Free Radical Reactions of the Phenothiazine, Metiazinic Acid

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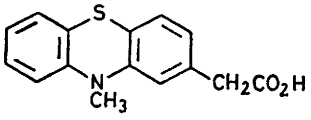
Absolute rate constants have been measured for the reaction of the anionic conjugate base of metiazinic acid, MZ^-, in the pH range 8—11, with hydrated electrons (k 1.5 × 10^9 l mol^{-1} s^{-1}), and for the oxidation of MZ^- by $OH^\bullet$, $Br_2^{-\bullet}$, $(SCN)_2^{-\bullet}$, $CO_3^{-\bullet}$, $Tl(OH)_2$, $N_3^\bullet$, $^\bullet CH_2CHO$, $(CH_3SCH_3)_2^{+\bullet}$, $CH_3SSCH_3^{+\bullet}$ and lipoate$^{+\bullet}$. The k values for these oxidations are found to be in the 10^9—10^{10} l mol^{-1} s^{-1} range, except for $k(^\bullet CH_2CHO + MZ^-)$ 2 × 10^8 l mol^{-1} s^{-1}. The oxidation of metiazinic acid leads to $MZ^{\pm\bullet}$ radical zwitterions with yields ranging from 50 to 100% with the minimum value referring to the oxidation by $^\bullet CH_2CHO$. The $MZ^{\pm\bullet}$ radical zwitterion is characterized by two strong optical absorption bands with maxima at 530 (ε 11 000 l mol^{-1} cm^{-1}) and 270 nm (51 500). Other primary oxidation products are indicated, but only a transient absorption around 350 nm ($OH^\bullet$ adduct and/or hydrogen abstraction product) could qualitatively be identified in the reaction of metiazinic acid with hydroxyl radicals.

METIAZINIC ACID (I) (MZ; 10-methylphenothiazin-2-ylacetic acid; Soripal; Rhône Poulenc) has been shown to be an effective anti-inflammatory agent in animals. It also has analgesic properties.[1,2]



(I)

Preliminary pulse radiolysis studies, reported briefly elsewhere,[3] have shown that the drug, like other phenothiazine derivatives such as promazine, chlorpromazine, and promethazine, can be readily oxidized to form a radical cation.[4-13] Attention has recently been focused on the possibility that free radicals, particularly those related to oxygen such as $OH^\bullet$, $O_2^{-\bullet}$, and peroxyl radicals, are involved in the development of inflammation and in neural disorders.[14-19] The free radical reactions of the drug are thus of interest and in this paper we describe extensive optical as well as conductivity pulse radiolysis studies. Particular emphasis has been given to the mechanism of the drug's one-electron oxidation. Studies have been restricted to the pH range 8—11 because of the limited solubility of the drug in its protonated form (pK_a 7.3).[20]

EXPERIMENTAL

Pulse radiolysis experiments have been undertaken using the Hahn–Meitner 1.6 and 4 MeV Van de Graaff accelerators and associated equipment for optical and conductometric analysis.[21,22] The propan-2-ol–tetranitromethane system was used for dosimetry.[23] Doses generally were in the range 1—10 J kg^{-1} per pulse.

Changes in absorption and conductance were quantified in terms of $\Sigma\Gamma_x\varepsilon_x$ and $\Sigma\Gamma_x\Lambda_x$, respectively, where Γ_x is the number of μmols of a particular species x formed or destroyed per joule of energy absorbed by the system, ε_x its extinction coefficient in units of l mol^{-1} cm^{-1}, and Λ_x its equivalent conductance in units of Ω^{-1} cm^2.

Metiazinic acid, as a yellow powder, was supplied by May and Baker Ltd. Other chemicals were of AnalaR grade and were used without further purification. Solutions were prepared using Millipore-filtered distilled water, deoxygenated by bubbling with N_2, and the pH was adjusted using NaOH and $HClO_4$. Except in studies with t-butyl alcohol all solutions were saturated with nitrous oxide.

RESULTS

(1) *Nitrogen-saturated Solutions containing t-Butyl Alcohol.*—On pulse radiolysis of N_2-saturated solutions containing t-butyl alcohol (0.5M) (to scavenge hydroxyl radicals) and metiazinic acid (0.1—1mM) at pH 10, the characteristic absorption of the solvated electron was observed immediately after the radiation pulse. The absorption at 600 nm decayed exponentially and by a first-order process in metiazinic acid concentration, corresponding to equation (1)

$$e_{aq}^- + MZ^- \longrightarrow \text{products} \qquad (1)$$

with k_1 1.5 × 10^9 l mol^{-1} s^{-1}. A weaker absorption ($\lambda_{max.}$ 285 nm) attributable to the reaction products, presumably $MZ^{2-\bullet}$, remained and subsequently decayed over a period of milliseconds.

