Excited States of Six-membered Aza-aromatic Rings. Part VIII.[1] Photochemical Reactions, Fluorescence, and Protolytic Equilibria in *N*-Alkylated Phenazinium Ion–Phenazyl Free Radical Systems

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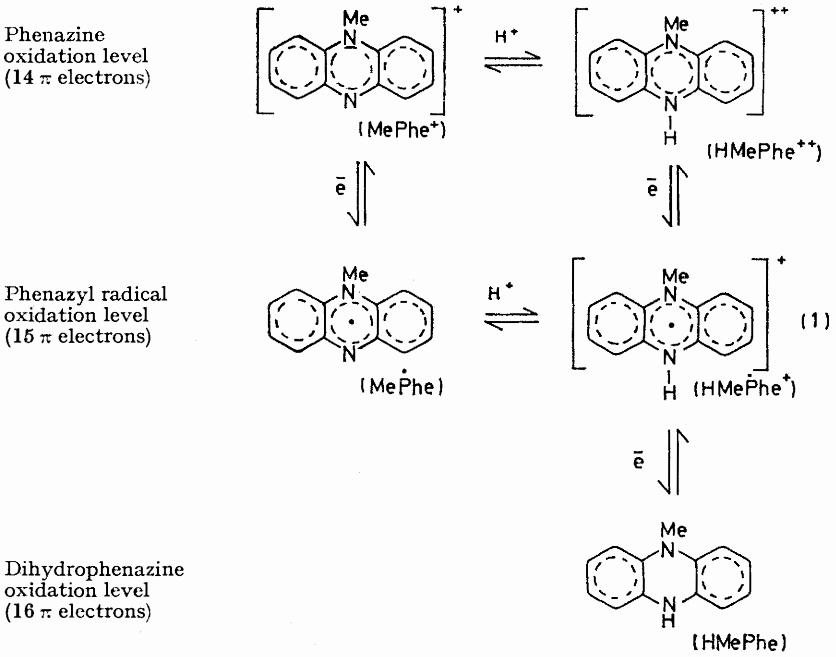
Electronic fluorescence spectra of the *N*-methylphenazyl free radical and its protonated form are reported. The protolytic p*K* values for the ground and first excited states of the species have been determined and compared with data on a series of related compounds. A hitherto obscure difference in the reduction mechanism of *N*-methyl- and *N*-ethyl-phenazinium ions is explained by steric inhibition of the reaction path leading to dealkylation.

THE redox system (1) involving *N*-methylphenazinium cation * ($\mathrm{MePhe^+}$) and *N*-methylphenazyl free radical ($\mathrm{Me\dot{P}he}$) [2,3] is often used in biochemistry as an electron-transfer catalyst.[4,5]



(1)

Only a few e.s.r. or u.v. spectra of the components of system (1) or homologous systems have been published.[3,5-7] $\mathrm{MePhe^+}$ is known to be sensitive to light, whereby the photoreduction to the free radical $\mathrm{Me\dot{P}he}$ is often accompanied by dealkylation to phenazine (Phe); unexpectedly, in the closely related $\mathrm{EtPhe^+}$–$\mathrm{Et\dot{P}he}$ system no dealkylation occurs.[8]

The aim of the work was to find the reason for this difference in behaviour by studying the photochemical reactions, spectra, and especially the protolytic equilibria in systems of type (1), on two oxidation levels in the ground and excited state, *i.e.* to gain insight into the electronic structure of the corresponding electronic states, as already achieved for some of the non-alkylated heterocycles.[1,9]

EXPERIMENTAL

N-Methylphenazinium methosulphate was synthesised by quaternisation of phenazine [8] and thrice crystallised from methanol–ether. Samples were stored in the cool and dark, and the 10^{-4}—10^{-5}M solutions required were prepared immediately before use. Water, ethanol (96 or 99·8%), methanol, and isopropanol (AnalaR) were used as solvents. All solvents were tested for fluorescence.

The free radicals $\mathrm{Me\dot{P}he}$ or $\mathrm{HMe\dot{P}he^+}$ were prepared from $\mathrm{MePhe^+}$ salts (i) by cathodic reduction in isopropanol, (ii) by photoreduction (λ 405 nm) in anhydrous alcohol, or (iii) by chemical reduction with oxalic acid.

