free radicals in 10^{-2} *M* solutions of the seven representative free radical salts in 50% H_2SO_4 and in solid samples of three of the salts are presented in Table III. Assuming that the solution giving the greatest esr double integral represents 100% free radical, six of the seven representative free radical salts were found to yield 70–100% free radical upon solution in 50% H_2SO_4. The fact that the bromopromethazine free radical salt yielded only 63% free radical is consistent with the relative instability of this radical. Concentrations of free radicals in the several solid samples studied suggested more variable free radical concentrations (compared to a solid sample of diphenylpicrylhydrazyl) than were found in the study of the H_2SO_4 solutions of the free radical salts.

Table III. Concentration of Free Radical

Compound	Double integral, mm^3	% present as free radical[a]
A. Measurements on 10^{-2} *M* Solutions in 50% H_2SO_4		
Phenothiazine[b]	169,044	70
I	228,131	94
VII	218,719	90
XVI	194,240	80
XX	239,876	99
XXI	241,137	100
XXII	171,661	71
XL	153,934	63
B. Measurements as Solid Samples (Calculated for 5 × 10^{-5} mole)		
1,1-Diphenyl-2-picrylhydrazyl	41,514	81
I	28,312	55
VII	18,122	35
XVI	51,300	100

[a] These figures are calculated assuming the largest double integral in each category to represent 100% free radical. [b] Phenothiazine, previously shown to exist in free radical form under these conditions,[4] was employed as a reference.

Measurements of Free Radical Decay. Early experiments with the chlorpromazine free radical demonstrated that the free radical was stable in 50% H_2SO_4, decayed very slowly in 9 *N* H_2SO_4, and decayed more rapidly in more dilute H_2SO_4 solutions. The results of one such experiment are presented in Figure 4. The reciprocal of the concentration of the free radical remaining at each time interval, when plotted as a function of time, yields a straight line between 5 and 18 min (Figure 5). Because preliminary experiments demonstrated that k' was not independent of the initial free radical concentration, free radical decay was always measured from an initial concentration of 10^{-4} *M*.

To measure the precision of the estimate of k', 22 pairs of duplicate determinations of k' were performed for several radicals at several $[H^+]$. The average difference between duplicate measurements was 10% for $1 \leqslant k' \leqslant 50$ l. $mole^{-1}$ sec^{-1}.

In general, free radical decay was more rapid in less concentrated H_2SO_4 solutions; a plot of $\log k'$ as a function of H_0 yielded a straight line. The k' for each of the 57 free radicals studied is listed in Table IV, as are the slopes of these lines, $\Delta \log k'/\Delta H_0$, and their intercepts, $\log k'$, at $H_0 = 0$; the slopes of most of the lines are approximately equal to 1.0, suggesting a direct relationship between the rate of radical decay and H_0. The values of $\log k'$ for $H_0 = 0$,

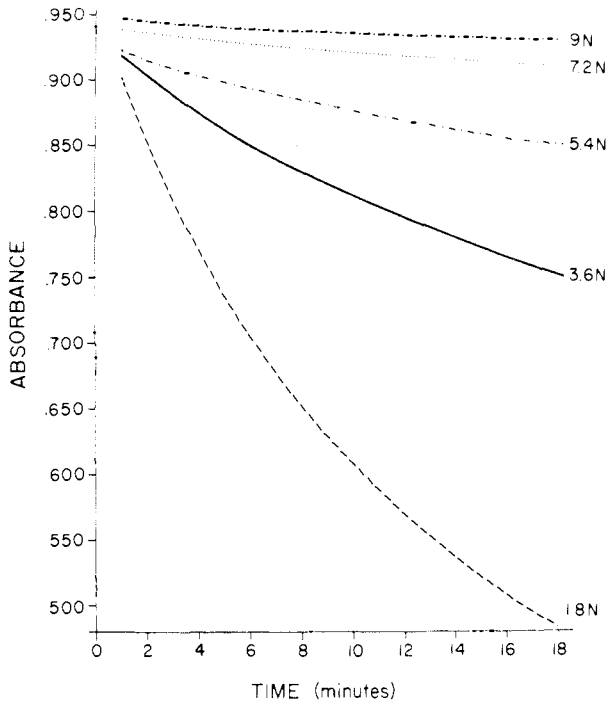

Figure 4. Decay of the chlorpromazine free radical in several concentrations of H_2SO_4. Initial concentration of the free radical is 10^{-4} M; $T = 25 \pm 0.5°$. The change in absorbance at 525 nm is shown as a function of time.