(2) *Nitrous Oxide-saturated Solutions.*—On pulse radiolysis of N_2O-saturated solutions of metiazinic acid (5 × 10^{-5}M; pH 10) a large absorption change ($\lambda_{max.}$ 270, 350, and 530 nm) was observed after 1 μs (Figure 1). Over the next 20 μs the absorption at 270 and 530 nm continued to increase whereas at 350 nm the absorption decreased simultaneously. The conductivity of the system throughout this period increased concurrently with the absorption at 270 and 530 nm, *i.e.* a fast change is followed by a second slower change. Kinetic studies with metiazinic acid concentrations in the range 5—10μM showed that the rate of the rapid increases increases in conductivity and absorption at 530 nm was first order in metiazinic acid concentration with a rate constant of 8.4 × 10^9 l mol^{-1} s^{-1}, derived from the slope of the plot of first-order rate constants against concentration in Figure 2.

The rate of the subsequent slower changes in absorption and conductivity were not affected by changes in metiazinic acid concentration, *i.e.* refer to a pure first-order process. From the half-life of 5.3 μs a rate constant k of 1.3 × 10^5 s^{-1} is calculated. Separation of the two processes is, however, only possible at higher metiazinic acid concentrations (>2 × 10^{-4}M) where the fast process is completed during the duration of the 1 μs pulse.

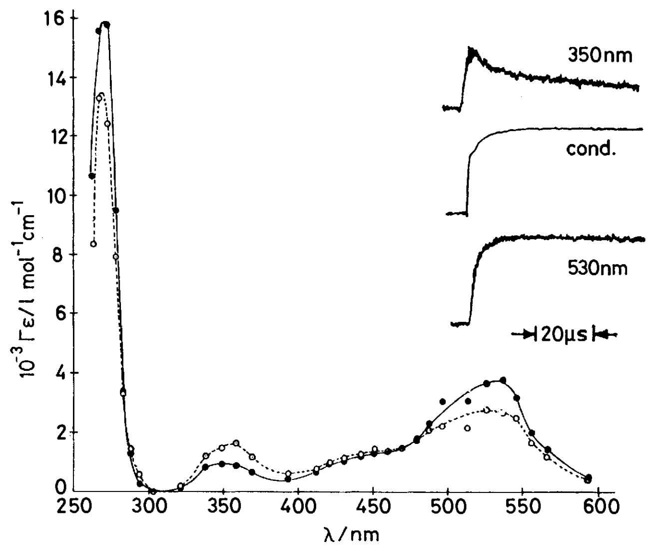


FIGURE 1 Spectra taken 1 μs (○) and 20 μs (●) after pulse irradiation of an N_2O-saturated solutions containing 5 × 10^{-5}M-metiazinic acid at pH 10. Inserts: optical and conductivity changes observed in the same system

With a radiation dose of 3 J kg^{-1} and a metiazinic acid concentration of 10^{-4}M the increase in absorption immediately and 20 μs after the pulse corresponded, respectively, to $\Sigma\Gamma_x\varepsilon_x$ 2 800 ± 100 and 3 600 ± 100 at 530 nm and 12 400 ± 500 and 15 700 ± 500 at 270 nm. The respective changes in conductance were $\Sigma\Gamma_x\Lambda_x$ 44 ± 2 and 55 ± 2.

(3) *Bromide, Thiocyanate, or Carbonate Solutions.*—On pulse radiolysis of N_2O-saturated solutions of metiazinic acid (10—100 μM) and excess potassium bromide, potassium thiocyanate, or sodium carbonate (0.02, 0.1, or 1M, respectively) strong transient absorptions at 360, 460, and 600 nm, respectively, were observed immediately after the radiation pulse.[24,25] These subsequently decayed exponentially and a strong visible absorption with broad similarities to that observed in the absence of these inorganic salts appeared simultaneously. This is demonstrated in the inserts of Figure 3 for thiocyanate-containing solutions. The changes in absorptions, measured at 460 and 270 nm, occur exponentially with the first half-lives being inversely proportional to the MZ concentration. Absolute bimolecular rate constants were derived from the slopes of appropriate kinetic plots as is shown for the reaction of $(SCN)_2^{-\bullet}$ with MZ in Figure 4. The kinetic data are summarized in the Table. Also listed in the Table are the intensities of the absorptions at the 530 and 270 nm maxima expressed in terms of $\Sigma\Gamma_x\varepsilon_x$.

