Aminopyrine and Antipyrine Free Radical-cations: Pulse Radiolysis Studies of One-electron Transfer Reactions

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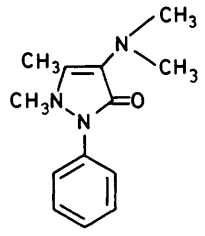
Absolute rate constants for the reaction of a variety of electrophilic free radicals with the pyrazoline derivatives aminopyrine and antipyrine have been measured by pulse radiolysis. In the case of aminopyrine the resulting radical cation is a particularly stable species (ε_{325} 5.35 × 10^3 dm^3 mol^{-1} cm^{-1}). Both compounds are readily oxidised to their respective radical-cations with the one-electron oxidation potential of antipyrine ($E°$ 1.1—1.6 V) being higher than that of aminopyrine ($E°$ 0.26—0.5 V). Studies of the reaction of the radical-cations with reducing agents suggest that aminopyrine in particular may prove to be a useful reference compound in studies of free radical one-electron oxidations.

Free-radical intermediates have now been detected during the metabolism of many xenobiotics.[1] In particular, the chemical and enzymatic oxidation of both antipyrine (2,3-dimethyl-1-phenyl-Δ^3-pyrazoline-5-one, AT) (**2**) and aminopyrine (the 4,4-dimethylamino derivative, AP) (**1**) have been studied exten-



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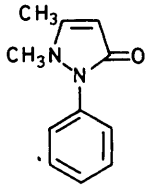

(**2**)

sively *in vitro*, by both u.v.–visible absorption spectroscopy and e.s.r. techniques. Indeed, in several enzyme systems such as those containing horseradish peroxidase and prostaglandin synthetase the stable radical cation ($AP^{+\bullet}$) has been observed.[2–4]

In the light of these observations it was decided to investigate further the reactions of various electrophilic free radical species with both aminopyrine and antipyrine as well as to assess the redox and kinetic properties of the related radical-cations.

The technique of pulse radiolysis has provided considerable information on the rates of formation and chemical properties of a large number of radical-cations in solution. Such long-lived radical species are proving particularly useful as reference solutes in pulse radiolysis studies of one-electron transfer reactions. Several redox couples where the radical serves as the one-electron donor have been investigated and the one-electron reduction potential, E_7^1, determined using the semiquinone equilibrium method.[5–7]

In couples where the free radical is the electron acceptor, reference compounds have included various phenothiazines, 2,2′-azino-di-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), and *NNN′N′*-tetramethyl-*p*-phenylenediamine (TMPD).[8–12] We now describe studies in which pulse radiolysis has been used to investigate several one-electron transfer reactions of the aminopyrine and antipyrine radical-cations and to measure the associated spectral changes. Since the bulk of these studies were completed a brief report on the reaction of the aminopyrine radical cation with glutathione has been published.[13]

Experimental

Pulse radiolysis experiments were undertaken using the Brunel University 4 MeV linear accelerator and associated equipment for kinetic spectroscopy and computer analysis of data as described previously.[14,15] An electron pulse (200 ns) producing a radiation dose of *ca.* 2—10 J kg^{-1} in an irradiation cell of 1.5 cm optical path length was used. Solutions were prepared in doubly distilled or Millipore-filtered water and exposed to the minimum of light prior to experimentation. Aqueous solutions were purged and saturated with oxygen-free nitrogen or nitrous oxide (British Oxygen Corporation) using the syringe bubbling technique.[16] Chemicals were supplied by Sigma, Aldrich, or Boehringer Mannheim and used without further purification. The phenothiazines were a gift from May and Baker. t-Butyl alcohol (B.D.H.) was purified by fractional crystallisation.

Results and Discussion

(i) *Reaction of Hydroxyl Radical* (HO•).—The radiolysis of water leads to the formation of hydroxyl radicals (HO•) and solvated electrons (e^-_{aq}) each with a yield of *ca.* 0.28 μmol J^{-1} and hydrogen atoms (H•) with a yield of *ca.* 0.06 μmol J^{-1}. In the presence of nitrous oxide (saturation concentration *ca.* 200μM in water at normal room temperature and pressure) solvated electrons react rapidly effectively to double the yield of hydroxyl radicals.[14] [reaction (1), k_1 5.8 × 10^9 l mol^{-1} s^{-1}].

$$e^-_{aq} + N_2O \xrightarrow{k_1} N_2 + OH^- + HO^\bullet \qquad (1)$$

On pulse radiolysis of a nitrous oxide-saturated solution containing 1mM-aminopyrine at pH 9 a strong absorption was observed with $G\varepsilon$ 2.90 × 10^{-4} M^2 J^{-1} at 325 nm (Figure 1a). The product formed decayed rapidly through a bimolecular process ($2k$ *ca.* 2 × 10^8 l mol^{-1} s^{-1}). With antipyrine a similar but weaker absorption spectrum was obtained (Figure 1b).

