Kinetics, spectral and redox behaviour of OH adducts of methylxanthines: a radiation chemical study[1]

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Received (in Cambridge) 23rd September 1998, Accepted 14th December 1998

The reactions of $^{\bullet}OH$, $O^{\bullet-}$ and $SO_4^{\bullet-}$ with mono- (MX), di- (DMX) and tri- (TMX) methylxanthines were investigated by pulse radiolysis. The second order rate constants for the $^{\bullet}OH$ reaction range from 8.5×10^9 dm^3 mol^{-1} s^{-1} for 1,3,7-TMX to 2.4×10^9 dm^3 mol^{-1} s^{-1} for 1-MX, the order being 1,3,7-TMX > 1,3-DMX ≈ 3,7-DMX ≈ 7-MX > 3-MX > 1-MX. The second order rate constants ($k \sim 3.2$–4.5×10^9 dm^3 mol^{-1} s^{-1}) for the $SO_4^{\bullet-}$ reaction are comparable to those obtained in the $^{\bullet}OH$ reaction which are considerably reduced in the case of $O^{\bullet-}$ reaction ($k = 0.33$–1×10^9 dm^3 mol^{-1} s^{-1}). The transient absorption spectra of the OH adducts of methylxanthines (X–4OH$^{\bullet}$ and X–8OH$^{\bullet}$) have exhibited maxima at 320–330 nm and a broad peak around 490 nm whereas the latter peak was not observed in the spectra obtained in the $O^{\bullet-}$ and $SO_4^{\bullet-}$ reactions. The rates of decay of absorption around 500 nm due to the dehydration of the X–4OH$^{\bullet}$ adducts are in the range 1.5–4 × 10^4 s^{-1}. In contrast to the behaviour observed with 1,3,7-TMX, the X–8OH$^{\bullet}$ adducts of mono- and dimethylxanthines undergo ring opening with $k = (4$–$11) \times 10^4$ s^{-1}. The addition of O_2 ($N_2O:O_2$ (4 : 1 v/v)) to the X–4OH$^{\bullet}$ adduct of methylxanthines ($k = 1 \times 10^9$ dm^3 mol^{-1} s^{-1}) is much more effective than its dehydration. While O_2 addition predominates over the ring opening in 1-MX, 3-MX and 1,3-DMX, the X–8OH$^{\bullet}$ adducts of 7-MX and 3,7-DMX were found to be relatively unreactive.

Introduction

Studies on free radical chemistry of nucleic acid components by radiation chemical methods have significantly contributed to our understanding of the chemical basis of radiation induced DNA damage (see references 2–5 for recent reviews). The work on the reactions of oxidising[6–9] (*e.g.* $^{\bullet}OH$, $SO_4^{\bullet-}$) and reducing[10,11] (*e.g.* e^-_{aq}, $H^{\bullet}$, $CH_3{}^{\bullet}CHOH$, $^{\bullet}CH_2(CH_3)_2COH$) radicals with nucleobases[5–7] has been particularly significant. Though the rates for the reactions of both e^-_{aq} and $^{\bullet}OH$ with pyrimidines and purines are, in general, diffusion-controlled,[12] a variation in the rates for the $^{\bullet}OH$ reaction by more than an order of magnitude was reported by Steenken and co-workers[7] in the case of C(6) and N(9) substituted purines with $k = 3 \times 10^8$ for purine to 8.4×10^9 dm^3 mol^{-1} s^{-1} for *N*^{6},*N*^{6},9-trimethyladenine.

The $^{\bullet}OH$ reaction pattern in purines has also been established by the pioneering work of Steenken's group.[7] It has been demonstrated by them that $^{\bullet}OH$ reacts mainly by addition to C(4) and C(8) of these compounds forming the corresponding isomeric OH adducts: A–4OH$^{\bullet}$ and A-8OH$^{\bullet}$ (A = adenine). In contrast to pyrimidines,[12] these radical adducts were shown to undergo unimolecular transformation reactions of dehydration of A–4OH$^{\bullet}$ and ring opening of A–8OH$^{\bullet}$. Further, these transformation reactions were characterised by the spectral changes: the first order increase in absorption around 360 nm was attributed to the opening of the imidazole ring while the decrease in absorption in the higher wavelength region (400–480 nm) was assigned to OH^- elimination. Furthermore, it has been reported[7] that the transformation rates are influenced by the nature of the substituents at the C(6) and N(9) positions. The rate of the dehydration reaction was relatively more influenced by the substituent than that found for the ring opening. Its rate was increased by electron donating substituents at C(6) whereas a decrease was seen if the N(9) position had an electron withdrawing group. For example, in the case of *N*^{6},*N*^{6},9-trimethyladenine—the fully alkylated adenine—the *k* values for the OH^- elimination and ring opening reactions are 2.2×10^6 and 2.3×10^5 s^{-1} respectively while the corresponding rates for *N*^{6},*N*^{6}-dimethyladenosine are 4.2×10^5 and 9.5×10^4 s^{-1}. This variation has been explained on the basis of different contributions of the enthalpic and entropic factors to the dehydration and ring opening reactions.

Methylxanthines[13,14] (Table 1) represent an interesting class of non-DNA purines for the study of their reaction with oxidising and reducing radicals to examine the effect of the substituents on the transformation rates of the OH adducts formed. Studies[15,16] on radiation chemical reactions with xanthine (purine-2,6(1*H*,3*H*)-dione) derivatives have been limited. Guanine derivatives also have an oxygen attached to C(6) as in xanthines. The rates of transformation in the OH adducts of many xanthines and guanine derivatives are still unknown. Our work on the reactions of $^{\bullet}OH$, $O^{\bullet-}$ and $SO_4^{\bullet-}$ with 1,3,7-trimethylxanthine (1,3,7-TMX) has recently been published.[17a] We have reported earlier[17b,c] our results on the reactions of e^-_{aq} with di- and trimethylxanthines.