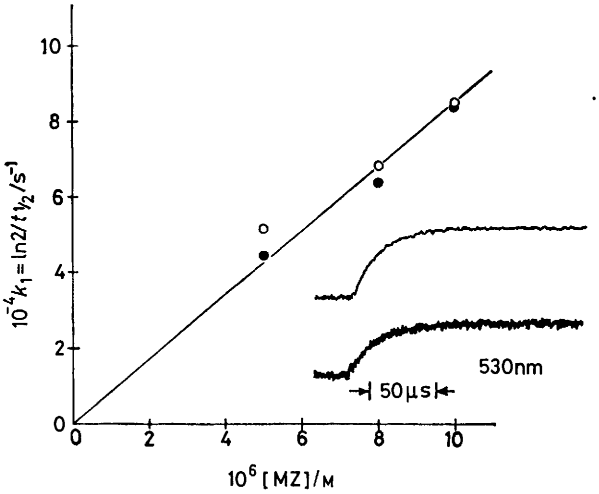


FIGURE 2 Plot of first-order rate constant k_1 obtained from the initial increase in conductivity (○) and at 530 nm (●) against MZ concentration in N_2O-saturated solutions at pH 10. Inserts: conductivity and optical changes at 530 nm, observed in a solution containing 5 × 10^{-6}M-MZ; radiation dose 1.3 J kg^{-1}

(4) *Thallium*(II) *Sulphate, Sodium Azide, or Ethylene Glycol Solutions.*—On pulse radiolysis of N_2O-saturated solutions containing an excess of thallium(II) sulphate (3mM), sodium azide (10mM), or ethylene glycol (1M) and metiazinic acid little change in absorption in the region 300—650 nm was observed immediately after the radiation pulse. However, absorptions broadly similar to those previously observed in the presence of excess thiocyanate, carbonate, and bromide grew subsequently in an exponential manner. The first-order rate constants varied linearly with MZ concentration and bimolecular rate constants were derived from the slopes of the kinetic plots. An example is shown for the reaction of $N_3^\bullet$ with MZ in Figure 4. The respective values and the maximum intensities at the absorption maxima are recorded in the Table.

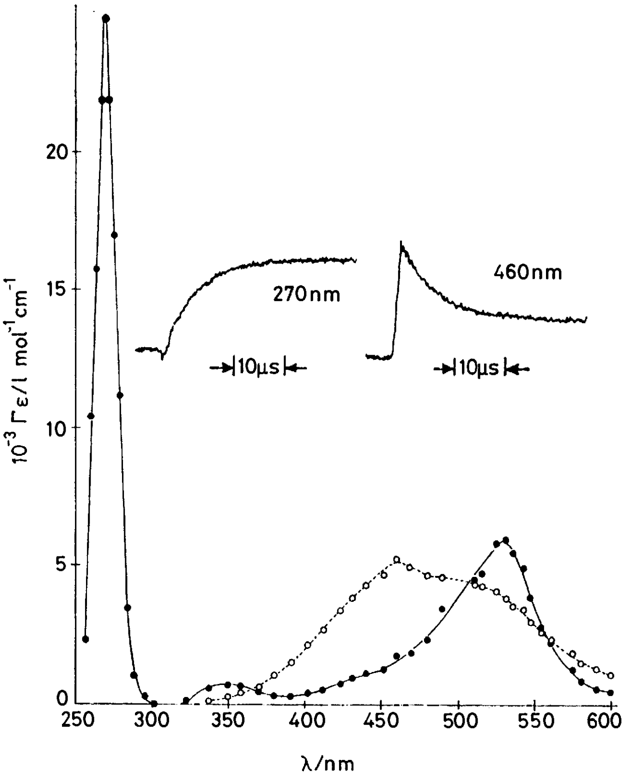


FIGURE 3 Spectra obtained 1 μs (○) and 20 μs (●) after pulse irradiation of an N_2O-saturated solution containing 0.1M-KSCN and 5 × 10^{-5}M-MZ at pH 10. Inserts: optical traces at 270 and 460 nm

(5) *Dimethyl Sulphide or Dimethyl Disulphide Solutions.*—On pulse radiolysis of N_2O-saturated solutions containing excess dimethyl sulphide or disulphide (1mM) and metiazinic acid transient absorptions ($\lambda_{max.}$ 480 and 420 nm,

respectively) previously observed in the absence of metiazinic acid and assigned to sulphur-centred radical cations [26, 27] were apparent immediately after the pulse. These subsequently decayed exponentially and a new absorption again broadly similar to that observed in the presence of excess thiocyanate formed simultaneously. This is shown for the dimethyl sulphide solutions at 450 and 270 nm in Figure 5. The rates of decay and formation of the respective absorptions were similar, *i.e.* second-order rate constants were calculated from the linear first-order rate constants against metiazinic acid concentration plots. The values are listed in the Table.