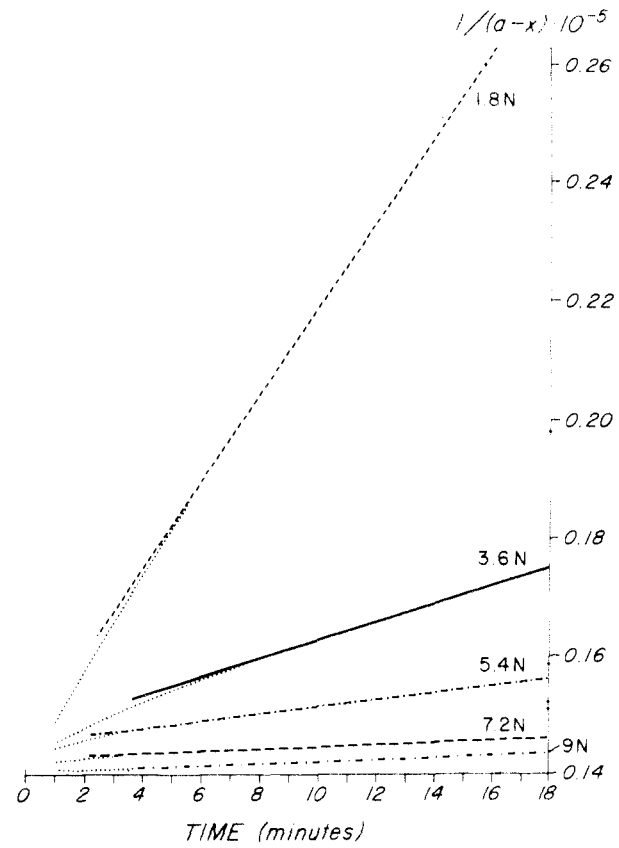

Figure 5. Reciprocal plots of the remaining free radical concentration in several concentrations of H_2SO_4 as a function of time. The measurements of the concentration of free radical remaining at time t, $(a - x)$, shown in Figure 4, have been transformed. The change with time of the reciprocal of the remaining free radical concentration is linear after the first 5 min or so.

on the other hand, permit comparison of the decay rates of most of the free radicals. Absorption spectra recorded during free radical decay suggested that only a single radical species was present.

In Table V are the data obtained by pooling the k' for each of several of the radicals with important structural features in common which appear to decay similarly. Thus, a 2-Cl composite has been derived employing the observations for all of the 2-Cl radicals except that for XXXI; a 2-CF_3 composite has been developed from the observations from the three 2-CF_3 radicals, excluding XLV; a 2-H composite considers five of the 14 2-H radicals. The radicals of XXI and XXIII behave sufficiently similarly to suggest that their common structural features are important in determining radical stability; the same relationship appears true of the radicals of XXII and XVIII, of XXVI and XXVII, and of XXVIII and XVII. Finally, there is reason to believe that the radical of LI is identical with the second radical of L; when both XLIX and L were oxidized, a green compound was produced by the addition of 1 equiv of oxidant, which was replaced by an orange compound after the addition of two more equivalents of oxidant. The orange compound was similar to the radical of LI by the criteria of absorption spectrum and rate of decay.

The rate of decay may be seen to vary widely, depending upon the nature of the substituents at positions 2 and 10 of the phenothiazine nucleus. The influence of the substituent in position 2 may be demonstrated by a consideration of the logarithm of the ratio of k' for each of the promazine-derivative radicals to that of the promazine radical (k'_0) at $H_0 = 0$. When the logarithms of the ratios of $k'/k'_{(H_0 = 0)}$, hereafter termed "Q" for convenience, are plotted as a function of the constants for meta substituents, a satisfactory relationship may be seen to exist for those promazine-derivative free radicals for which Hammett substituent constants are available[8] (Figure 6).