It has also been demonstrated[17] by us that the addition of $^{\bullet}OH$ to 1,3,7-TMX leads to the formation of both X–4OH$^{\bullet}$ and X–8OH$^{\bullet}$ (X = xanthine) isomeric radical adducts as was seen[7] with adenine and its derivatives. While the first order decay in absorption around 490 nm due to OH^- elimination from the X–4OH$^{\bullet}$ of 1,3,7-TMX was confirmed by both optical and conductance detection, the absence of a delayed increase in absorption at 360 nm even on longer timescales (~100 µs) was attributed to the lack of ring opening in the X–8OH$^{\bullet}$ adduct. This is in contrast to the behaviour observed[16] with its isomer 1,3,9-TMX where a reverse trend was observed, *i.e.* the X–8OH$^{\bullet}$ adduct underwent a unimolecular transformation due to the ring opening but the X–4OH$^{\bullet}$ adduct, even if it was formed, did not undergo dehydroxylation.

In this study, we report our results obtained from pulse radi-

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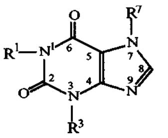
Table 1 Second order rate constants[a] ($k/10^9$ dm^3 mol^{-1} s^{-1}) obtained in the reactions of $^{\bullet}$OH, $O^{\bullet-}$ and $SO_4^{\bullet-}$ with methylxanthines

		$^{\bullet}$OH		$SO_4^{\bullet-}$		
	σ^{+b}	pH 7	pH ~ 13[c]	pH 3	pH 6	pH 10
1,3,7-Trimethylxanthine	−1.80	8.5	1.0	3.7	4.5	4.3
1,3-Dimethylxanthine (8.5)	−1.50	5.0	0.69	1.8	3.5	4.2
3,7-Dimethylxanthine (11.0)	−1.48	5.5	0.33	3.5	3.2	2.6
1-Methylxanthine (7.9, 11.8)	−0.85	2.4	0.56	nd	3.8	2.6
3-Methylxanthine (8.5, 11.5)	−1.40	4.0	0.77	2.4	3.4	3.1
7-Methylxanthine (8.4, 10.5)	−1.52	5.7	0.83	3.0	3.6	3.5

[a] The rate constant values are accurate to ±15%. [b] These values were determined from the data reported[7a] for substituted purines (see text for discussion). Values in parentheses are the pK_a values (reference 13). [c] At this pH, $O^{\bullet-}$ is the reacting species.

olysis on the kinetics, spectral and redox behaviour of the OH adducts of mono- and dimethylxanthines. In contrast to the behaviour observed with 1,3,7-TMX,[17a] mono- and dimethylxanthines undergo a ring opening reaction and the rates of transformation reactions are characteristic of the compound. The redox behaviour of the OH adducts and their yields are dependent on the structures of these derivatives. The present study demonstrates the effect of the number and the position of the methyl groups on the reactivity pattern of the OH radical in these systems.

Experimental

The methyl derivatives of xanthine (Sigma) and other chemicals (Qualigens) used in this study were of high purity and were used as received. The solutions were prepared in water obtained from the Millipore Milli-Q purification system. The reaction of the OH radicals was studied in N_2O saturated aqueous solutions of the solute (10^{-3} mol dm^{-3}) where e^-_{aq} is converted into $^{\bullet}$OH (reactions (1) and (2)), $N_2O:O_2$ (4:1 v/v) saturated solutions

$$H_2O \rightsquigarrow e^-_{aq}, {}^{\bullet}OH, {}^{\bullet}H, H^+, H_2O_2, H_2 \quad (1)$$

$$e^-_{aq} + N_2O \longrightarrow {}^{\bullet}OH + OH^- + N_2 \quad (2)$$

were used to record the OH adduct spectra in the presence of O_2. The reaction of $O^{\bullet-}$ was studied at pH ⩾ 13 where the radical anion is essentially the reacting species ($^{\bullet}OH \rightleftharpoons O^{\bullet-} + H^+$; p$K_a$ = 11.9).

The reaction of $SO_4^{\bullet-}$ was studied in N_2 saturated aqueous solutions of the xanthine derivatives (10^{-3} mol dm^{-3}) containing $K_2S_2O_8$ (1.5×10^{-2} mol dm^{-3}) and 0.2 mol dm^{-3} *tert*-butyl alcohol. The OH radicals are scavenged by *tert*-butyl alcohol while e^-_{aq} reacts quantitatively with $S_2O_8^{2-}$ to produce the $SO_4^{\bullet-}$ radical ion (reactions (3) and (4)).

$${}^{\bullet}OH + (CH_3)_3COH \longrightarrow H_2O + {}^{\bullet}CH_2(CH_3)_2COH \quad (3)$$

$$S_2O_8^{2-} + e^-_{aq}(H) \longrightarrow SO_4^{\bullet-} + SO_4^{2-}(HSO_4^-) \quad (4)$$

Pulse radiolysis experiments with optical detection were carried out using 7 MeV electron pulses (pulse width 50 ns) from a linear accelerator at the Bhabha Atomic Research Centre, Mumbai as described previously.[18] A KSCN dosimeter was used in the optical pulse radiolysis. The dose per pulse was between 10–15 Gy.

$SO_4^{\bullet-}$ was also produced using the laser flash photolysis technique[19] to obtain the transient absorption spectra for the reaction of $SO_4^{\bullet-}$ with 3,7-MX. The experiments were carried out using an in-house built set-up at the Tata Institute of Fundamental Research, Mumbai. An excimer laser with a KrF fill (λ = 248 nm) was used for the excitation and a xenon lamp as the monitoring light source. The signal was processed by a Biomation 8100 digitizer connected to a minicomputer. The signal-to-noise ratio was improved by averaging several traces. An N_2 saturated solution of the reactants was flowed through a quartz cuvette of 1 cm path length in order to avoid accumulation of reaction products during the measurements.

Results

Evaluation of rate constants

Reaction of $^{\bullet}$OH. The second order rate constants for the reaction of $^{\bullet}$OH were obtained from the dependence of k_{obs} on [solute] in the range (0.2–1) × 10^{-3} mol dm^{-3} at neutral pH and are tabulated in Table 1. A typical trace for the case of 1,3-DMX depicting the increase in absorption at 335 nm is given in Fig. 1A.