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* Often quoted in the literature as PMS (phenazinium methosulphate).

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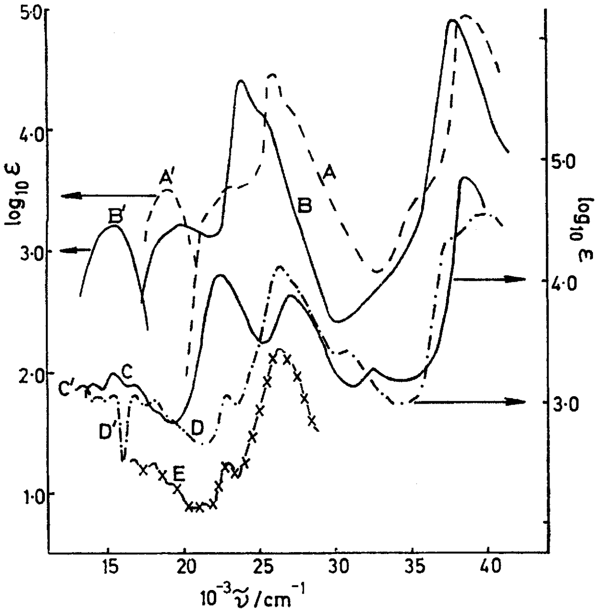
Electrolysis was carried out in isopropanol, in the absence of O_2 and light, using a potentiostat[10] in a three-electrode system with a reference saturated calomel electrode (SCE). $MePhe^+$ was reduced on a Pt gauze cathode at $-0{\cdot}3$ V in neutral 0·1M-LiCl or in acid solutions (0·1M-LiCl—10^{-3}M-$HClO_4$) at $+0{\cdot}1$ V *vs.* SCE.

Absorption spectra were measured immediately after electrolysis, in the absence of air, on Optica CF4 or Unicam SP 700 spectrophotometers. The reported absorption coefficients are the mean values from five electrosyntheses. Luminescence and excitation spectra were measured by means of spectrofluorimeters,[9,11] and the lifetimes by means of a phase fluorimeter.[12] E.s.r. spectra were recorded on a JEOL JES-Me3X spectrometer.

RESULTS

Spectra.—For systems analogous to (1) the electronic absorption spectra, the luminescence spectra, and the e.s.r. spectra were measured at both the phenazine and phenazyl free radical oxidation levels. To isolate the spectra of different chemical species, the pH of the solutions (and in some cases the temperature and concentration of the solutions) was systematically varied. Examples of hitherto unreported spectra, in particular the fluorescence spectra of the free radical species, are shown in the Figure. A comparison of spectral data for a number of chemical species is presented in Table 1. The electronic spectra within each homologous series of isoelectronic (π and n) compounds (*e.g.* $HPhe^+$, $MePhe^+$, $EtPhe^+$) are remarkably similar.



Absorption and fluorescence (primed) spectra of A,A′, $MePhe^+$ in isopropanol; B,B′, $HMePhe^{++}$ in 72% $HClO_4$; C,C′, $HMe\dot{P}he^+$ in acidified isopropanol; D,D′, MeṖhe in isopropanol; E, fluorescence excitation spectrum of MeṖhe in ethanol

Special attention was paid to the spectra of the free radicals. The first absorption (and luminescence) bands of both MeṖhe and $HMe\dot{P}he^+$ showed marked vibronic structure. Solutions of MeṖhe were reddish and we observed the red fluorescence of MeṖhe in anhydrous alcohol on excitation at 405 or 435 nm. Because of doubts whether the fluorescence arose from an oxidation product of the free radical,[13b] we checked its origin. The fluorescence spectrum was virtually a mirror image of the first free radical absorption band, and the excitation spectrum matched closely the absorption spectrum of MeṖhe (Figure). Fluorescence appeared in samples of MeṖhe prepared either photochemically (even in the presence of O_2) or by electrolysis of isopropanol solutions of $MePhe^+$ thoroughly degassed by a repeated vacuum freeze–thaw technique. The EtṖhe free radical emitted very similar fluorescence. The vibrational spacing is *ca.* 1300 cm^{-1} for absorption and *ca.* 1000 cm^{-1} for fluorescence of MeṖhe. The fluorescence lifetime (τ) of the electrochemically prepared MeṖhe in isopropanol is 5·1 ns in the absence of O_2, and 4·5 ns in an air-saturated solution. The natural lifetime (τ_e), as calculated from the integrated first absorption band,[14] is 340 ns. Thus, the fluorescence quantum yield may be estimated as $\eta = \tau/\tau_e = 0{\cdot}015$ in the absence of O_2. The fluorescence had very low anisotropy, even on excitation to the first absorption band, and in a rigid low-temperature glass. Remarkably, the fluorescence was quenched efficiently by water.