The rates of growth of these transients appeared proportional to AP or AT concentration, but were too fast for an accurate direct kinetic analysis with the Brunel instrumentation, k_2 and k_3 being *ca.* 10^{10} l mol^{-1} s^{-1}. However, it was possible to measure these rate constants using the standard thiocyanate

$$HO^\bullet + AP \xrightarrow{k_2} \text{products} \qquad (2)$$

$$HO^\bullet + AT \xrightarrow{k_3} \text{products} \qquad (3)$$

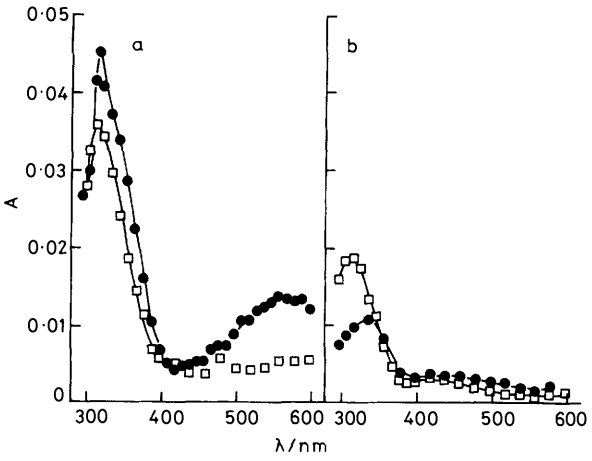

Figure 1. a, Transient absorption spectra observed 5 μs after pulse radiolysis of an N_2O-saturated solution containing 1mM-aminopyridine (□) and plus 0.2M-bromide (●): pH 9, dose 10 Gy. b, Transient absorption spectra observed 10 μs after pulse radiolysis of an N_2O-saturated solution containing 1mM-antipyrine (□) and plus 0.2M-bromide (●): pH 8.5, dose 10 Gy

Table 1. Bimolecular rate constants for the reaction of various free radicals with aminopyrine (AP) and antipyrine (AT)

	Aminopyrine		Antipyrine	
Radical	pH	k/l mol^{-1} s^{-1}	pH	k/l mol^{-1} s^{-1}
HO• (SCN^- competition)	7.0	2.3×10^{10}	7.0	1.1×10^{10}
$Br_2^{-\bullet}$	9.0	1.6×10^9	6.2	3.9×10^8
$N_3^\bullet$	7.3	3.7×10^9	8.5	NR[a]
$(SCN)_2^{-\bullet}$	9.0	3.6×10^8	5.8	NR
$I_2^{-\bullet}$	7.0	4.9×10^7	7	NR
$Cl_3COO^\bullet$	*ca.* 6	4.9×10^8	*ca.* 6	NR
RS• (cysteine)	6	2.6×10^8		NR
(cysteamine)	6.5	3.9×10^8		NR
(glutathione)	6	2.5×10^8		NR
$NO_2^\bullet$	7	5.3×10^6		NR
$CZ^{+\bullet}$	6	2.9×10^8		NR
$ABTS^{+\bullet}$	6	1.6×10^8		NR
RO• (phenol)	8	7×10^7		
(*m*-cresol)	8	6×10^7		
(*p*-cresol)	8	1.6×10^7		
(*p*-methoxyphenol)	8	$<10^5$		

[a] NR = no reaction observed.

competition method.[14] On pulse radiolysis of nitrous oxide-saturated solutions containing 1mM-SCN^- at pH 7 the initial maximum absorbance due to $(SCN)_2^{-\bullet}$ at 480 nm decreased with increasing AP or AT concentration. Plots of $A°/A$ against [AP]/[SCN^-] and [AT]/[SCN^-] (where $A°$ is the absorbance immediately after the pulse in the absence of AP or AT and A the absorbance in their presence) were linear in both cases. Taking k_4 as 1.0×10^{10} l mol^{-1} s^{-1} values of k_2 2.3×10^{10} and

$$HO^\bullet + 2SCN^- \xrightarrow{k_4} HO^- + (SCN)_2^{-\bullet} \quad (4)$$

k_3 1.1×10^{10} l mol^{-1} s^{-1} were obtained from the slopes. [Although $(SCN)_2^{-\bullet}$ reacts with AP its rate was sufficiently lower than that of •OH to make the competition method possible.]