In order to quantify the substituent effect on reaction rates in terms of σ^+ values, we used a normalisation procedure by superimposing our data on the Hammett plots obtained for C(6) substituted purines by Vieira and Steenken.[7a] This analysis seems to be justified as the OH radical also adds to the C(4) and C(8) positions of methylxanthines. A ρ^+ value of −0.59 was obtained from our data (Fig. 2) which is lower than the value ($\rho^+ = -1.0$) reported[7a] for adenine derivatives; but it is consistent with that observed[20] for substituted benzenes ($\rho^+ = -0.5$). The order of degree of activation among the methylxanthines thus obtained is 1,3,7-TMX > 3-DMX ≈ 3,7-DMX ≈ 7-MX > 3-MX > 1-MX.

The lower degree of activation for 1-MX suggests that the methyl substituent at the N(1) position does not seem to influence the rate of addition of OH at the C(4) and C(8) positions compared to that of the CH_3 substituent at the adjacent positions in its isomers 3-MX and 7-MX. The increase in reactivity in the case of 1,3,7-TMX can be explained on the basis of larger electron density due to the number of the electron donating methyl groups.

Reaction of $O^{\bullet-}$. The rates of the reaction of $O^{\bullet-}$ with methylxanthines were determined at the λ_{max} ~ 320 nm as a function of [solute] in N_2O saturated solutions containing 0.1 M NaOH. The observed k values are lower than the diffusion-controlled limit. The second order rate constants listed in Table 1 are reduced by more than a factor of five ($k = 1 \times 10^9$ for 1,3,7-TMX to 3.3×10^8 dm^3 mol^{-1} s^{-1} for 3,7-DMX) compared to those measured for the $^{\bullet}$OH reaction in neutral solution.

A similar decrease in the rates of the reaction of $O^{\bullet-}$ compared to those of the $^{\bullet}$OH reaction was also reported[9] earlier for pyrimidine and purine bases. This reduction results from a decrease in the electron affinity and from the electrostatic repulsion following the deprotonation of the parent molecule at the pH used in these experiments. The rate constant values in the case of dimethylxanthines are lower as the anion formed after

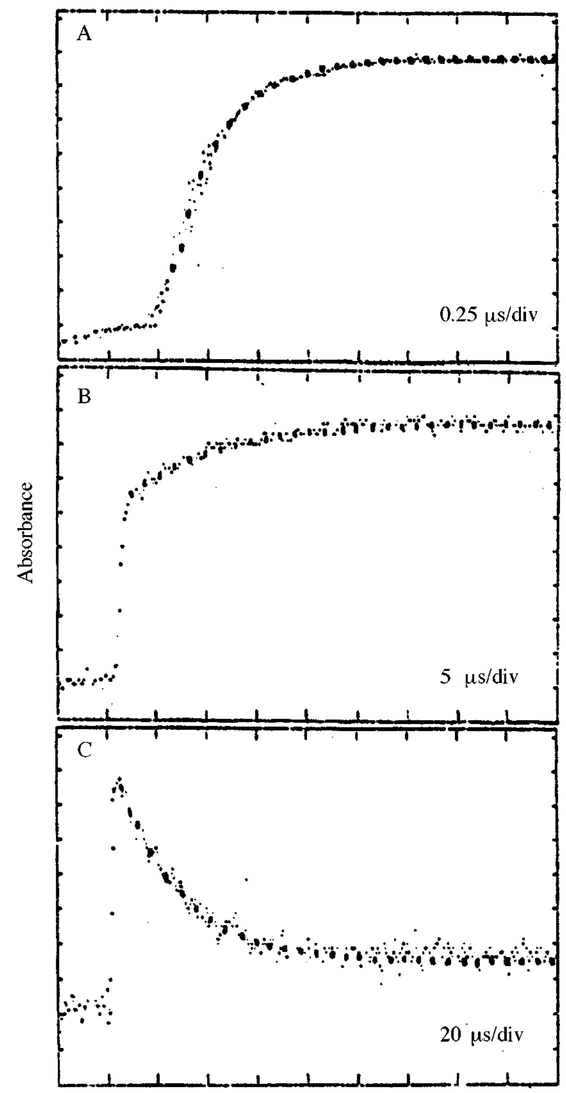


Fig. 1 Time dependent absorption changes at A) 330 nm, B) 370 nm, C) 490 nm recorded in N_2O saturated neutral solutions of 1,3-dimethylxanthine (10^{-3} mol dm^{-3}). Dose per pulse = 15 Gy.

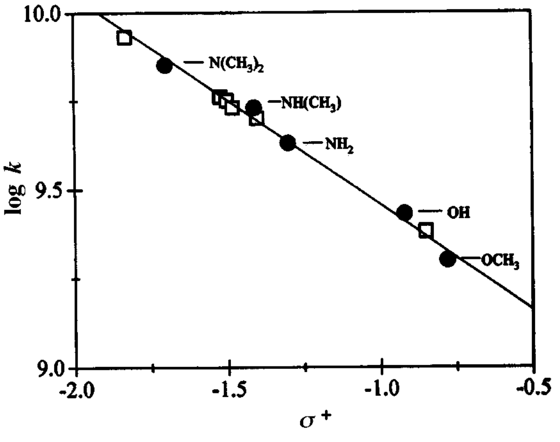


Fig. 2 Hammett plot (●) for C(6) substituted purines (taken from reference 7*a*). (□) Values obtained for methylxanthines (Table 2) by superimposition.

the $O^{\bullet -}$ addition is further destabilised by the two methyl groups.

Table 2 Rate constants ($k/10^4$ s^{-1}) for the transformation reactions of the OH adducts of methylxanthines at neutral pH

Substrate	Build-up at ~360 nm (ring opening)	Decay at ~490 nm (dehydration)
1-Methylxanthine	6.6	1.5
3-Methylxanthine	4.0	3.4
7-Methylxanthine	4.8	1.6
8-Methylxanthine	15.0	Not observed
1,3-Dimethylxanthine	10.0	3.9
3,7-Dimethylxanthine	11.0	1.9
1,3,7-Trimethylxanthine	<1.0	4.0
1,3,9-Trimethylxanthine	1.7	Not observed
Adenine	13.0	13.0
*N*⁶-Methyladenine	17.0	23.0
*N*⁶,*N*⁶-Dimethyladenine	23.0	260.0
*N*⁶,*N*⁶-Dimethyladenosine	9.5	42.0
*N*⁶,*N*⁶,9-Trimethyladenine	23.0	220.0

Values for adenine and its derivatives, 1,3,7-trimethylxanthine and its isomer are taken from references 7*a*, 17*a* and 16*c* respectively.