The influence on free radical stability of the substituent at position 10 is characterized by the Q for each of 13 promazine-analog radicals (those not substituted at position 2), by the Q for each of 10 2-chlorophenothiazine radicals (k'_0 in this case that of VII), and by the Q for each of two 2-trifluoromethyl phenothiazine radicals (k'_0 in this case that of XVI) (Table VI). A variety of 10 substituents do not influence free radical stability; that is $Q = 0$. Substitution of ethyl for methyl groups and the removal of one or both methyl groups have no effect ($-0.37 \leq Q \leq 0.24$). Similarly, substitution on carbon atom 3 of the n-propyl moiety of side chains containing a variety of saturated N-containing rings, including piperazine, homopiperazine, and pyrrolidine, exerts no influence on free radical stability ($-0.34 \leq Q \leq 0.14$).

Branching of the carbon chain between the ring and side-chain nitrogens produces radicals much less stable than those with an n-propyl moiety between the two nitrogen atoms ($0.94 \leq Q \leq 2.00$). Similarly, the free radical is less stable if the nitrogen of the 10-alkyl substituent is separated from the ring nitrogen by only two carbon atoms ($Q = 1.06$ and 1.17), and more stable if the two nitrogens are separated by an n-butyl moiety ($Q = -0.83$). The radical with three carbon atoms separating the two nitrogen atoms is somewhat less stable if two of the carbon atoms form a portion of a piperidine or pyrrolidine ring, than if three carbon atoms are present as an n-propyl moiety ($Q = 0.47$ and 0.52).

Activation Energy of the Decay Process. Decay of seven free radicals was also studied over a temperature range from 15 to 40° The log k' was then plotted as a function of the reciprocal of the absolute temperature, and the activation energy for the decay process of each radical was calculated