(ii) *Reactions of* $Br_2^{-\bullet}$, $N_3^\bullet$, $(SCN)_2^{-\bullet}$, *and* $Cl_3COO^\bullet$.—The spectra of products formed on pulse radiolysis of similar solutions but containing in addition 0.2M-bromide ion are also shown in Figure 1. Under these conditions the oxidising radical is $Br_2^{-\bullet}$ formed according to reaction (5). Clearly in the case of

$$HO^\bullet + 2Br^- \longrightarrow HO^- + Br_2^{-\bullet} \quad (5)$$

aminopyrine the products obtained on oxidation by HO• and $Br_2^{-\bullet}$ differ. The product from $Br_2^{-\bullet}$ was completely stable in the time scale of these experiments, no decay of the transient being observed. This product is considered to be the radical-cation of aminopyrine formed by reaction (6).

$$Br_2^{-\bullet} + AP \longrightarrow 2Br^- + AP^{+\bullet} \quad (6)$$

The spectra obtained compare favourably with that recorded on one-electron oxidation of aminopyrine by cyclic voltammetry.[17] Assuming $Br_2^{-\bullet}$ reacts quantitatively with AP, the molar absorptivity of $AP^{+\bullet}$ corresponds to ε_{325} 5 350 and ε_{580} 1 820 l mol^{-1} cm^{-1}. This compares favourably with the value ε_{570} 1 760 l mol^{-1} cm^{-1} recently obtained.[13] A similar spectrum was observed on pulse radiolysis of N_2O-saturated solutions containing excess of thiocyanate or azide ions or air-saturated solutions containing excess of t-butyl alcohol and carbon tetrachloride where reactions (4) or (7) or (8)—(10) occur initially and the appearance can be attributed to reactions (11)—(13), respectively.

$$OH^\bullet + N_3^- \longrightarrow OH^- + N_3^\bullet \quad (7)$$

$$OH^\bullet + (CH_3)_3COH \longrightarrow H_2O + {}^\bullet CH_2(CH_3)_2COH \quad (8)$$

$$e^-_{aq} + CCl_4 \longrightarrow Cl^- + CCl_3^\bullet \quad (9)$$

$$CCl_3^\bullet + O_2 \longrightarrow CCl_3O_2^\bullet \quad (10)$$

$$(SCN)_2^{-\bullet} + AP \longrightarrow 2SCN^- + AP^{+\bullet} \quad (11)$$

$$N_3^\bullet + AP \longrightarrow N_3^- + AP^{+\bullet} \quad (12)$$

$$CCl_3O_2^\bullet + AP \longrightarrow CCl_3O_2^- + AP^{+\bullet} \quad (13)$$

In all instances where measurable, the $AP^{+\bullet}$ absorption formed and the oxidising radical absorption decayed exponentially with time, with pseudo-first-order rate constants being proportional to AP concentration. The corresponding absolute rate constants are shown in Table 1. As with aminopyrine the spectra of the products of the reactions of HO• and $Br_2^{-\bullet}$ with antipyrine differ from one another, presumably because the former involves addition and the latter electron transfer. The rate constant for the reaction with $Br_2^{-\bullet}$ [(14)] is considerably lower than that with aminopyrine (Table 1) and $N_3^\bullet$ and $(SCN)_2^{-\bullet}$ failed to oxidise antipyrine at a detectable rate.

$$Br_2^{-\bullet} + AT \longrightarrow \text{Products} \quad (14)$$

This decrease in reactivity shown by antipyrine may indicate that $E°(AP^+/AP) < E°(AT^+/AT)$. Experimental evidence for this is presented later.

(iii) *Reaction of Thiyl Radicals* (RS•).—Thiyl radicals (RS•) have been shown to be moderate one-electron oxidising agents.[18–21] On pulse radiolysis of nitrogen-saturated solutions containing propan-2-ol–propanone (1M) and thiol (20mM) at pH 6, thiyl radicals are generated according to reactions (15)—(19).

$${}^\bullet OH + (CH_3)_2CHOH \longrightarrow H_2O + (CH_3)_2\dot{C}OH \quad (15)$$

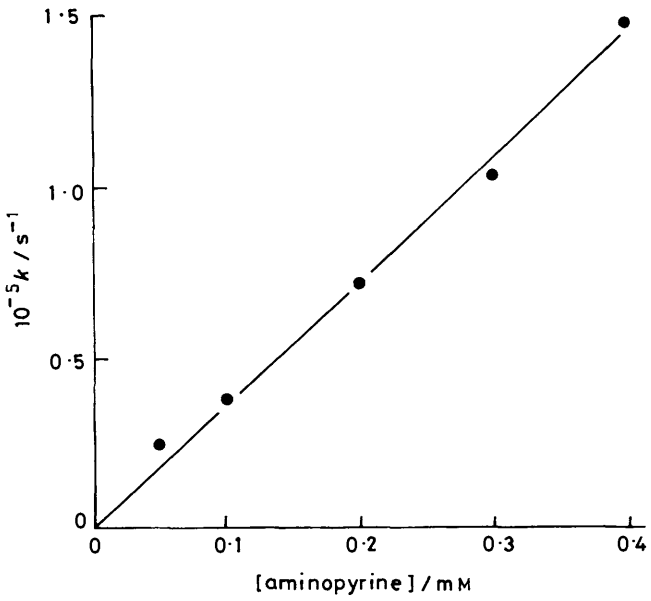

Figure 2. Plot of first-order rate constant against aminopyrine concentration for the formation of absorption at 325 nm on pulse radiolysis of nitrogen-saturated solutions containing 1M-propan-2-ol, 1M-propanone, 20mM-cysteamine, and aminopyrine at pH 6.5. Dose = 5 Gy