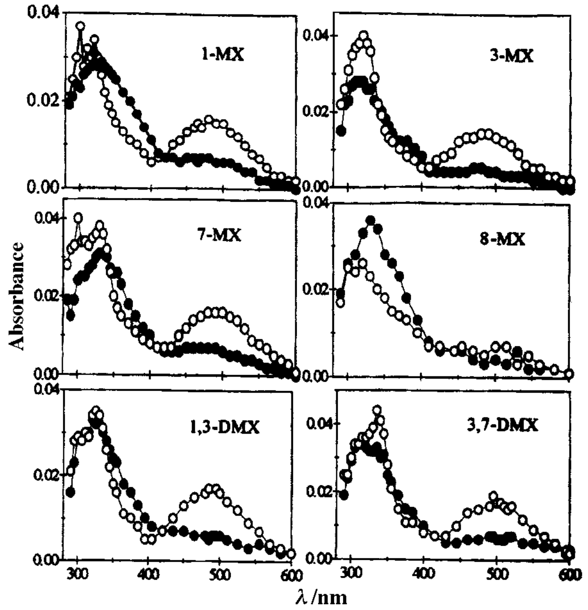


Fig. 3 Transient absorption spectra measured in N_2O saturated neutral solutions of mono- and dimethylxanthines (1 × 10^{-3} mol dm^{-3} and 1 × 10^{-4} mol dm^{-3} in the case of 8-methylxanthine) at 2 (○) and 20 μs (●) after the pulse. Dose per pulse = 15 Gy.

Reaction of $SO_4^{\bullet -}$. The rate constants for the reaction of $SO_4^{\bullet -}$ with the purines were obtained from the traces recorded at the absorption maximum in the wavelength range 320–340 nm. This wavelength rather than 460 nm, where $SO_4^{\bullet -}$ absorbs, was chosen so as to obtain a good signal-to-noise ratio. The second order rate constants ($k \sim (3.2\text{–}4.5) \times 10^9$ dm^3 mol^{-1} s^{-1}) given in Table 1 are as high as those observed ($(2.4\text{–}8.5) \times 10^9$ dm^3 mol^{-1} s^{-1}) for the corresponding $^{\bullet}OH$ reaction.

The observed high rate constants for one electron oxidation of methylxanthines by $SO_4^{\bullet -}$ ($E^0 = 2.4$ V) are in accord with the lower reduction potentials ($E^0 \leqslant 1.2$ V) reported by Faraggi *et al.*[8] for methylxanthines. The E^0 values for these compounds are comparable to that for $N_3^{\bullet}$ ($E^0 = 1.3$ V). The unreactive nature ($k \leqslant 1 \times 10^6$ dm^3 mol^{-1} s^{-1}) of $N_3^{\bullet}$ with 7-MX was explained[8] by them on the basis of its lower reduction potential. The rate constants measured for the $SO_4^{\bullet -}$ reaction with methylxanthines at pH 3 and 10 are nearly the same as at neutral pH.

Absorption spectra of OH adducts

N_2O saturated solutions. All the transient absorption spectra exhibited maxima at 320–330 nm and a broad peak around 490 nm (Fig. 3). The time-resolved spectra recorded at 45 μs revealed an increase in absorption around 360–380 nm and a

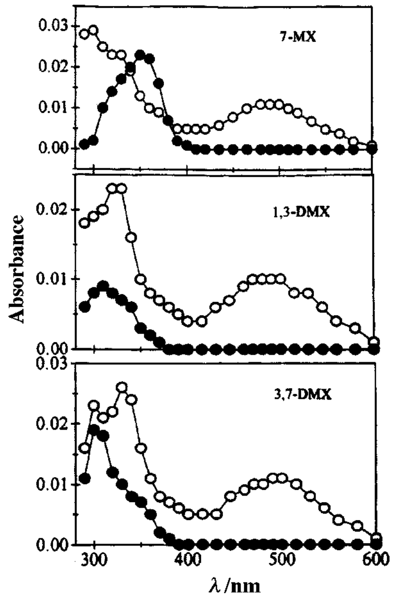


Fig. 4 Transient absorption spectra recorded in $N_2O:O_2$ saturated neutral solutions of 7-methylxanthine and 1,3- and 3,7-dimethylxanthines at 2 (○) and 20 μs (●) after the pulse. Dose per pulse = 15 Gy.

significant decrease at 500 nm. An exception to this behaviour is 8-MX where the intensity of the broad peak in the higher wavelength region was very low which remained unchanged on a longer timescale. Furthermore, the rates for the two optical changes were found to be different (Table 2) indicating that they represent two independent processes. In Fig. 1, the traces depicting the initial fast build-up at 325 nm and the time dependent absorption changes for the delayed increase at 370 nm and decay at 490 nm for 1,3-DMX are shown.

The decrease in absorbance at 500 nm is consistent with the decay of the X–4OH$^•$ adduct by the OH^- elimination as was demonstrated[17a] by us in the case of 1,3,7-TMX. Similarly, the increase in absorption around 360 nm, in analogy with the behaviour observed with its isomer 1,3,9-TMX[16] and other substituted purines,[7] is assigned to the ring opening of the X–8OH$^•$ adduct.

The observed rates of dehydration (decay at 500 nm) are in the range $(1.5–4) \times 10^4$ s^{-1} (Table 2), the values of which are 5–6 times lower than those reported[7a] for the corresponding methyladenines. Though the variation in the rates among the methyl derivatives of xanthine is not large, the number and even the position of the -CH_3 substituent does seem to influence to some extent the reaction rate. For example, the rate for 3-MX is twice ($k = 3.4 \times 10^4$ s^{-1}) that of its isomers 1-MX and 7-MX ($k = {\sim}1.5 \times 10^4$ s^{-1}). A value of about 4×10^4 s^{-1}, similar to that found for 3-MX, was observed for 1,3-DMX and 1,3,7-TMX. Thus, the -CH_3 substituent at the N(3) position seems to enhance the rate of dehydration, though a slightly lower rate ($k = 2 \times 10^4$ s^{-1}) was observed for 3,7-DMX (Table 2). The rates of the ring opening as manifested by the increase in absorption at 370 nm are higher for dimethylxanthines ($k = 1 \times 10^5$ s^{-1}) than those found for monomethyl derivatives ($k = (4–7) \times 10^4$ s^{-1}).