Table IV. Apparent Second-Order Decay Constants (k') as a Function of $[H^+]$

	k' (L mole^{-1} sec^{-1})									
	1 N H_2SO_4							$-\frac{\Delta \log k'}{\Delta H_0}$	$\log k'$	
Compound	10.8	9.0	7.2	5.4	3.6	1.8	0.9		($H_0 = 0$)	r^a
I		0.05	0.21	0.37	0.83	1.99		0.80	0.48	0.98
II		0.07	1.86	2.69	3.97	9.13	15.3	0.90	1.23	0.90
III		0.10	0.27	0.87	2.80	11.3	18.4	1.09	1.22	1.00
IV			0.07	0.26	0.51	1.02		0.77	0.20	0.97
V		2.52	2.89	3.11	4.18	11.5	15.2	0.38	1.06	0.94
VI	1.50	4.73	3.60	2.90	3.54	7.97	11.9	0.21	0.87	0.71
VII		0.23	0.34	1.02	2.65	12.2		0.93	1.15	0.99
VIII		1.02	3.47	6.16	10.4	21.5	32.4	0.65	1.48	0.98
IX		0.22	0.74	2.42	7.50	18.6		1.00	1.52	1.00
X		0.07	2.56	4.61	6.12	11.4	18.6	0.91	1.37	0.87
XI		0.48	1.15	3.24	7.06	19.6		0.85	1.45	1.00
XII		0.23	0.97	2.56	5.49	11.6	16.1	0.83	1.25	0.98
XIII				0.16	2.42	6.19	9.91	1.36	1.03	0.96
XIV			0.40	0.15	0.75	1.05	1.83	0.49	0.14	0.82
XV		2.80	3.43	3.25	4.42	10.5	18.8	0.37	1.09	0.91
XVI		0.23	0.65	1.80	5.97	16.0		0.97	1.40	1.00
XVII		0.37	1.32	3.63	10.3	28.6	46.2	0.97	1.65	1.00
XVIII		3.88	14.4	40.0	93.6	201	273	0.85	2.48	0.99
XIX		0.14	0.15	0.34	0.70	1.92	3.46	0.69	0.40	0.99
XX		0.10	0.08	0.13	0.20	0.35	0.60	0.40	−0.35	0.98
XXI		0.21	0.56	1.75	5.67	16.2		1.02	1.42	1.00
XXII	0.61	1.96	6.74	23.3	52.0	141		1.09	2.47	0.98
XXIII		0.13	0.48	1.56	5.13	18.9	33.3	1.13	1.48	1.00
XXIV				0.12	0.30	0.80	1.64	0.88	0.11	0.99
XXV				0.24	0.66	1.71	1.95	0.75	0.30	0.99
XXVI		0.22	0.26	0.54	1.60	5.87	15.5	0.89	0.95	0.98
XXVII		0.24	0.34	0.79	2.35	7.15	13.4	0.86	1.00	0.99
XXVIII		0.24	0.68	2.22	7.61	22.1	36.8	1.04	1.54	1.00
XXIX			0.15	0.17	0.41	0.94	1.97	0.66	0.14	0.97
XXX			0.24	0.84	3.04	10.3		1.10	1.22	1.00
XXXI		0.20	0.25	0.58	1.64	5.11	10.7	0.85	0.87	0.99
XXXII		0.04	0.19	0.82	3.12	4.97	16.3	1.15	1.12	0.98
XXXIII		0.14	0.30	1.30	4.66	14.9		1.11	1.39	1.00
XXXIV		0.11	0.34	0.75	2.37	9.95	19.5	1.05	1.18	1.00
XXXV			0.21	0.72	2.43	9.83		1.12	1.17	1.00
XXXVI		0.17	0.34	0.74	2.54	8.11	14.7	0.93	1.07	1.00
XXXVII		0.10	0.30	0.86	2.87	10.2	14.4	1.05	1.17	0.99
XXXVIII		0.07	0.34	0.96	2.54	9.11	18.1	1.09	1.19	0.99
XXXIX		0.11	0.28	0.89	3.41	11.4		1.08	1.25	1.00
XL		6.92	28.2	81.1	151	592		0.97	2.94	0.99
XLI		21.3	6.00	107	119	123		0.59	2.34	0.75
XLII	1.65	7.70	20.4	46.9	130			1.13	3.01	0.97
XLIII		17.2	29.5	66.3	202	576	464	0.76	2.76	0.99
XLIV			0.42	5.02	19.0	44.0	53.0	1.17	1.85	0.95
XLV		0.98	3.38	9.80	22.3	54.0	89.2	0.89	1.93	0.99
XLVI		0.12	0.58	1.73	5.24	16.8		1.11	1.47	0.99
XLVII		0.30	0.75	2.27	7.77	22.2		1.01	1.54	1.00
XLVIII		0.38	0.95	3.27	9.94	28.6		1.01	1.66	1.00
XLIX-I				2.97	15.3	30.5	29.6	0.79	1.55	0.95
XLIX-II		1.22	2.79	5.79	7.60	13.9	17.7	0.52	1.25	0.98
L-I				0.75	10.4	33.1		1.54	1.86	0.97
L-II		0.24	0.68	1.76	5.26	13.4	24.5	0.94	1.33	1.00
LI		0.24	0.67	1.58	4.56	12.6	21.6	0.91	1.28	1.00
LII		0.14	2.22	3.48	4.61	10.2		0.83	1.28	0.88
LIII		0.72	3.38	5.95	8.62	16.2		0.65	1.40	0.94
LIV		6.03	7.97	12.2	21.0	39.0		0.44	1.64	1.00
LV	0.52	2.49	2.96	3.26	5.68	14.0		0.53	1.21	0.91

[a] r = correlation coefficient.

from the slope of the log k' *vs.* $1/T$ plot. The results reveal that the energies of activation of the decay of the radicals in 3.6 or 7.2 N H_2SO_4 are quite similar, ranging from 9.4 to 18.8 kcal/mole. The decay of the seventh free radical (XX) was so slow at the temperatures studied that accurate measurement of its energy of activation could not be accomplished.