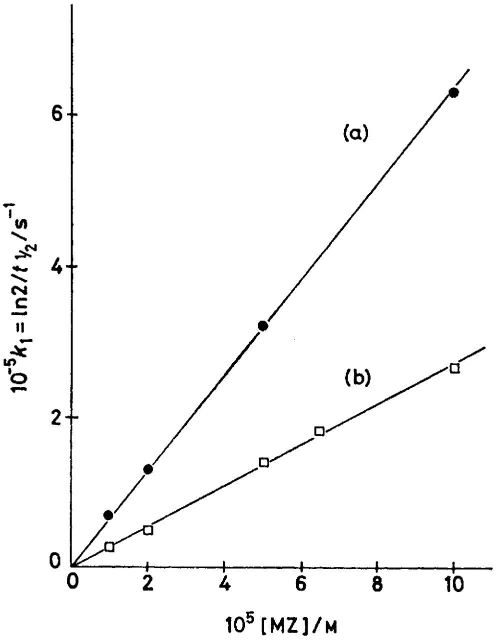


FIGURE 4 Plots of the mean values of the first-order rate constants k_1 against MZ concentration obtained for the increase in absorption of $MZ^{\pm\bullet}$ (270 and 530 nm) for the reaction $N_3^{\bullet} + MZ^- \longrightarrow MZ^{\pm\bullet} + N_3^-$ (●) and for the build-up at 270 nm and the decay at 460 nm for the reaction $(SCN)_2^{-\bullet} + MZ^- \longrightarrow MZ^{\pm\bullet} + 2SCN^-$ (□). Solutions: N_2O-saturated, pH 10 (a) 0.01M-NaN_3; (b) 0.1M-KSCN

(6) *Lipoate Solutions containing Excess Bromide.*—On pulse radiolysis of N_2O-saturated solutions (pH 10) containing potassium bromide (0.02M), lipoic acid (10^{-4}M), and metiazinic acid (1×10^{-5}M) a transient absorption ($\lambda_{max.}$ 420 nm), similar to that previously observed in the absence of the phenothiazine was apparent at the end of the 1 µs

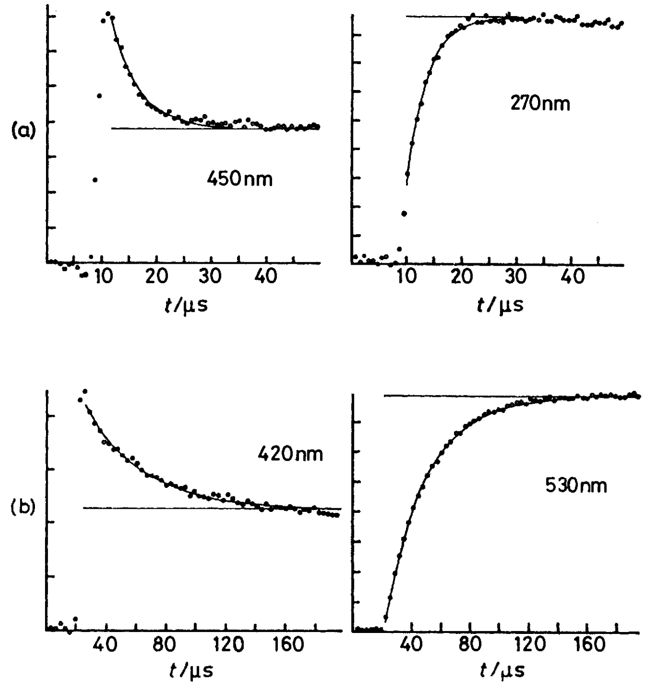


FIGURE 5 Electron transfer to organic radicals. Changes in optical absorptions with time involving a first-order fit. Solutions: N_2O-saturated, pH 10: (*a*) 1×10^{-3}M-CH_3SCH_3, 5×10^{-5}M-MZ; (*b*) 0.02M-KBr, 1×10^{-4}M-lipoic acid, 1×10^{-5}M-MZ

Rate constants and yields of $MZ^{+\bullet}$ zwitterion radicals (expressed in terms of $\Gamma\varepsilon$ and percentage relative to the yield of the oxidant)

Oxidizing agent X	$k(X + MZ^-)$/ l mol^{-1} s^{-1}	Observation at λ/nm	$\Gamma\epsilon$/ l mol^{-1} cm^{-1}	$MZ^{\pm\bullet}$ formation (%)
$Br_2^{-\bullet}$	3.4×10^9	529	5 100	84
		270	15 700	
$(SCN)_2^{-\bullet}$	2.6×10^9	525	5 900	97
		273	21 700	
$CO_3^{-\bullet}$	3.2×10^9	527	6 200	100
		273	20 600	
$Tl(OH)_2^{\bullet}$	2.5×10^9	522	4 300	71
		271	15 100	
$N_3^{\bullet}$	6.4×10^9	522	6 200	100
		274	21 200	
$\dot{C}H_2CHO$	0.2×10^9	529	3 100	51
$(CH_3SCH_3)_2^{+\bullet}$	5.9×10^9	529	3 800	78 [a]
		273	15 200	
$CH_3SSCH_3^{+\bullet}$	5.0×10^9	531	4 100	100 [b]
		278	11 300	
Lipoate$^{+\bullet}$	3.3×10^9	529	6 100	100
		274	20 900	
$OH^{\bullet}$	8.4×10^9	530	3 600 [c]	59 [c]
		270	15 700 [c]	