$$H^{\bullet} + (CH_3)_2CHOH \longrightarrow H_2 + (CH_3)_2\dot{C}OH \quad (16)$$

$$e^-_{aq} + (CH_3)_2CO \longrightarrow (CH_3)_2\dot{C}O^{-\bullet} \quad (17)$$

$$(CH_3)_2CO^{-\bullet} + H^+ \longrightarrow (CH_3)_2\dot{C}OH \quad (18)$$

$$(CH_3)_2\dot{C}OH + RSH \longrightarrow (CH_3)_2CHOH + RS^{\bullet} \quad (19)$$

Inclusion of aminopyrine (0.1—0.4mM) in such systems resulted in the exponential appearance of the $AP^{+\bullet}$ absorption at 325 nm. A plot of first-order rate constant against AP concentration for the reaction of the cysteamine thiyl radical is shown (Figure 2), from which a value k_{20} 3.9 × 10^8 l mol^{-1} s^{-1} is

$$CyS^{\bullet} + AP \longrightarrow CyS^- + AP^{+\bullet} \quad (20)$$

derived. When the pH of the system was raised to above the pK_a of the thiol no $AP^{+\bullet}$ could be detected. This is in accord with reaction (21) competing for the thiyl radicals. The rate constant

$$RS^{\bullet} + RS^- \longrightarrow RSSR^{-\bullet} \quad (21)$$

for the oxidation of AP by the glutathione thiyl radicals (k 2.5 × 10^8 l mol^{-1} s^{-1}, is slightly lower than that recently reported (k 5 × 10^8 l mol^{-1} s^{-1}) and is possibly due to the high organic content, 20%, of the present solutions.[13] Furthermore, when aminopyrine was replaced by antipyrine in the thiyl-generating systems, no absorption due to $AT^{+\bullet}$ could be seen, indicating that the rate constant for the analogous reaction is considerably smaller.

(iv) *Reaction of Nitrogen Dioxide* ($NO_2^{\bullet}$).—Recent studies have shown that nitrogen dioxide, $NO_2^{\bullet}$, can oxidise many substrates by a one-electron transfer mechanism, but that it is generally less reactive than the radicals $Br_2^{-\bullet}$, $(SCN)_2^{-\bullet}$, $N_3^{\bullet}$, and CCl_3OO.[22,23] On pulse radiolysis of nitrogen-saturated solutions containing 0.1M-$NaNO_3$ and 1M-t-butyl alcohol, $NO_2^{\bullet}$ is rapidly generated ($t_{\frac{1}{2}}$ 3 μs) according to reactions (8) and (22)—(24).

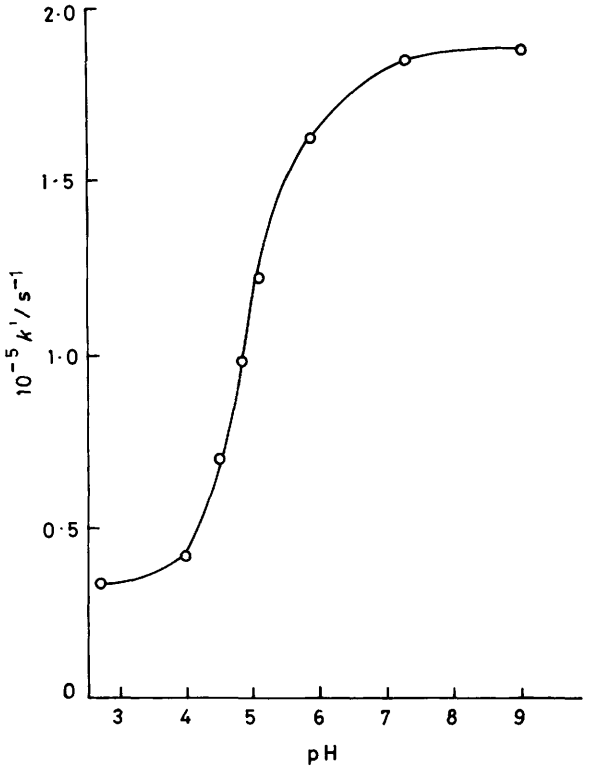

Figure 3. Plot of first-order rate constant against pH for the formation of absorption at 325 nm on pulse radiolysis of nitrous oxide-saturated solutions containing 0.2M-KSCN and 0.5mM-aminopyrine

$$e^-_{aq} + NO_3^- \longrightarrow NO_3^{2-\bullet} \quad (22)$$

$$NO_3^{2-\bullet} + H^+ \longrightarrow NO_3H^{-\bullet} \quad (23)$$

$$NO_3H^{-\bullet} \longrightarrow NO_2^{\bullet} + OH^- \quad (24)$$

In the additional presence of aminopyrine the absorption due to $AP^{+\bullet}$ again appeared exponentially and was first order in AP, in agreement with reaction (25) with k_{25} 5.3 × 10^6 l

$$NO_2^{\bullet} + AP \xrightarrow{k_{25}} NO_2^- + AP^{+\bullet} \quad (25)$$

mol^{-1} s^{-1} (Table 1). Again, no analogous reaction was detected with antipyrine.