Discussion

The mechanism by which phenothiazine free radicals decay has been studied by a number of workers.[4,9-11] This mechanism depends upon two reactions: (1) the disproportionation of the semiquinone free radical, and (2) the further reactions undergone by the oxidized form of the phenothiazine. A 10-alkylphenothiazine semiquinone free radical, S^+, disproportionates to the phenothiazine, R, and to T^{2+}, the phenazothionium ion, according to the reversible reaction

$$2S^+ \rightleftharpoons R + T^{2+}$$

the disproportionation equilibrium. Billon[11] has proposed that the phenazothionium ion is reversibly attacked by water, leading eventually to the phenothiazine sulfoxide.

Table V. Parameters Calculated for the Decay of Several Classes of Radicals

Class	Components	$\frac{\Delta \log k'}{\Delta H_0}$	$\log k'$ $(H_0=0)$	r
2-Cl	VII, XXX, XXXII, XXXIX	1.05	1.17	0.99
2-CF_3	XVI, XLVI, XLVII	1.02	1.46	0.99
2-Acetyl	II, LII, LIII	0.78	1.28	0.85
2-Propionyl	XV, LIV	0.35	1.28	0.71
2-Butyryl	V, L	0.46	1.12	0.91
2-H	I, XIX, XXIV, XXV, XXIX	0.62	0.26	0.82
2-H, 10-isobutyl	XXI, XXIII	1.08	1.46	1.00
2-H, 10-isopropyl	XVIII, XXII	1.01	2.50	0.97
XXVI, XXVII		0.87	0.98	0.98
XVII, XXVIII		1.01	1.60	0.99
L-II, LI		0.92	1.31	1.00

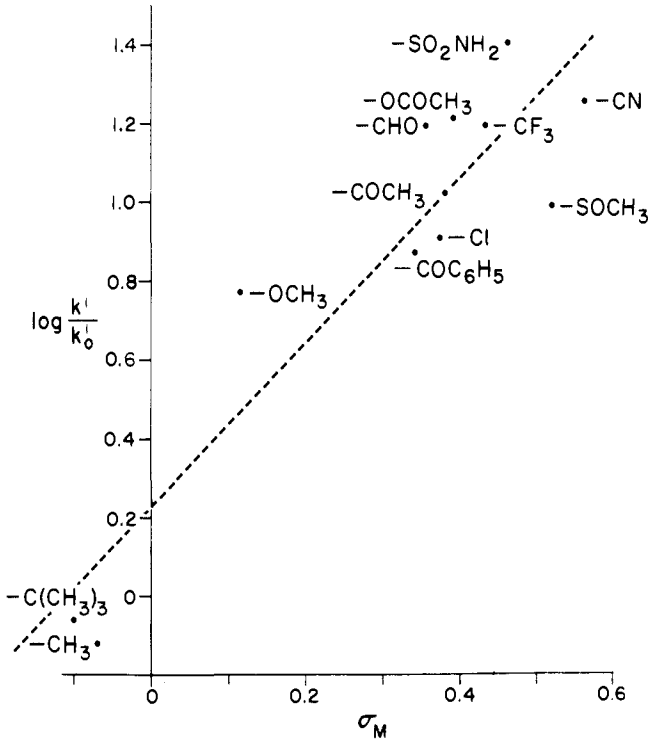

Figure 6. Hammett plot of logarithms of the ratios of the apparent second-order decay constants at $H_0 = 0$ of 12 2-substituted promazine radicals with that of the promazine radical, using the meta-substituent constant.

Although decay proceeds by second-order kinetics, it is probably not the disproportionation reaction which is rate limiting. Disproportionation and its reverse, free radical formation from equimolar concentrations of the reduced and oxidized forms of the phenothiazine, appear to be instantaneous reactions. The reduced 10-alkylphenothiazine that results from disproportionation is stable under the conditions of this reaction. Tozer and Tuck[4] have demonstrated that the rates of the hydrolytic reactions undergone by the phenazothionium ion of phenothiazine itself determine the rate of decay of the phenothiazine free radical. It appears likely that the rate-limiting reactions in the process of decay of the 10-alkylphenothiazine free radicals are the analogous hydrolytic reactions of their phenazothionium ions. Tozer[12] has shown that hydrolysis of the phenazothionium ion proceeds by first-order kinetics. The decay of the free radical results from hydrolysis of the phenazothionium ion followed by disproportionation of the radical. Because 2 equiv of radical disappear for every equivalent of phenazothionium hydrolyzed, it is the disproportionation reaction