[a] $(CH_3SCH_3)_2^{+\bullet}$ is formed with only 80% efficiency from $OH^{\bullet}$ under experimental conditions ([CH_3SCH_3] 1mM).[26] [b] $CH_3SSCH_3^{+\bullet}$ is formed with only 45% efficiency from $OH^{\bullet}$ radicals.[27] The fact, however, that the yield of $MZ^{\pm\bullet}$ is higher than 45% of the primary, available $OH^{\bullet}$ suggests that other radicals formed during the primary attack of $OH^{\bullet}$ on dimethyl disulphide exhibit oxidizing properties. [c] Measured 20 µs after the pulse; values include $MZ^{+\bullet}$ formation through reactions (5a) and (6).

pulse. This subsequently decayed and a new absorption ($\lambda_{max.}$ 530 and 270 nm), similar to that observed in solutions in the absence of lipoic acid, grew in simultaneously. Two absorption–time curves recorded at 420 and 530 nm are shown in Figure 5. The rate of both the appearance and the decay of these absorptions varied in a first-order manner with the metiazinic acid concentration. The slopes of the kinetic plots, *i.e.* the bimolecular rate constants and the maximum intensities are also given in the Table.

(7) *Ground State Absorption of Metiazinic Acid.*—The u.v.-visible absorption spectrum of a solution of metiazinic acid (5×10^{-5}M; pH 10) was measured using a Variscan spectrophotometer and a 1 cm cell. Absorption maxima were observed at 305 nm ($\varepsilon_{max.}$ 3 800 l mol^{-1} cm^{-1}) and in the u.v. ($\leqslant$ 250 nm). The extinction coefficient at 270 nm was 1 800 l mol^{-1} cm^{-1}. There was no absorption in the visible region.

DISCUSSION

It is now well established that any rapid chemical changes occurring after nanosecond or microsecond pulse radiolysis of dilute aqueous solutions are due principally to solvated electrons, hydroxyl radicals, and protons formed within the pulse, each in a yield of *ca.* 0.3 μmol per joule of energy absorbed by the solution.

$$\begin{array}{lccccc} H_2O \xrightarrow{1J} & e^-_{aq} \; + & OH^\bullet \; + & H_3O^+ \; + & H^\bullet & (2) \\ & 0{\cdot}26 & 0{\cdot}29 & 0{\cdot}26 & 0{\cdot}06 & \\ & \mu mol & \mu mol & \mu mol & \mu mol & \end{array}$$

Nitrous oxide reacts rapidly with hydrated electrons to yield additional hydroxyl radicals. In this process also hydroxide ions are formed which immediately interact with the H_3O^+ formed during the pulse (k_3 8.7×10^9 l mol^{-1} s^{-1}). In N_2O-saturated systems containing MZ^- in

$$e_{aq}^- + N_2O \longrightarrow OH^\bullet + OH^- + N_2 \qquad (3)$$

$$OH^- + H_3O^+ \longrightarrow 2H_2O \qquad (4)$$

a concentration much lower than the saturation concentration of N_2O (*ca.* 20mM) any changes observed can quantitatively be attributed to the reaction of $OH^\bullet$ radicals with metiazinic acid, the rate constant of which (k 8.4×10^9 l mol^{-1} s^{-1}) indicates a fast and practically diffusion-controlled process. The rapid increase in conductance occurring simultaneously with the appearance of the 270 and 530 nm absorption bands in such solutions suggests that an ion pair is formed in the electron transfer reaction (5a). The radical $MZ^{\pm\bullet}$ is effectively a zwitterion-radical with the negative charge on the carboxy-group

$$MZ^- + OH^\bullet \longrightarrow MZ^{\pm\bullet} + OH^- \qquad (5a)$$

and the positive charge most likely delocalized between the sulphur and the nitrogen atom of the phenothiazine ring.[4] Since the zwitterion radical, $MZ^{\pm\bullet}$, does not contribute to any change in conductivity the yield of the increase in conductance amounts to $\Sigma\Gamma_x\Lambda_x = -\Gamma_{MZ^-}\Lambda_{MZ^-} + \Gamma_{OH^-}\Lambda_{OH^-} = 44 \pm 2$. Anions of the size of MZ^- typically exhibit equivalent conductances Λ of 25 Ω^{-1} cm^2 (at 25 °C),[28] and for the hydroxide ion $\Lambda_{25°C}$ 197 Ω^{-1} cm^2 has been determined.[28] Since $\Gamma_{MZ^-} = \Gamma_{OH^-}$, $44 = -\Gamma_{OH^-}25 + \Gamma_{OH^-}197$, *i.e.* $\Gamma_{OH^-} = 44/172 = 0.26 \pm 0.01$ μmol J^{-1}.