(v) *Oxidation of Aminopyrine by Phenoxyl Radical* (PhO$^{\bullet}$).—Phenoxyl radicals have been shown to undergo one-electron transfer,[20,24–26] and it was of interest to see if they could also oxidise aminopyrine. On pulse radiolysis of an N_2O-saturated solution containing 0.1M-NaN_3, 10mM-phenol, and 0.1mM-aminopyrine at pH 8 the characteristic absorption of the phenol phenoxyl free radical[27] was observed immediately after the pulse. This was subsequently replaced by that of $AP^{+\bullet}$ in agreement with reaction (26).

$$RO^{\bullet} + AP \xrightarrow{k_{26}} RO^- + AP^{+\bullet} \quad (26)$$

The reaction was found to be first order in AP concentration with k_{26} 7.0 × 10^7 l mol^{-1} s^{-1}. Similar results were found when phenol was replaced by *m*- and *p*-cresol (Table 1). Interestingly, no reaction could be seen when *p*-methoxyphenol was used, the presence of the electron-donating groups clearly reducing the rate of $AP^{+\bullet}$ oxidation. This must be due to kinetic rather than thermodynamic control as the redox potential of the

p-$CH_3OC_6H_4O^{\bullet}$–p-$CH_3C_6H_4OH$ couple is *ca.* 600 mV[25] and we show below that the redox potential for the $AP^{+\bullet}$–AP couple is considerably less than this.

(vi) *Variation of Rate of Aminopyrine Oxidation with* pH.—Clearly the introduction of the dimethylamino group at C-4 of the heterocyclic ring of AP is important to both oxidation and stability of the $AP^{+\bullet}$ as shown above. Figure 3 shows the effect of pH on the second-order rate constants derived from the slopes of a series of plots of observed first-order rate constants against AP concentration at different pH values for oxidation of AP by $(SCN)_2^{-\bullet}$. Similar results were obtained where $Br_2^{-\bullet}$ was used as the one-electron oxidant. The effect observed is in agreement with the protonation of the amino nitrogen atom of aminopyrine, decreasing the ease of oxidation, with pK_a *ca.* 4.9.

(vii) *Redox Potentials of $AT^{+\bullet}$–AT and $AP^{+\bullet}$–AP Couples.*—The kinetic results shown in Table 1 suggest that $E°(AT^{+\bullet}–AT)$ is greater than $E°(AP^{+\bullet}–AP)$. This was confirmed by showing reaction (27) occurs. Although Br_2^- oxidises AP faster than

$$AT^{+\bullet} + AP \xrightarrow{k_{27}} AT + AP^{+\bullet} \qquad (27)$$

AT, the antipyrine radical cation $AT^{+\bullet}$ can be formed selectively in the presence of aminopyrine by pulsing an N_2O-saturated solution in which $[Br^-] \gg [AT] \gg [AP]$, the $HO^{\bullet}$ radical selectively oxidising Br^- and the $Br_2^{-\bullet}$ radical selectively oxidising AT. Using 0.2M-KBr, 10mM-AT, and 50—200 μM-AP an instantaneous absorbance at 300 nm was observed followed by an exponential grow-in. The first-order rate constant for this slower reaction was proportional to the AP concentration, giving k_{27} 1.4 × 10^9 l mol^{-1} s^{-1}.

By investigating the occurrence and direction of reactions (28) and (29) for a series of substrate couples $S^{+\bullet}$–S it was

$$AT^{+\bullet} + S \rightleftharpoons AT + S^{+\bullet} \qquad (28)$$

$$AP^{+\bullet} + S \rightleftharpoons AP + S^{+\bullet} \qquad (29)$$

possible to establish limits for the two redox potentials of interest. For example ABTS is readily oxidised by a number of electrophilic radical species[18] to form the long-lived radical cation $ABTS^{+\bullet}$ with ε_{415} 36 000 l mol^{-1} cm^{-1}. When the above experiment was repeated using ABTS in place of AP, $ABTS^{+\bullet}$ was observed to form exponentially with a pseudo-first-order rate constant proportional to the ABTS concentration giving k_{30} 5 × 10^9 l mol^{-1} s^{-1}. However if the experiment was repeated

$$AT^{+\bullet} + ABTS \xrightarrow{k_{30}} AT + ABTS^{+\bullet} \qquad (30)$$

with AP in place AT, no grow-in of $ABTS^{+\bullet}$ was observed. However on generating $ABTS^{+\bullet}$ rapidly in the presence of a low concentration of AP [using N_2-saturated 1M-propan-2-ol, 1M-propanone, 5 × 10^{-2}M-cysteamine (RSH), 1mM-ABTS, and 100—250 μM-AP] through reaction (31), the absorption at 410