Table VI. Influence of the 10-Substituent on Free Radical Decay

Group	Q^a	Group	Q^a
A. Comparison of Promazine Analogs			
Isopropyl chain		2 of 3 carbon atoms included in piperidyl or pyrrolidinyl moiety	
XVIII	2.00		
XXII	1.99		
Ethyl or isobutyl chain		XXVI	0.47
XVII	1.17	XXVII	0.52
XXI	0.94	*n*-Propyl chain	
XXIII	1.00	IX	−0.08
XXVIII	1.06	XXIV	−0.37
n-Butyl chain		XXV	−0.18
XX	−0.83	XXIX	−0.34
B. Comparison of Chlorpromazine Analogs			
n-Propyl chain			
XXX	0.07	XXXV	0.02
XXXI	−0.28	XXXVI	−0.07
XXXII	−0.03	XXXVII	0.02
XXXIII	0.24	XXXVIII	0.04
XXXIV	0.03	XXXIX	0.10
C. Comparison of Triflupromazine Analogs			
n-Propyl chain			
XLVI	0.07	XLVII	0.14

$^aQ = \log k'/k'_{0(H_0=0)}$.

which accounts for the apparent second-order kinetics. Further evidence that decay of the free radical is a more complex process than disproportionation alone is suggested by the observations that k' varied inversely with the initial concentration of free radical present, and that the plots of the reciprocals of the concentration of remaining free radical as a function of time did not yield a straight line during the first few minutes of decay.

Hydrogen ion increases the stability of the free radical by stabilizing the phenazothionium ion, probably by two mechanisms: (1) H^+ decreases the rate of hydrolytic degradation of the phenazothionium ion, and (2) H^+ protonates the sulfoxide. That the rate of free radical decay is inversely proportional to the H^+ *activity* rather than to the H^+ *concentration* suggests that H^+ does not stabilize the free radical as a reactant. Rather, this suggests that H^+ influences the stability of the free radical by entering into an equilibrium which precedes the rate-limiting reaction.[13] This equilibrium may well be the dissociation of water.

As was shown by Tozer and Tuck[4] for a smaller number of compounds, substituents on the 2 position of the phenothiazine nucleus have a predictable effect on the rate of free radical decay. The most stable radicals were the promazine radical and the phenothiazine radicals substituted with an *n*-alkyl moiety. The effects of substituents on the 10 position could not be similarly predicted because Hammett substituent constants are not available for the substituents studied.

Considering the effects of the 10 substituents, it is apparent that the greater the number of carbon atoms between the nitrogen atoms at the 10 position and that in the 10-alkyl substituent the more stable the radical; a branched-chain aliphatic moiety yielded a more unstable radical than did a straight-chain moiety. Villalonga, *et al.*,[14] have suggested that molecules of promazine and diethazine have the same surface area, with coplanar phenothiazine nuclei, whereas the promethazine molecule has a smaller surface area. Bloom and Laubach,[15] using molecular models, reported that in promethazine there is important steric repulsion between the methyl moiety on the β-carbon atom of the side chain and the hydrogen atoms at positions 1 and 8 of the phenothiazine nucleus, which is consistent with the

surface area measurements of Villalonga; these same authors speculate that the loss of coplanarity resulting from the repulsion interferes with resonance stabilization of the free radical. Such an explanation is consistent with the finding here that radicals with isopropyl or isobutyl moieties between the N atom at the 10 position and that in the 10-alkyl substituent are less stable than those radicals with *n*-propyl and *n*-butyl moieties. The slowly decaying radical which appears most appropriate for further study as an enzyme inhibitor is that of XX; one could not design a more stable radical, unless stability should continue to increase with progressive lengthening of the "inter-N" distance. An appropriate unstable free radical may be found among the radicals prepared from the promethazine derivatives.