Solutions of $HMe\dot{P}he^+$ were green; its fluorescence on the borderline of the i.r. could be detected only with a high sensitivity instrument.[11] The fluorescence was about two orders of magnitude weaker than that of MeṖhe, and only its first vibronic band could be recorded because of the spectral sensitivity limitations. The vibronic spacings in the first absorption band of $HMe\dot{P}he^+$ are *ca.* 1500 and 1100 cm^{-1}.

The hyperfine multistructures of the e.s.r. spectra of both MeṖhe and $HMe\dot{P}he^+$ radicals have been resolved and analysed;[15] in this paper, however, the e.s.r. spectra are mentioned only as one of the analytical tools for identification of the radical species.

Formation and Reactivity of the Free Radicals.—A freshly synthesised crystalline sample of $MePhe^+$ methosulphate did not contain any detectable free radical; the radical appeared after crystallisation from methanol and then disappeared slowly during storage of the crystals for a few months. The formation of MeṖhe on crystallisation of $MePhe^+$ salts from methanol has not previously been taken into account.[16]

$MePhe^+$ irradiated in aqueous solutions at 405 or 435 nm, especially in the presence of air, principally underwent photo-oxidation to pyocyanine [32,000 (s) and 14,000 (w) cm^{-1}]. Neither dealkylation nor free radical formation were observed.

The free radicals were synthesised by chemical, cathodic, or photochemical reduction of *N*-alkylphenazinium ions. In numerous samples of chemically prepared $HMe\dot{P}he^+$ the e.s.r. spectra revealed the presence of another free radical, different from MeṖhe. The cathodic reduction was the cleanest, and was used to prepare samples for the

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study of the properties of the free radicals. From the point of view of parallel dealkylation, however, photoreduction of MePhe^{+} and its homologues seemed the most intriguing.

Dealkylation.—The reactions occurring in the photoreduction experiments showed differences characteristic of the specific alcohol used as solvent. On irradiation of 10^{-5}M-MePhe^{+} in anhydrous ethanol (even in the presence of O_2) at 405 or 435 nm, MeṖhe was formed as the main product. It was, however, accompanied by phenazine (Phe) which could be identified by its absorption and fluorescence spectrum.[9] On irradiation at 365 nm, Phe was the main product. In isopropanol, however, the photochemical reaction was clean, leading to MeṖhe or HMeṖhe^{+}, as the solution was acidified in the course of reaction (2).

$$\mathrm{Pr^iOH + 2MePhe^+ \xrightarrow{h\nu} Me_2CO + 2Me\dot{P}he + 2H^+} \quad (2)$$

TABLE 1

Electronic transitions for homologues of phenazine at two oxidation levels at room temperature. Wavenumbers given in 1000 cm^{-1}