$$RS^{\bullet} + ABTS \longrightarrow RS^- + ABTS^{+\bullet} \qquad (31)$$

nm decayed rapidly consistent with reaction (32) occurring with

$$ABTS^{+\bullet} + AP \longrightarrow ABTS + AP^{+\bullet} \qquad (32)$$

k_{32} 1.6 × 10^8 l mol^{-1} s^{-1}. The results thus indicated that $E°(AP^{+\bullet}–AP) < E°(ABTS^{+\bullet}–ABTS) < E°(AT^{+\bullet}–AT)$. The apparent anomaly that $k_{30} > k_{27}$ can perhaps be attributed to the different charges on the reactants. ABTS is in fact a doubly charged anion, hence reaction (30) is between a singly charged radical cation and a doubly charged anion, whereas in reaction (27) the substrate is a neutral molecule.

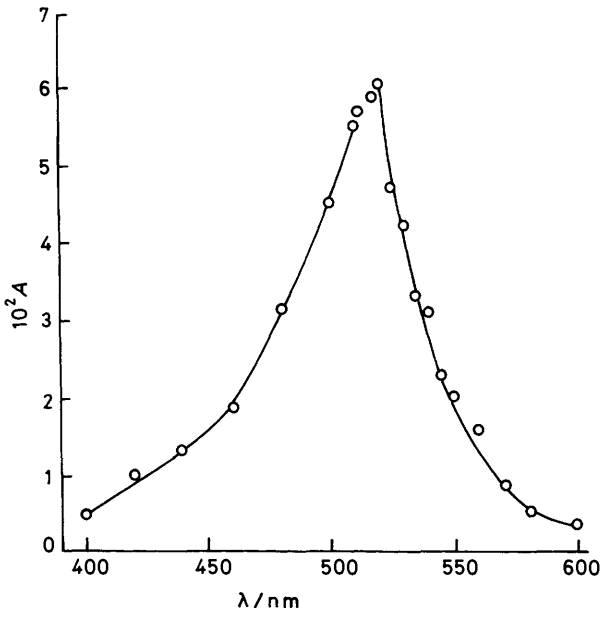

Figure 4. Transient absorption spectrum observed 10 μs after pulse radiolysis of an N_2O-saturated solution containing 0.2M-KBr, 25mM-antipyrine, and 0.25mM-promethazine, pH 3, dose 10 Gy

Figure 4 shows the spectrum obtained 10 μs after pulse-radiolysis of an N_2O-saturated (pH 3) 0.2M-KBr–25mM-AT solution also containing the phenothiazine promethazine (PZ). This spectrum is identical to that previously assigned to the promethazine radical cation[9] showing reaction (33) between $AT^{+\bullet}$ and promethazine occurs by simple electron transfer. The rate of formation of this radical cation corresponded to k_{33} 1.6 × 10^9 l mol^{-1} s^{-1} in agreement with a recent report.[28] Similar results were obtained with the

$$AT^{+\bullet} + PZ \xrightarrow{k_{33}} AT + PZ^{+\bullet} \qquad (33)$$

phenothiazines chloropromazine and trimeprazine and with the water-soluble vitamin E analogue Trolox C at neutral pH, the spectra of the products being that of the radical cations of the phenothiazines and the phenoxyl radical of Trolox C indicating simple electron-transfer reactions. The rate constants for these reactions are given in Table 2. From the $E°(S^{+\bullet}–S)$ values given in this table it follows that $E°(AT^{+\bullet}–AT) > 860$ mV. However, the fact that $Br_2^{-\bullet}$ did oxidise AT whereas $(SCN)_2^{-\bullet}$ did not [section (ii)] allows a more positive lower limit to be assigned, 1.29 V, with an upper limit of 1.69 V.

With AP in place of AT, chloropromazine (CZ) was found to behave in the same way as ABTS, *i.e.* reaction (29) proceeds in the reverse directions, with k_{34} 2.9 × 10^8 l mol^{-1} s^{-1}, showing

$$CZ^{+\bullet} + AP \xrightarrow{k_{34}} CZ + AP^{+\bullet} \qquad (34)$$

$E°(AP^{+\bullet}–AP) < 780$ mV. An upper limit considerably less than this, 480 mV, was indicated by the fact that $AP^{+\bullet}$ did not oxidise Trolox C (TxOH) in neutral solution, whereas $AT^{+\bullet}$ did under similar conditions. However if the pH of the solution was raised to 13 there was a rapid grow-in at 435 nm corresponding to the formation of the phenoxyl radical form Trolox C[29] in 65% yield and with k_{35} 6.1 × 10^8 l mol^{-1} s^{-1} (Figure 5). The 65% yield