In N_2O-saturated systems it is generally accepted that $\Gamma_{OH^\cdot} = 0.55$ μmol J^{-1}. Thus only 47% of the primary available $OH^\bullet$ react by this electron transfer mechanism. The absorption band present after 1 μs at 350 nm is, at least partially, attributed to the $OH^\bullet$ radical adduct formed in reaction (5b). Hydrogen atom abstraction (5c)

$$MZ^- + OH^\bullet \longrightarrow MZ^-(OH)^\bullet \qquad (5b)$$

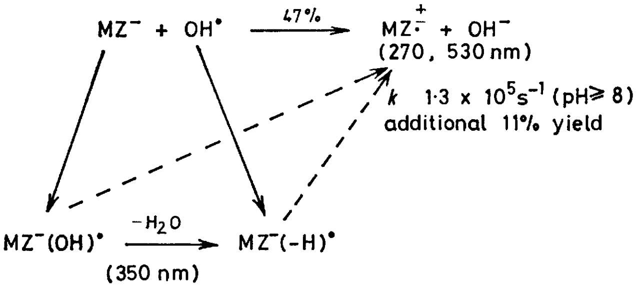
$$MZ^- + OH^\bullet \xrightarrow{-H_2O} MZ^-(-H)^\bullet \qquad (5c)$$

has, however, also to be taken into consideration. A subsequent first-order decay *via* process (6) then yields

$$\left.\begin{array}{l} MZ^-(OH)^\bullet \\ MZ^-(-H)^\bullet \end{array}\right\} MZ^{\pm\bullet} + OH^- \qquad (6)$$

additional radical zwitterions The rate constant k_6 1.3×10^5 s^{-1}, did not change within the accessible pH range 8—11. The total yield of $MZ^{\pm\bullet}$ determined from $\Sigma\Gamma_x\Lambda_x$ after completion of reactions (5a) and (6) amounts to $\Gamma(MZ^{\pm\bullet})$ 0.32 ± 0.01 which corresponds to 58% of the initially formed $OH^\bullet$ radicals. It is suggested that the remaining 42% of the hydroxyl radicals either undergo abstraction reactions or *via* addition end up in other channels. The formation of radical cations *via* different pathways has also been reported for the $OH^\bullet$ radical induced oxidation of *NN*-dimethylaniline[29a] and *NN'*-tetramethyl-*p*-phenylenediamine (TMPD).[29b] Specifically, it has been suggested that the indirect route may involve a $\geqslant N-\dot{C}H_2$ radical. A detailed mechanism could, however, not be provided. Such a radical [denoted as $MZ^-(-H)^\bullet$] could also be envisaged for metiazinic acid.

The overall reaction process for the $OH^\bullet$ radical reaction with metiazinic acid shown in the Scheme accounts for the observations. Since $MZ^-(-H)^\bullet$ may have an absorption similar to that of the $OH^\bullet$ addition radical $MZ^-(OH)^\bullet$, and furthermore in principle could be formed *via* the $OH^\bullet$ adduct as well as by direct hydrogen atom abstraction, it is not possible to assign quantitative yields and extinction coefficients to these species.



SCHEME

The u.v. absorption of $MZ^{\pm\bullet}$ is characterized by an extremely strong and relatively narrow band at 270 nm. Assuming that all the hydroxyl radicals which react with MZ^- lead to a loss of one equivalent of ground state absorption (ε270 nm 1 800 l mol^{-1} cm^{-1}) means that the observed absorption changes after 1 μs correspond to:

$$\varepsilon_{MZ^{\pm\cdot}} = \frac{\Gamma_{OH^{\cdot}}\varepsilon_{270\,nm} + (\Gamma\varepsilon)_{observed}}{\Gamma_{obs.,\,cond.}} = \frac{(1\,800 \times 0.55) + (12\,400 \pm 500)}{(0.26 \pm 0.01)}$$

$$\varepsilon_{MZ^{\pm\cdot}} = 51\,500 \pm 2\,000 \text{ l mol}^{-1}\text{ cm}^{-1} \text{ at } 270 \text{ nm}$$

A similar value is obtained using the data after 20 μs, *i.e.* when additional $MZ^{\pm\bullet}$ is formed *via* the $OH^{\bullet}$ radical adduct in reaction (6). The absorptions at 530 nm correspond to $\Sigma\Gamma_x\varepsilon_x$ 2 800 ± 100 and 3 600 ± 150 after 1 and 20 μs, respectively. Assuming that this absorption band is solely due to $MZ^{+\bullet}$ then $\varepsilon^{MZ^{+\bullet}} = 11\,000 \pm 500$ l mol^{-1} cm^{-1} at 530 nm.