N_2O/O_2 saturated solutions. The spectra measured in oxygenated solutions are different from those obtained in deoxygenated solutions and the intermediates show high reactivity for O_2 as can be seen from the spectra shown in Fig. 4 for 7-MX, 1,3-DMX and 3,7-DMX. The general features of these spectra are the inhibition of the delayed increase in absorption at 370 nm except for 7-MX where the rate of the delayed increase at 370 nm was accelerated in the presence of oxygen. This is evident from the spectrum recorded at 20 μs after the pulse in the case of 7-MX which is shown in Fig. 4. The time-resolved spectra at 40 μs after the pulse obtained with 1-MX and 3-MX are featureless. The spectral nature of 3,7-DMX in the presence of oxygen on a longer timescale (~20 μs) is different from the remaining mono- and dimethylxanthines with a peak around 305 nm (Fig. 4). This spectral nature of 3,7-DMX is similar to that observed for 1,3,7-TMX.

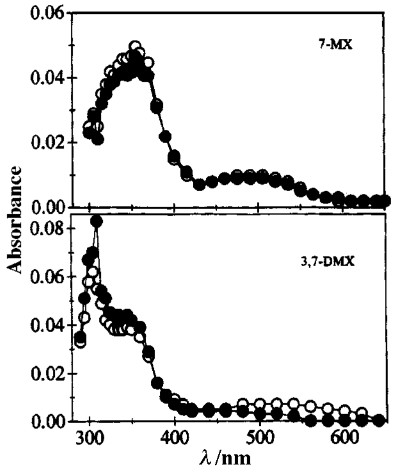


Fig. 5 Transient absorption spectra for the $O^{•-}$ reaction recorded in N_2O saturated basic solutions ($pH > 13$) of 7-methyl and 3,7-dimethylxanthines (10^{-3} mol dm^{-3}) at 4 (○) and 18 μs (●) after the pulse. Dose per pulse = 15 Gy.

The spectral behaviour in the higher wavelength region (400–600 nm) in the presence of oxygen is identical for all the derivatives with the complete disappearance of the peak at 500 nm within 5 μs of the pulse.

Basic solutions. The transient absorption spectra obtained in the reaction of $O^{•-}$ with mono- and dimethylxanthines exhibited maxima in the range 300–360 nm with only a weak absorption above 450 nm. The broad maxima seen in the higher wavelength region (490 nm) in the OH adduct spectra were not noticeable. In contrast, the delayed increase in absorption around 350 nm corresponding to the ring opening was seen in all cases, the exception again being 7-MX. The rates of ring opening estimated from the time resolved spectra at 20 μs appear to be lower than those found for the $^•OH$ reaction. A different behaviour with 3,7-DMX where an enhancement in the rate ($k = 1.8 \times 10^5$ s^{-1}) of ring opening as compared to the $^•OH$ reaction ($k = 1.1 \times 10^5$ s^{-1}) was observed. The intensity of absorption at the λ_{max} is nearly the same as that observed for the $^•OH$ reaction except for 3,7-DMX where it was almost doubled in the $O^{•-}$ reaction. Fig. 5 shows the transient absorption spectra obtained in the $O^{•-}$ reaction with 7-MX and 3,7-DMX.

$SO_4^{•-}$ reaction. The absorption spectra of the transients formed in the $SO_4^{•-}$ reaction with the methylxanthines in neutral solutions ($pH = 6$) were characterised by a single broad peak centered around 320–350 nm with negligible absorption in the higher wavelength region in all the derivatives except in 1-MX. An additional peak around 470 nm was only clearly observed in the case of 1-MX. The spectrum obtained in the reaction of $SO_4^{•-}$ with 3,7-DMX by laser flash photolysis of $S_2O_8^{2-}$ (energy per pulse = 60 mJ, pulse width 20 ns) is similar (not shown in Fig. 6) to that measured in pulse radiolysis experiments. A typical absorption trace at 330 nm obtained in laser flash photolysis of 3,7-DMX solutions is shown in the inset of Fig. 6.

The spectra for 1-MX, 7-MX and 3,7-DMX are shown in Fig. 6. In contrast to the spectral behaviour of the $^•OH$ adducts, the absorption spectra recorded in all the derivatives at 45 μs revealed neither a delayed increase at 360 nm nor a first order decrease at 490 nm in 1-MX.

The time-resolved spectra were also recorded in basic solu-

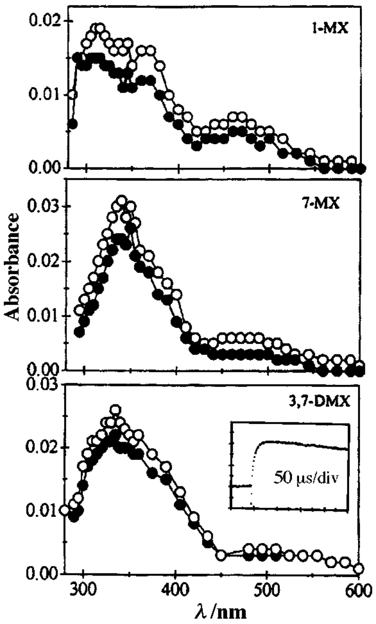


Fig. 6 Transient absorption spectra recorded in N_2 saturated neutral solutions of 1-methyl, 7-methyl and 3,7-dimethylxanthines (10^{-3} mol dm^{-3}) containing $K_2S_2O_8$ (1.5×10^{-2} mol dm^{-3}) and 0.2 M *tert*-butyl alcohol at 2 μs (○) and 20 μs (●) after the pulse. Inset: Absorption trace at 330 nm obtained in laser flash photolysis of 3,7-DMX solutions. $[K_2S_2O_8] = 5 \times 10^{-3}$ mol dm^{-3}.