$$AP^{+\bullet} + TxO^- \xrightarrow{k_{35}} AP + TxO^{\bullet} \qquad (35)$$

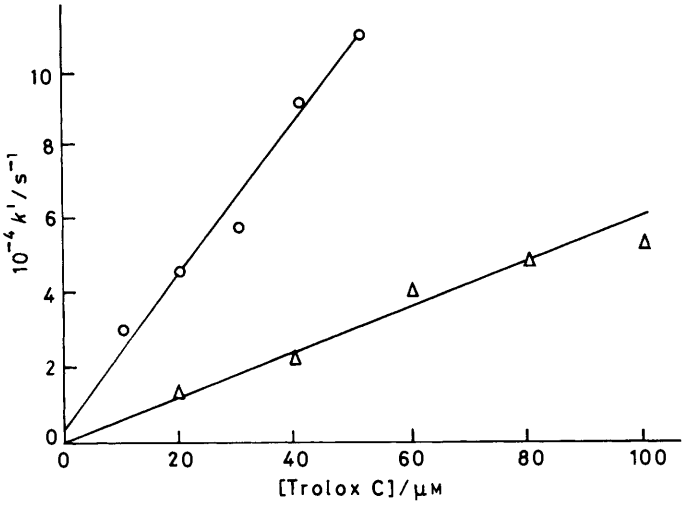

Figure 5. Plot of first-order rate constant against Trolox C concentration for the increase in absorption at 435 nm following pulse radiolysis of nitrous oxide-saturated solutions containing 0.2M-KBr, Trolox C, and 25mM-antipyrine (○, pH 7) or 25mM-aminopyrine (△, pH 13)

suggests reaction (35) is an equilibrium and therefore $E°(AP^{+\bullet}$–AP) is close to $E°(TxO^{\bullet}$–$TxO^{-})$, *ca.* 200 mV. However, this was shown not be the case in experiments in which S is *NNN'N'*-tetramethyl-*p*-phenylenediamine (TMPD) which has $E°(S^{+\bullet}$–S) 270 mV. $AP^{+\bullet}$ converted TMPD into $TMPD^{+\bullet}$ stoicheiometrically with k_{36} 4.6×10^8 l mol^{-1} s^{-1}, no evidence for

$$AP^{+\bullet} + TMPD \xrightarrow{k_{36}} AP + TMPD^{+\bullet} \qquad (36)$$

equilibria being found in experiments with varying AP and TMPD concentrations.

Table 2 summarizes this work, from which 1.25 V < $E°(AT^{+\bullet}$–AT) < 1.69 V and 0.27 V < $E°(AP^{+\bullet}$–AP) < 0.48 V.

(viii) *Reactions of Hydroxy Adducts of AP + AT.*—It is clear from the spectra shown in Figures 1a and b that the reaction of $^{\bullet}OH$ with both aminopyrine and antipyrine leads to products, probably OH adducts, which differ spectrally from those obtained when $Br_2^{-\bullet}$ is the oxidant. They also differ in terms of stability; whereas $AP^{+\bullet}$ is extremely long-lived, the OH adduct decays slowly. The OH adduct of antipyrine decays faster than its dimethyl analogue.

On pulse radiolysis of a nitrous oxide-saturated solution containing antipyrine (0.01M) and ABTS (0.1mM) the ABTS radical cation is formed. However, the yield of $ABTS^{+\bullet}$ is only 42% of that when bromide rather than $^{\bullet}OH$ is the initial oxidant. Where bromide is used the yield corresponds to a 100% yield of $^{\bullet}OH$ radicals assuming the initial yield is 0.55 μM J^{-1}. Therefore, we conclude that the reaction of $^{\bullet}OH$ with AT produces two products, the radical-cation which oxidises ABTS

$$^{\bullet}OH + AT \longrightarrow OH^{-} + AT^{+\bullet} \qquad (37)$$

$$^{\bullet}OH + AT \longrightarrow (AT\text{-}OH)^{\bullet} \qquad (38)$$

(Table 2) and the OH adduct which is not oxidising. The spectra shown in Figure 1b are consistent with this interpretation.

Conclusions.—The results presented here indicate that the pyrazoline analogues aminopyrine and antipyrine may be useful as reference chromagens in pulse radiolysis studies. In this respect, aminopyrine would prove the most useful given the relatively high extinction coefficient of the radical cation (ε_{325} 5.25×10^3 l mol^{-1} cm^{-1}) as well as the increased stability of $AP^{+\bullet}$. Antipyrine was found to be far less readily oxidised by the free radicals investigated. Indeed, only $Br_2^{-\bullet}$ and $OH^{\bullet}$ were found to oxidise AT and therefore the one-electron redox potential for ($AT^{-\bullet}$–AT) must have a value between 1.2 and 1.6 V. The dimethyl derivative aminopyrine is oxidised by a variety of electrophilic free radical species including that of the antipyrine radical cation, and we suggest that $E(AP^{+\bullet}$–AP) lies within the range 0.27—0.48 V, much lower than that for antipyrine.