Confirmatory evidence that the absorption bands at 530 and 270 nm are due to $MZ^{\pm\bullet}$ is provided by the results of the studies undertaken with solutions containing excess bromide, thiocyanate, carbonate, azide, or thallium(I) ions or ethylene glycol, dimethyl sulphide, dimethyl disulphide, or lipoate, where no secondary processes as in reaction (6) are occurring.

The strong visible absorptions seen initially in solutions containing bromide, thiocyanate, carbonate, $(CH_3)_2S$, or CH_3SSCH_3 are attributed to $Br_2^{-\bullet}$, $(SCN)_2^{-\bullet}$, $CO_3^{-\bullet}$, $[(CH_3)_2S]_2^{+\bullet}$, and $CH_3SSCH_3^{+\bullet}$, respectively,[24–27] formed according to reactions (7)—(11).

$$OH^{\bullet} + Br^{-} \longrightarrow Br^{\bullet} + OH^{-} \tag{7a}$$

$$Br^{\bullet} + Br^{-} \longrightarrow Br_2^{-\bullet}\ (\lambda_{max.}\ 360\text{ nm}) \tag{7b}$$

$$OH^{\bullet} + SCN^{-} \longrightarrow SCN^{\bullet} + OH^{-} \tag{8a}$$

$$SCN^{\bullet} + SCN^{-} \longrightarrow (SCN)_2^{-\bullet}\ (\lambda_{max.}\ 480\text{ nm}) \tag{8b}$$

$$OH^{\bullet} + CO_3^{2-} \longrightarrow CO_3^{-\bullet} + OH^{-}\ (\lambda_{max.}\ 600\text{ nm}) \tag{9}$$

$$OH^{\bullet} + (CH_3)_2S \longrightarrow (CH_3)_2\dot{S}OH \tag{10a}$$

$$(CH_3)_2\dot{S}(OH) + (CH_3)_2S \longrightarrow ((CH_3)_2S)_2(OH)^{\bullet} \tag{10b}$$

$$((CH_3)_2S)_2(OH)^{\bullet} \longrightarrow OH^{-} + ((CH_3)_2S)^{+\bullet}\ (\lambda_{max.}\ 480\text{ nm}) \tag{10c}$$

$$((CH_3)_2S)_2^{+\bullet} \rightleftharpoons (CH_3)_2S^{+\bullet} + (CH_3)_2S \tag{10d}$$

$$CH_3SSCH_3 + OH^{\bullet} \longrightarrow CH_3SSCH_3^{+\bullet} + OH^{-}\ (\lambda_{max.}\ 420\text{ nm}) \tag{11}$$

These radicals then react with MZ^{-} to form $MZ^{\pm\bullet}$ with the rate constants and yields given in the Table. The respective reactions are (12)—(16).

$$Br_2^{-\bullet} + MZ^{-} \longrightarrow 2Br^{-} + MZ^{\pm\bullet} \tag{12}$$

$$(SCN)_2^{-\bullet} + MZ^{-} \longrightarrow 2SCN^{-} + MZ^{\pm\bullet} \tag{13}$$

$$CO_3^{-\bullet} + MZ^{-} \longrightarrow CO_3^{2-} + MZ^{\pm\bullet} \tag{14}$$

$$((CH_3)_2S)_2^{+\bullet} + MZ^{-} \longrightarrow 2(CH_3)_2S + MZ^{\pm\bullet} \tag{15a}$$

$$(CH_3)_2S^{+\bullet} + MZ^{-} \longrightarrow (CH_3)_2S + MZ^{\pm\bullet} \tag{15b}$$

$$CH_3SSCH_3^{+\bullet} + MZ^{-} \longrightarrow CH_3SSCH_3 + MZ^{\pm\bullet} \tag{16}$$

In case of the sulphide solutions the high rate constant (5.9 × 10^{9} l mol^{-1} s^{-1}) indicates that the complex radical cation $(CH_3SCH_3)_2^{+\bullet}$ can oxidize metiazinic acid directly. However, oxidation of MZ^{-} through the molecular radical cation $CH_3SCH_3^{+\bullet}$ which is in equilibrium with the complexed species,[26] may contribute to the overall process. For the dimethyl disulphide solutions it has to be taken into account that reaction (11) accounts only for *ca.* 45% of the $OH^{\bullet}$ radicals (the remainder leads to nonionic products).[27]