		Low energy absorption bands					Fluorescence		
	Compound	$\tilde{\nu}_{max.}$ ($\log_{10}\varepsilon$)					$\tilde{\nu}_{max.}$	$\tilde{\nu}_{oo}$	Remarks
Phenazine oxidation level	HPhe^{+}		23·5 (3·43)	26·2 (4·27)		38·7 (4·94)	19·0	21·25	*b*
	MePhe^{+}		22·9 (3·53)	26·0 (4·48)		38·6 (5·03)	18·9	20·6	*a*
	EtPhe^{+}		23·0 (3·55)	26·0 (4·45)		38·7 (4·97)	18·3	20·3	*a*
	H$_2$Phe^{++}		19·8 (2·98)	24·3 (4·33)			15·2	17·3	*b*
	HMePhe^{++}		18·6 *19·7* (3·23) 21·1	23·8 (4·43)		37·74 (4·90)	15·3	17·5	*a*
	HEtPhe^{++}		18·5 *19·8* (3·25) 21·1	23·8 (4·30)		37·7 (4·95)	15·15	17·4	*a*
Phenazyl oxidation level	HṖhe	16·65		27·4					*c*
	MeṖhe	*16·67* (3·06) 17·9 19·2 20·6	22·8 (3·13)	26·3 (4·12)	30·3 (3·48)	37·05 *39·80* (4·62)	*15·2* 14·2	15·9	*a*
	EtṖhe	*16·5* (3·3) 17·8 19·0	22·8 (3·2)	26·2 (4·2)	30·5 (3·5)	37·0 *39·5* (4·7)	*15·7* 14·7	16·1	*a, d*
	H$_2$Ṗhe^{+}	*15·65* 17·1	22·4	27·0	32·3				*e*
	HMeṖhe^{+}	14·0 *15·50* (3·26) 16·6 18·2	22·5 (4·07) 23·0	27·0 (3·89)	32·3 (3·27)	38·5 (4·91)	13·4	13·7	*a,f*

[a] Present work. [b] Ref. 9. [c] Ref. 23. [d] Refs. 16 and 17. [e] Ref. 3*b*. [f] In fluorescence only the first vibronic maximum.

In a special experiment, HMeṖhe^{+} electrochemically generated in the absence of O_2 was transferred under vacuum to an optical cell and also to an e.s.r. sample cell and both cells were sealed. After many months of storage in the dark, neither sample showed any change in spectral features. Similar experiments revealed that no Phe was formed during storage of MeṖhe in solution. Hence, dealkylation must occur in the course of, or parallel to, the photochemical (or chemical) reduction of MePhe^{+}.

The different reactivities in various alcohols suggest that dealkylation may be subject to some kind of steric hindrance in the course of reduction: isopropanol, a bulky reagent, reduces MePhe^{+} cleanly to MeṖhe, with no dealkylation. If this is true, then the size of the alkyl substituent on Phe should also influence the course of reaction; the results found (Table 2) support this hypothesis.

Association.—Hausser *et al.*[17] found that EtṖhe loses its paramagnetism at low temperatures, and a new absorption band appears at 12,000 cm^{-1}, ascribed to the formation of a π-complex. Later, it was shown by Serafimov and Zimmermann to be due to crystallites.[13a] MeṖhe in dilute aqueous solutions does not associate at room temperature. In ethanol, for 10^{-4}—10^{-2}M-MeṖhe or -HMeṖhe^{+}, we did not find any decrease in the e.s.r. signal intensity, even at temperatures as low as 120 K.

In the case of 2-cyano-MeṖhe, the e.s.r. signal intensity depends on pH, which is ascribed to the formation of diamagnetic association complexes of the type radical–H^{+}–radical.[18] We observed similar behaviour in the MeṖhe–HMeṖhe^{+} system. The effect may be due either to association or to disproportionation equilibria such as those included in equation (1); each equilibrium constant should exhibit an extremum at pH = pK of the free radical.

TABLE 2

Occurrence of parallel dealkylation in photochemical reaction of *N*-alkylphenazinium ions with alcohols to give free radicals

	Alcohol		
Alkyl	MeOH	EtOH	Pr^{i}OH
Me	Yes	Yes	No
Et	No	No	No

Protolytic Equilibria.—The protolytic pK of HMeṖhe^{+} was measured by spectrophotometry: MeṖhe was electrolytically prepared in isopropanol, some $HClO_4$ in the same solvent added, and the absorbance measured at several $\tilde{\nu}$

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[17] K. H. Hausser, *Z. Naturforsch.*, 1956, **11A**, 20; K. H. Hausser and J. N. Murrell, *J. Chem. Phys.*, 1957, **27**, 500.