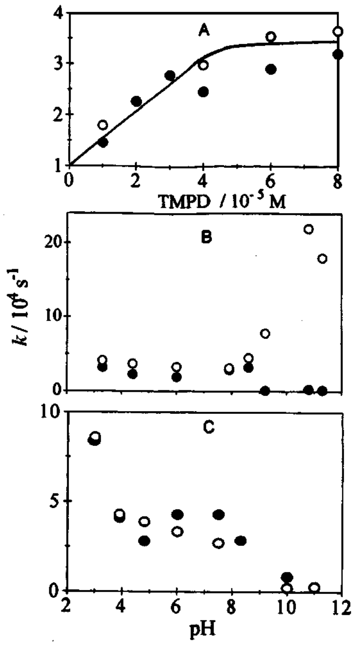


Fig. 7 A) A dependence of the rate (k_{obs}) of increase in absorption at 610 nm on [TMPD] for 3-methylxanthine (○) and 1,3-dimethylxanthine (●). Dependence of the rate constants for the increase in absorption at 370 nm (○) and the decay at 490 nm (●) on pH in B) 3-methylxanthine; C) 1,3-dimethylxanthine. For A, B and C, [solute] = 10^{-3} mol dm^{-3}.

tions (pH ~ 10) where the monomethylxanthines and 1,3-DMX exist as monoanions whereas 3,7-DMX reacts as a neutral molecule (Table 1). The reactivity of $SO_4^{\bullet -}$ in basic medium was found to be nearly the same as in neutral solutions (Table 1). The absorption spectra recorded in neutral and basic solutions were nearly identical for all the compounds except in the case of 1-MX. The weak peak at 470 nm observed in neutral solutions of 1-MX has completely vanished in basic solutions. The absorption spectra in acidic (pH ~ 3) solutions are nearly identical with those measured in a neutral medium in all systems.

Redox titration

The rates of formation of $TMPD^{\bullet +}$ monitored at 610 nm as a function of [TMPD] in the case of 3-MX and 1,3-DMX show a linear dependence for the low concentrations of the reductant, reaching a plateau value of 3.5×10^4 s^{-1} at 4×10^{-5} mol dm^{-3} TMPD (Fig. 7A). This plateau value corresponds to the rate of the dehydration found from the decay at 490 nm. The second order rate constant evaluated from the linear part of the plots (Fig. 7A) in both the systems is ~1×10^9 dm^3 mol^{-1} s^{-1}. The k values for $TMPD^{\bullet +}$ formation for other methylxanthines (1-MX, 7-MX and 3,7-DMX) were measured only at two concentrations of TMPD ((2 and 5) × 10^{-5} mol dm^{-3}). In the case of 1-MX and 3,7-DMX at 2×10^{-5} mol dm^{-3} TMPD these values are about 2×10^4 s^{-1} which are comparable to the rate of decay at 490 nm. However, when [TMPD] = 5×10^{-5} mol dm^{-3}, the observed k values are 6.6×10^4 and 4.5×10^4 s^{-1} respectively. The yield of the oxidising radicals determined from the maximum absorbance at 610 nm was about 37% $^{\bullet}OH$ in the case of 3-MX, 1,3-DMX and 3,7-DMX whereas it was about 25–28% $^{\bullet}OH$ for other methylxanthines.

In contrast, the variation in the yields of $TMPD^{\bullet +}$ in the $O^{\bullet -}$ reaction is much more significant. For example, a maximum yield of $TMPD^{\bullet +}$ corresponding to 80% $O^{\bullet -}$ was observed with 1-MX ([TMPD] = 5×10^{-5} mol dm^{-3}), while its yield was only 20% $O^{\bullet -}$ with 3,7-DMX. The yields of $TMPD^{\bullet +}$ were 50–60% $O^{\bullet -}$ with 7-MX and 3-MX and 1,3-DMX. The second order rate constants (k = (2.6–3.4) × 10^9 dm^3 mol^{-1} s^{-1}) for $TMPD^{\bullet +}$ formation in the $O^{\bullet -}$ reaction estimated from the traces recorded with 5×10^{-5} mol dm^{-3} TMPD are nearly the same with all the derivatives.