Table 2. Summary of electron-transfer reactions (28) and (29)

Radical	Substrate	pH	$10^{-9}k$/l mol^{-1} s^{-1}	$E°(S^{+\bullet}$–S)/V	Ref.
$Br_2^{-\bullet}$	AT	6	0.39	1.69	29
	AP	9	1.6		
$(SCN)_2^{-\bullet}$	AT	6	NR[a]	1.25	29
	AP	9	0.36		
$AT^{+\bullet}$	Ascorbate	8	2.0	0.3	25
	Trolox C	7	2.1	0.48	25
	Trimeprazine	3	3.4		
	Chlorpromazine	3	2.9	0.78	27
	Promethazine	3	1.6	0.86	27
	ABTS	8	5		
	AP	8	5.0		
$CZ^{+\bullet}$	AP	3	0.29	0.78	27
$ABTS^{+\bullet}$	AP	7	0.16		
$AP^{+\bullet}$	Trolox C (TxO^{-})	13	0.61	0.2	25
	TMPD	8	0.46	0.27	25
	Trolox C	7	NR[a]	0.48	

[a] No reaction observed.

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References

1 T. F. Slater, 'Biochemical Mechanisms of Liver Injury,' Academic Press, London and New York, 1978.
2 I. S. Forrest, F. M. Forrest, and M. Berger, *Biochim. Biophys. Acta*, 1958, **29**, 441.
3 R. P. Mason, in 'Free Radicals in Biology,' ed. W. A. Pryor, Academic Press, New York, 1982, vol. V, p. 161.
4 T. E. Eling, R. P. Mason, and K. Sivarajah, *J. Biol. Chem.*, 1985, **260**, 1601.
5 K. B. Patel and R. L. Willson, *J. Chem. Soc., Faraday Trans. 1*, 1973, **69**, 814.
6 Y. A. Ilan, D. Miesel, and G. Czapski, *Isr. J. Chem.*, 1974, **12**, 89.
7 P. M. Wood, *FEBS Lett.*, 1974, **44**, 22.
8 D. Bahnemann, K.-D. Asmus, and R. L. Willson, *J. Chem. Soc., Perkin Trans. 2*, 1981, 890.
9 D. Bahnemann, K.-D. Asmus, and R. L. Willson, *J. Chem. Soc., Perkin Trans. 2*, 1983, 1669.
10 S. Steenken, *J. Phys. Chem.*, 1979, **83**, 595.
11 S. Fujita and S. Steenken, *J. Am. Chem. Soc.*, 1981, **103**, 2540.
12 L. G. Forni and R. L. Willson, *Methods Enzym.*, 1984, **105**, 179.
13 I. Wilson, P. Wardman, G. M. Cohen, and M. D'Arcy Doherty, *Biochem. Pharmacol.*, 1986, **35**, 21.
14 R. L. Willson; in 'Biochemical Mechanisms of Liver Injury,' ed. T. F. Slater, Academic Press, London and New York, 1978, p. 123.
15 R. L. Willson; 'Free Radicals, Lipid Peroxidation and Cancer,' eds. D. C. H. McBrien and T. F. Slater, Academic Press, London, 1982, p. 275.

16 R. L. Willson, *Int. J. Radiat. Biol.*, 1970, **17**, 349.
17 H. Sayo and M. Masui, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1640.
18 B. S. Wolfenden and R. L. Willson, *J. Chem. Soc., Perkin Trans. 2*, 1982, 805.
19 L. G. Forni, V. O. Mora-Arellano, J. Monig, and R. L. Willson, *J. Chem. Soc., Perkin Trans. 2*, 1983, 981.
20 L. G. Forni and R. L. Willson, *Biochem. J.*, 1986, **240**, 897.
21 L. G. Forni and R. L. Willson, *Biochem. J.*, 1986, **240**, 905.
22 L. G. Forni, V. O. Mora-Arellano, J. E. Packer, and R. L. Willson, *J. Chem. Soc., Perkin Trans. 2*, 1986, 1.
23 W. A. Prutz, H. Monig, J. Butler, and E. J. Land, *Arch. Biochem. Biophys.*, 1985, **243**, 125.
24 R. Schuler, *Radiat. Res.*, 1977, **69**, 417.
25 S. Steenken and P. Neta, *J. Phys. Chem.*, 1982, **86**, 3661.
26 J. E. Packer, T. F. Slater, and R. L. Willson, *Nature* (*London*), 1979, **278**, 737.
27 E. J. Land and M. Ebert, *Trans. Faraday Soc.*, 1967, **63**, 1181.
28 S. V. Jovanovic, P. Neta, and M. G. Simic, *Mol. Pharmacol*, 1985, **28**, 377.
29 M. J. Davies, L. G. Forni, and R. L. Willson, *Biochem. J.*, in the press.

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