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at *ca.* 22,500 cm^{-1}. An apparent pH value (p$H^{\mathrm{Pr^iOH}}$) was measured by means of the glass electrode. To relate this to the aqueous pH scale the indicator method was used, with acridinium (A) and quinolium (Q) ions chosen as suitable indicators. On the aqueous scale, pK(A) = 5·58, pK(Q) = 4·90.[19] We found, with the glass electrode and spectrophotometry p$K^{\mathrm{Pr^iOH}}$(A) = 2·46, and p$K^{\mathrm{Pr^iOH}}$(Q) = 1·60. Thus, the correspondence of both scales is established as $\mathrm{p}K = \mathrm{p}K^{\mathrm{Pr^iOH}} + 3{\cdot}2\ (\pm\ 0{\cdot}1)$. For the free radical $\mathrm{HMe\dot{P}he^{+}}$ we obtained $\mathrm{p}K = 5{\cdot}65\ (\pm 0{\cdot}15)$, already recalculated for the aqueous pH scale.

The acid–base equilibrium of the parent compound (at the oxidation level of phenazine), $\mathrm{HMePhe^{++}} \rightleftharpoons \mathrm{MePhe^{+}} + \mathrm{H^{+}}$, was studied by spectrophotometry at various concentrations of $\mathrm{HClO_4}$, using the Hammett acidity function H_0.[20a] As the slope of a test diagram $\mathrm{d}\log_{10}([\mathrm{MePhe^+}]/[\mathrm{HMePhe^{++}}])/\mathrm{d}H_0$ was 0·8 instead of 1·0, we evaluated a possibly thermodynamically meaningful pK value from the Bunnet-Olsen equation[20b] by plotting $\log_{10}([\mathrm{HMePhe^{++}}]/[\mathrm{MePhe^+}]) + H_0$ *vs.* $[H_0] + \log_{10}[\mathrm{H^+}]$; from the intercept, $\mathrm{p}K = -3{\cdot}5 \pm 0{\cdot}5$.

TABLE 3

pK Values of protolytic equilibria at the oxidation level of phenazine or of phenazyl radical in both the ground and first electronic excited states

Oxidation level	Equilibrium	pK^0	ΔpK^*	pK^*	Remarks
Phe	$\mathrm{H_2Phe^{++}} \rightleftharpoons \mathrm{HPhe^{+}} + \mathrm{H^{+}}$	−4·3	8·4	4·1	*b*
	$\mathrm{HMePhe^{++}} \rightleftharpoons \mathrm{MePhe^{+}} + \mathrm{H^{+}}$	−3·5	6·8	3·3	*a*
$\mathrm{\dot{P}he^{-}}$	$\mathrm{H_2\dot{P}he^{+}} \rightleftharpoons \mathrm{H\dot{P}he} + \mathrm{H^{+}}$	3·8	(4·7)	(8·5)	*c*
	$\mathrm{HMe\dot{P}he^{+}} \rightleftharpoons \mathrm{Me\dot{P}he} + \mathrm{H^{+}}$	5·7	4·7	10·4	*a*

[a] Present work. [b] Ref. 9. [c] −pK^0 value taken from ref. 24; ΔpK^* assumed to be equal to that for $\mathrm{Me\dot{P}he}$ due to the great similarity of the absorption spectra.

To determine the pK^* values for the lowest excited state

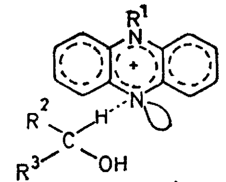
$$\mathrm{p}K^* - \mathrm{p}K = \Delta \mathrm{p}K^* = (\tilde{\nu}_{00}{}^{\mathrm{B}} - \tilde{\nu}_{00}{}^{\mathrm{HB}})\, hc/RT\ (\ln 10) \qquad (3)$$

the Förster cycle (3)[21] is applied where $\tilde{\nu}_{00}{}^{\mathrm{B}}$ and $\tilde{\nu}_{00}{}^{\mathrm{HB}}$ are the 0–0 transition wavenumbers for the first electronic transition in the basic and acidic species, respectively, measured as the intersection points of the first absorption bands with the normalised fluorescence spectra. The cycle assumes that ΔS_0 for the protolytic equilibrium is nearly the same for the ground and excited state, as should indeed be the case in the systems under study. All data on acid–base equilibria are collected in Table 3.