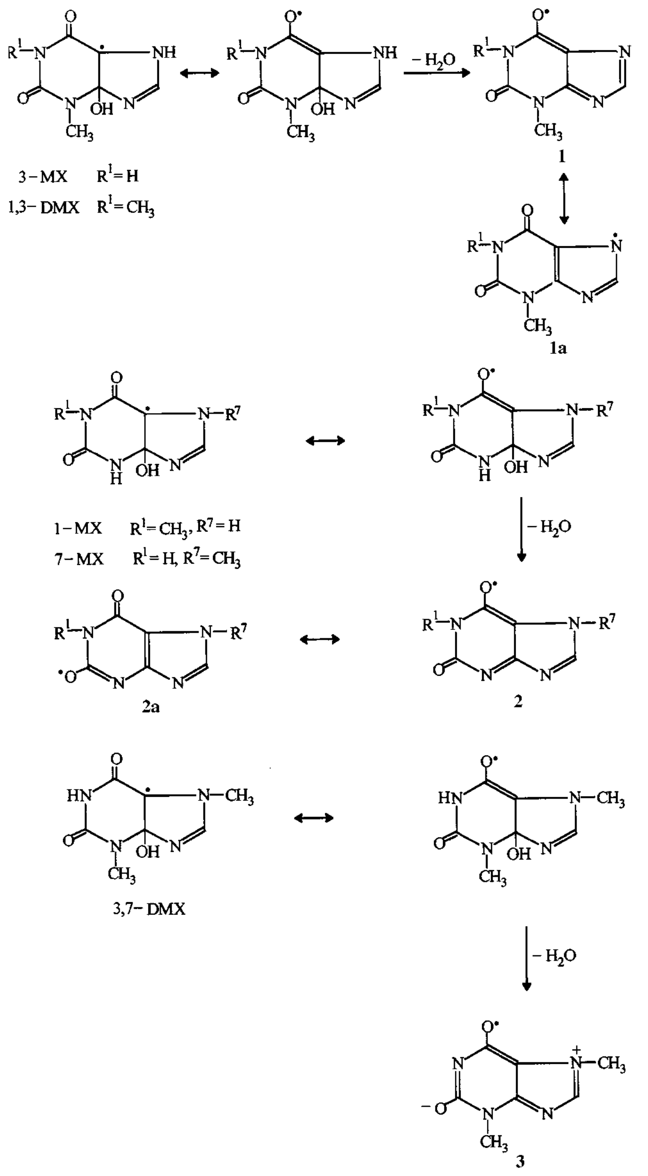
Discussion

Dehydration reaction of X–4OH$^{\bullet}$ adducts

As in the case of adenines, the OH radical adds to the C(4) and C(8) positions of mono- and dimethylxanthines. Due to the methyl substituent at the C(8) position in 8-MX, the addition of the OH radical seems to occur mainly at this position. Even if, at all, the X–4OH$^{\bullet}$ adduct of 8-MX is formed, its yield must be very low which is evident from its absorption spectrum (Fig. 2). As was demonstrated[7] with methyladenines, the OH^- elimination in the case of mono- and dimethylxanthines must be followed by rapid deprotonation of the initial radical cation leading to a neutral radical centered at the heteroatom. The observed lower rates of dehydration (k = (1.5–4) × 10^4 s^{-1}) with methylxanthines than the corresponding values[7a] for methyladenines ($k \geqslant 1 \times 10^5$ s^{-1}) are because of the relatively higher stability of their X–4OH$^{\bullet}$ adducts due to the delocalisation of the unpaired spin on oxygen bonded to C(6). Such a rate retarding effect ($k = 2.4 \times 10^4$ s^{-1}) was noticed[7a] in the case of hypoxanthine which also has the C=O group at the same position.

The transformation reactions involving dehydration from the X–4OH$^{\bullet}$ adducts for both mono- and dimethylxanthines are depicted in Scheme 1. The electron donating $-CH_3$ substituent at the N(3) position seems to enhance the rate of OH^- elimination from the X–4OH$^{\bullet}$ adduct as can be seen from the higher rates of dehydration found for 3-MX and 1,3-DMX ($k \sim 3.8 \times 10^4$ s^{-1}). Furthermore, the resulting radical, formed following the deprotonation at N(7) (radical **1**, Scheme 1), is stabilised due to the conjugation.

In contrast, the dehydration products (radical **2**, Scheme 1) of the X–4OH$^{\bullet}$ adducts of 1-MX and 7-MX, where the deprotonation takes place from N(3), are less stable due to the cross-conjugation with the double bond in the imidazole ring. The radical **2** (Scheme 1), however, has an extended conjugation with the C(2)=O. A comparison of the canonical forms **1a** and **2a** show that the unpaired spin in **1a** is on nitrogen whereas it is on oxygen in **2a**. As radicals are electrophilic, N$^{\bullet}$ (radical **1a**) is



Scheme 1 Dehydration reactions of X–4OH$^{\bullet}$ adducts.

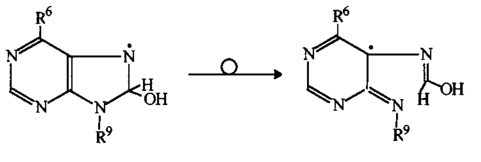
more preferred over O$^{\bullet}$ (radical **2a**) due to oxygen being more electronegative. Moreover, the lower rates ($k \sim 1.5 \times 10^4$ s^{-1}) of dehydration observed in the case of 1-MX and 7-MX indicate that the remote -CH$_3$ substituent at N(1) or N(7) does not have much inductive influence at the C(4) position as compared to that at the N(3) position. Although a similar k value is expected for 3,7-DMX, the relatively lower value ($k = 2 \times 10^4$ s^{-1}) found is perhaps due to the dipolar nature of the resulting dehydration product (radical **3**).

a) Effect of pH on dehydration rates. The dependence on pH in the range 3–11 of the rate constants of dehydration in 3-MX and 1,3-DMX is shown in Fig. 7B. It can be seen that the rates are constant in the pH range 4–8.5; but they are catalysed by H$^+$ at pH < 4 which was clearly noticeable in 1,3-DMX and inhibited by OH$^-$ above pH 9. The observed reduction in the rates of dehydration by H$^+$ for A–4OH$^{\bullet}$ adducts of methyladenines below pH 5 was explained[7b] by the decrease in the electron density upon protonation of N(1) or N(6) of these adducts in acidic solutions. As methylxanthines are more acidic (p$K_a \leqslant 1$) than methyladenines (p$K_a \geqslant 3$), such protonation at the unsubstituted nitrogens of their OH adducts is not likely at pH $\geqslant 3$ employed in our experiments. The protonation of the OH group of the X–4OH$^{\bullet}$ adduct is more likely (reaction 5, Scheme 2) leading to the enhanced rate of dehydration. The pK_a value for this protonation is approximately 3.6 in the case of 1,3-DMX. In basic solutions ($8.5 \leqslant$ pH $\leqslant 11.5$), 3-MX and 1,3-DMX react as monoanions. The decrease in the rate of dehydration in basic solutions of these compounds can be explained in terms of deprotonation from the OH group of X–4OH adduct to give X–4O$^{\bullet -}$ which cannot easily eliminate OH$^-$ (reaction 6, Scheme 2).

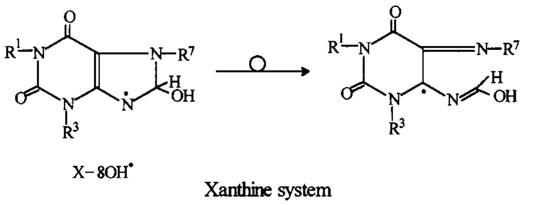
b) Redox behaviour of X–4OH$^{\bullet}$ adducts. The complete quenching of the dehydration reaction (decay at 490 nm) by 20% O$_2$ in about 5 µs—a general phenomenon—observed for all the derivatives suggests that the addition of O$_2$ to their X–4OH$^{\bullet}$ adducts is much more effective than the dehydration reaction (reaction 7, Scheme 2). The estimated second order rate constant for this reaction is about 1×10^9 dm^3 mol^{-1} s^{-1} which is similar to that found for 1,3,7-TMX. These values are slightly higher than those reported[7] for methyladenines ((4–5) $\times 10^8$ dm^3 mol^{-1} s^{-1}).