It has been suggested that oxidation of chlorpromazine to a free radical is a transformation essential both to the activity and the metabolism of the drug.[16] That a ranking of phenothiazine tranquilizers by the usual antipsychotic dose[17] appears much different from the ranking of these same drugs according to the stability of the free radicals generated from them cannot be taken as evidence against an important role of the free radical in drug action. The therapeutic activity of a drug depends on many factors—*e.g.*, the rate at which the drug is absorbed from the gastrointestinal tract and the degree of binding to plasma and tissue proteins—in addition to the potency of the drug at its site of action. The demonstration by Tozer and his coworkers[18] that the activity of phenothiazine anthelmintics is related to the free radical concentration is of interest in this context. It now appears important to compare the radicals described in this present report for their potency as inhibitors of an appropriate oxidoreductase.

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Relative Carcinogenicity of Some Diethylbenz[*a*]anthracenes†

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Four novel diethyl derivatives of benz[*a*]anthracene, 6,8-, 7,8-, 7,9-, and 8,12-diethylbenz[*a*]-anthracenes, were synthesized and tested for sarcomagenic activity. The hypothesis that a dialkyl derivative of benz[*a*]anthracene would be active only if the thickness of either the convex side of the molecule (positions 6, 7, 8) or of the concave one (positions 1, 11, 12) did not exceed 4 Å, *i.e.*, the thickness of the methyl group, could not be confirmed.

7,12-Dimethylbenz[*a*]anthracene (7,12-DMBA) is the most potent of the known carcinogenic polynuclear aromatic hydrocarbons. Conversely, 7,12-diethylbenz[*a*]-anthracene (7,12-DEBA) is completely devoid of carcinogenic properties. The activity of 7-ethyl-12-methylbenz[*a*]-anthracene is comparable to that of the 7,12-dimethyl derivative and 7-methyl-12-ethylbenz[*a*]anthracene is a potent carcinogen, although somewhat less so. These observations suggested the hypothesis that molecular thickness may be an important factor in eliciting carcinogenic activity so that a dialkyl derivative of benz[*a*]anthracene would be active only if the thickness of either the convex side of the molecule (positions 6, 7, 8) or of the concave one (positions 1, 11, 12) did not exceed 4 Å, *i.e.*, the thickness of the methyl group.[1] In order to test the validity of this hypothesis, we synthesized 6,8-, 7,9-, and 8,12-diethylbenz[*a*]anthracenes for biological evaluation.‡

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‡We included 7, 9-DEBA because in the trimethyl series, an additional methyl in position 9 seems to enhance sarcomagenic potency.[2]

6,8-DEBA was synthesized, starting from *p*-bromopropiophenone (**1**) which reacted with benzylmagnesium chloride (**2**) to give 1-ethyl-1-(*p*-bromophenyl)-2-phenylethylene (**3**). The stilbene derivative **3** was photochemically cyclized in the presence of iodine[3] to 3-bromo-10-ethylphenanthrene (**4**). The Grignard derivative of **4** gave with succinic anhydride 4-oxo-4-[3-(10-ethyl)phenanthryl]-butyric acid (**5**), albeit in very poor yield. Wolff-Kishner reduction converted **5** to 4-[3-(10-ethyl)phenanthryl]-butyric acid (**6**). Cyclization of **6** with anhydrous HF led to 6-ethyl-8-oxo-8,9,10,11-tetrahydrobenz[*a*]anthracene (**7**). Reaction of the ketone with ethylmagnesium bromide afforded 6,8-diethyl-8-hydroxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (**8**). Finally, 6,8-diethylbenz[*a*]anthracene (**9**) was obtained by simultaneous dehydrogenation-dehydration of the alcohol **8** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.[4]

The starting material for the synthesis of 7,9-DEBA was 4-ethylaniline (**10**) which was first brominated[5] to 2-bromo-4-ethylaniline (**11**). The amino group was substituted by the cyano group[6] to give 3-bromo-4-cyanoethylbenzene (**12**). Treatment of **12** with 1-naphthylmagnesium bromide