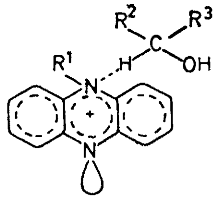
DISCUSSION

The results reveal the expected similarity of spectra and pK values for the isoelectronic (π and *n*) homologues. In addition, and more importantly, however, the results allow us to infer the reaction mechanism of the photochemical formation of the free radicals, and to compare the ground and excited state reactivities of the free radicals through observation of the newly discovered fluorescence spectra of these species.

In competitive photoreactions of *N*-alkylphenazinium ions, leading either to free radicals or *via* dealkylation to phenazine, the role of steric factors is evident (Table 2). Let us assume, by analogy with many other reductions with alcohols, an α-hydrogen transfer mechanism. There are two different reaction paths, the reactant approaching either (i) the tertiary nitrogen atom or (ii) the quaternary atom. In case (i) the nature of R^1 should have a negligible effect. In case (ii) the steric interaction of R^1 with R^2 and R^3 can inhibit the formation of the activated complex. Reduction to the free radical may occur in both ways. Dealkylation, however, probably *via* splitting R^{1+} off simultaneously with hydrogen atom (or hydrogen$^+$ ion?) transfer, can only occur by path (ii). The reasonable assumption of a type (ii) activated complex being preferred, *e.g.*, because of greater positive charge on the reaction site (as usual in reduction processes), clarifies all the observed facts.*



(i)



(ii)

The pK values of the free radicals formed by one electron transfer are higher by nearly 10 pK units than those of the parent compounds as might be expected on the basis of electron densities (and as already known for other cases[22]). In the free radicals of the phenazine series the changes of pK on excitation (Table 3) follow the tendency known for the parent compounds,[9] *i.e.*, $\mathrm{p}K^* \gg \mathrm{p}K^{\circ}$, as the π-electronic charge on the nitrogen atoms usually increases in the lowest excited state.

It seems worthwhile emphasising that the pK^* values of the free radicals studied are in the range typical for

* *Note added in proof*: Dr. P. Hemmerich (Konstanz) suggested that the effects summarised in Table 2 are due not to steric hindrance, but simply to competition between reduction and dealkylation treated as independent processes. In such cases, however, the photodealkylation of $\mathrm{MePhe^+}$ should occur in the non-reducing solvents as well. $\mathrm{MePhe^+}$ irradiated at 405 nm in very pure acetonitrile disappears with a quantum yield at least one order of magnitude less than in MeOH, and no demethylation product (phenazine) was found. The result supports our view that both processes have a common transition state (ii).

[19] D. D. Perrin, 'Dissociation Constants of Organic Bases in Aqueous Solution,' Butterworths, London, 1965.

[20] C. H. Rochester, 'Acidity Functions,' Academic Press, New York, 1970 (*a*) p. 43; (*b*) p. 89.

[21] Th. Förster, *Z. Elektrochem.*, 1950, **54**, 42.

[22] D. Rehm and A. Weller, *Israel J. Chem.*, 1970, **8**, 259.

[23] H. Masuhara, M. Okuda, and M. Koizumi, *Bull. Chem. Soc. Japan*, 1971, **44**, 38.

[24] R. C. Kaye and H. I. Stonehill, *J. Chem. Soc.*, 1952, 3240.

strong aliphatic amines. This may have important structural implications. Marginally, the high pK^* value in the lowest excited doublet state seems also to explain the unusual efficient quenching of $\text{Me}\dot{\text{P}}\text{he}$ fluorescence by H_2O: if proton transfer from H_2O to the strongly basic excited $\text{Me}\dot{\text{P}}\text{he}$ is fast enough, $\text{HMe}\dot{\text{P}}\text{he}^+$ is formed in the excited state, but its extremely weak fluorescence would be undetectable under the conditions of the experiment.

We thank Dr. A. Chodkowska, Dr. J. Zawadowska, and Mrs. H. Malec for help with e.s.r. experiments, the electrolytic experiments, and in numerous other measurements, respectively. Thanks are also due to Drs. K. Berens and J. Prochorow for use of their instruments, and to Professor H. Zimmermann for discussion. The work was sponsored by the Polish Academy of Sciences.

[3/1523 *Received, 20th July, 1973*]