When both lipoate and bromide are present first the lipoate radical cation is formed and this then reacts with MZ^{-} [reaction (17)]. The lipoate oxidation by $Br_2^{\bullet-}$

$$Br_2^{-\bullet} + \text{lipoate} \longrightarrow 2Br^{-} + \text{lipoate}^{+\bullet} \tag{17a}$$

$$\text{lipoate}^{+\bullet} + MZ^{-} \longrightarrow \text{lipoate} + MZ^{\pm\bullet} \tag{17b}$$

occurs with k_{17a} 3.9 × 10^{9} l mol^{-1} s^{-1} and seems to be almost quantitative, based on an extinction coefficient of lipoate$^{\bullet+}$ of *ca.* 2 × 10^{3} l mol^{-1} cm^{-1}.[30] The slow exponential formation of the absorptions at 530 nm in solutions containing azide, or thallium(II) ions, or ethylene glycol are similarly attributed to the reactions of $N_3^{\bullet}$, $Tl(OH)_2^{\bullet}$, and $\dot{C}H_2CHO$ which exhibit no or too small absorptions in the visible region.[31–33]

$$OH^{\bullet} + N_3^{-} \longrightarrow N_3^{\bullet} + OH^{-} \tag{18a}$$

$$N_3^{\bullet} + MZ^{-} \longrightarrow MZ^{\pm\bullet} + N_3^{-} \tag{18b}$$

$$OH^{\bullet} + Tl^{+} \longrightarrow Tl^{2+} + OH^{-} \tag{19a}$$

$$Tl^{2+} + 2OH^{-} \longrightarrow Tl(OH)_2^{\bullet} \tag{19b}$$

$$Tl(OH)_2^{\bullet} + MZ^{-} \longrightarrow MZ^{\pm\bullet} + Tl^{+} + 2OH^{-} \tag{19c}$$

$$OH^{\bullet} + HOCH_2CH_2OH \longrightarrow HO\dot{C}HCH_2OH + H_2O \tag{20a}$$

$$HO\dot{C}HCH_2OH + OH^{-} \rightleftharpoons {}^{-}O\dot{C}HCH_2OH + H_2O \tag{20b}$$

$${}^{-}O\dot{C}HCH_2OH \longrightarrow \dot{C}H_2CHO + OH^{-} \tag{20c}$$

$$\dot{C}H_2CHO + MZ^{-} \longrightarrow CH_2CHO^{-} + MZ^{\pm\bullet} \tag{20d}$$

The results show that metiazinic acid reacts readily with a large variety of oxidants. A practically quantitative formation of $MZ^{\pm\bullet}$ zwitterion radicals occurs in the oxidation of metiazinic acid by $(SCN)_2^{-\bullet}$, $CO_3^{-\bullet}$, $N_3^{\bullet}$, $CH_3SSCH_3^{+\bullet}$, and lipoate$^{+\bullet}$ radicals. The other oxidants react apparently less selectively and additional products are formed. A correlation between rate constants and $MZ^{\pm\bullet}$ yields is, however, not observed. This is not surprising, since all reactions occur with rate constants at or close to the diffusion limit, and particular selectivity is usually only observed at much lower rates.

It is interesting to compare our present results with the radical-induced oxidation of some other sulphur-containing compounds, particularly sulphides and disulphides. In the oxidation of R_2S by $Br_2^{-\bullet}$, for example, an intermediate $R_2S \therefore Br$ radical was observed.[34] No such stabilizing sulphur–halide interaction could be detected with metiazinic acid, probably because of the strong intramolecular resonance stabilization of the $MZ^{\pm\bullet}$ radical.

In the oxidation of disulphides it was noticed that the reaction of $OH^{\bullet}$ radicals leads to $RSSR^{+\bullet}$ radical cations with *ca.* 40—45% efficiency,[27] similar to the metiazinic acid system. Significantly different results are, however, obtained for the radical cation formation from metiazinic acid and disulphides, respectively, through other oxidants.[35] Thus $RSSR^{+\bullet}$ radical cations are formed with 100% efficiency by a large number of oxidants, including $(CH_3SCH_3)_2^{+\bullet}$ and $Br_2^{-\bullet}$ while $MZ^{\pm\bullet}$ formation through these oxidants is less efficient. $CO_3^{-\bullet}$ and $Tl(OH)_2$ which give appreciable yields of $MZ^{\pm\bullet}$, on the other hand, in their reaction with a disulphide yielded practically no $RSSR^{+\bullet}$ radical cations. In this respect it is also interesting to see how good an oxidant $Tl(OH)_2$, in particular, is towards metiazinic acid since in the presence of tetranitromethane $Tl(OH)_2$ has been found to even act as a mild reductant.[32c]

Metiazinic acid clearly undergoes fast reactions with oxidizing free radicals. Whether these reactions are responsible for the drug's reported anti-inflammatory properties remains, of course, a matter of debate. Previous studies have shown that the drug is unlikely to act by scavenging superoxide radicals.[3] The high rate constant determined for the reaction of $OH^{\bullet}$ would therefore be in agreement with the current ideas that many biological effects hitherto attributed to $O_2^{-\bullet}$ are more likely due to the reactions of $OH^{\bullet}$ or other electrophilic radicals.

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