The agreement between the rates of TMPD$^{\bullet +}$ formation and 480 nm decay in 3-MX and 1,3-DMX suggests that the radical formed by dehydration from the X–4OH$^{\bullet}$ adduct (radicals **1** and **2**, Scheme 1) is only involved in the oxidation process rather than the adduct itself. However, the behaviour is different with 1-MX, 7-MX and 3,7-DMX. At low [TMPD] (2×10^{-5} mol dm^{-3}) their dehydrated products are involved in the oxidation process (reactions 10, 11) as is evident from the identical rates of dehydration and oxidation ($k_{\text{dehy.}} \approx k_{\text{ox}} = 2 \times 10^4$ s^{-1}) (Scheme 3). With increasing [TMPD] (5×10^{-5} mol dm^{-3}), the oxidation process seems to predominate over the dehydration reaction of the X–4OH$^{\bullet}$ adducts of the three derivatives (reaction 12). Such a behaviour was also noticed[7a] in the case of adenosine 5′-phosphate and 2′-deoxyadenosine where the k_{plateau} (TMPD) $\approx k_{\text{decay}} = 1.2 \times 10^5$ s^{-1}.

Ring opening reaction of X–8OH$^{\bullet}$ adducts. As can be seen from Table 2, the rates for the ring opening in methyladenines[7a] are higher by 2–4 times than those found for methylxanthines. For example, the k values for the ring opening are (4–6) $\times 10^4$ s^{-1} for mono- and 1×10^5 s^{-1} for dimethylxanthines compared to 1.7×10^5 s^{-1} and 2.3×10^5 s^{-1} reported[7a] for mono- and dimethyladenines respectively. The radicals formed from the ring opening of the –8OH$^{\bullet}$ adducts of methyladenines and methylxanthines are given in reactions (13) and (14). The ring opening of the X–8OH$^{\bullet}$ adducts of methylxanthines occurs from the cleavage of the N(7)–C(8) bond unlike in adenine derivatives.

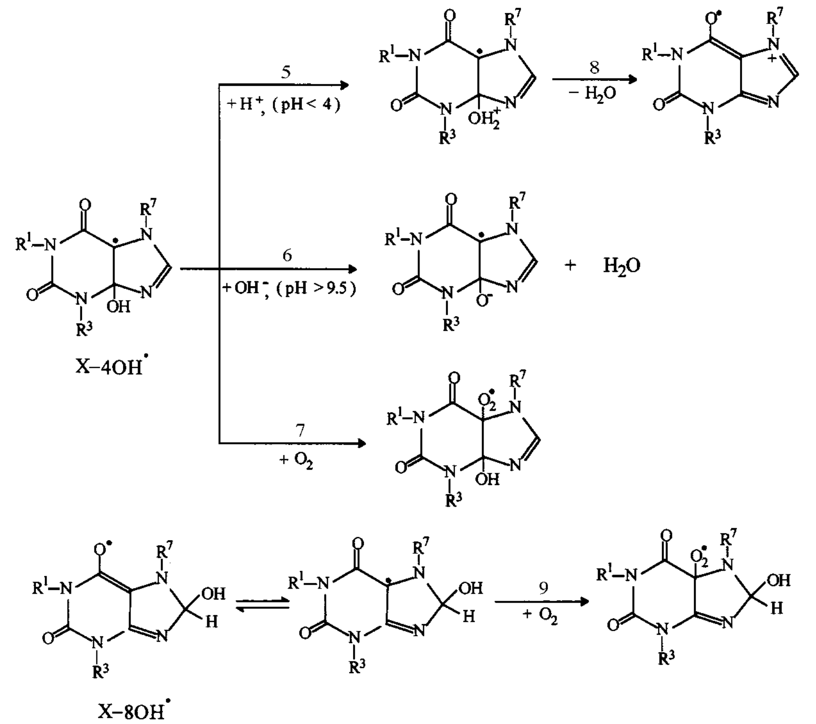


(13)



(14)

What is interesting to note is that, while the rates of the ring opening in dimethylxanthines are higher than those found for monomethyl derivatives, the X–8OH$^{\bullet}$ adduct of fully methylated xanthine (1,3,7-TMX) does not undergo ring opening within ~200 µs. Though the rate of the ring opening should be enhanced with increasing methylation, its absence in the case of the X–8OH$^{\bullet}$ adduct of 1,3,7-TMX is due to the extra stability gained by the location of the unpaired spin on the hetero-



Scheme 2 pH dependence of the dehydration reaction and the addition of O_2 to OH adducts.

$$\begin{tikzcd}[column sep=huge, row sep=huge]
(X\text{-}4OH)^\bullet \arrow[r, "(10)", "k = 2\times10^4\,s^{-1}"'] \arrow[d, "(12)\ TMPD"'] & (^\bullet X(\text{-}H)) \arrow[d, "TMPD"', "(11)\ k \sim 1\times10^9\,dm^3\,mol^{-1}\,s^{-1}"] \\
TMPD^{\bullet +} & TMPD^{\bullet +}
\end{tikzcd}$$

Scheme 3

atoms. Such a possibility, however, does not exist in the case of 1,3,9-TMX as the X–8OH• adduct is a carbon centered radical.

The dependence of the rate of the ring opening on pH in the range 3–11 in the case of 3-MX and 1,3-DMX is shown in Fig. 7C. Similar to the dehydration reaction, its rate is also independent of pH in the range 3–9.5. The OH^- induced enhancement of the rate was noticed above pH 9.5 in the case of 3-MX whereas its inhibition was observed in 1,3-DMX. The enhancement in the former is explicable in terms of the deprotonation at the N(7) and N(1) positions and possibly the X–8OH• adduct itself leading to the formation of unstable multiply charged species. Such an increase in the rate was also reported[7b] in the case of methyl derivatives of adenine and their nucleosides. In contrast, the H^+ induced enhancement and OH^- inhibition observed in the case of 1,3-DMX is not clearly understood.

As in the case of the X–4OH• adduct, the addition of O_2 to the X–8OH• adduct can only occur at C(5) (reaction 9, Scheme 2) as the radical site in the other mesomeric forms is on a heteroatom. The rates of O_2 addition to the X–8OH• adducts of 1-MX, 3-MX and 1,3-DMX are higher than those found for 7-MX and 3,7-DMX; but they are lower than those observed with their X–4OH• adducts. This is evident from the complete disappearance of the 490 nm peak in 5 µs in all derivatives suggesting that O_2 addition is much more effective than the dehydration reaction whereas there is a competition between O_2 addition to the X–8OH• adduct and its ring opening. Thus the rate of dehydration must be at least 4–5 times higher.

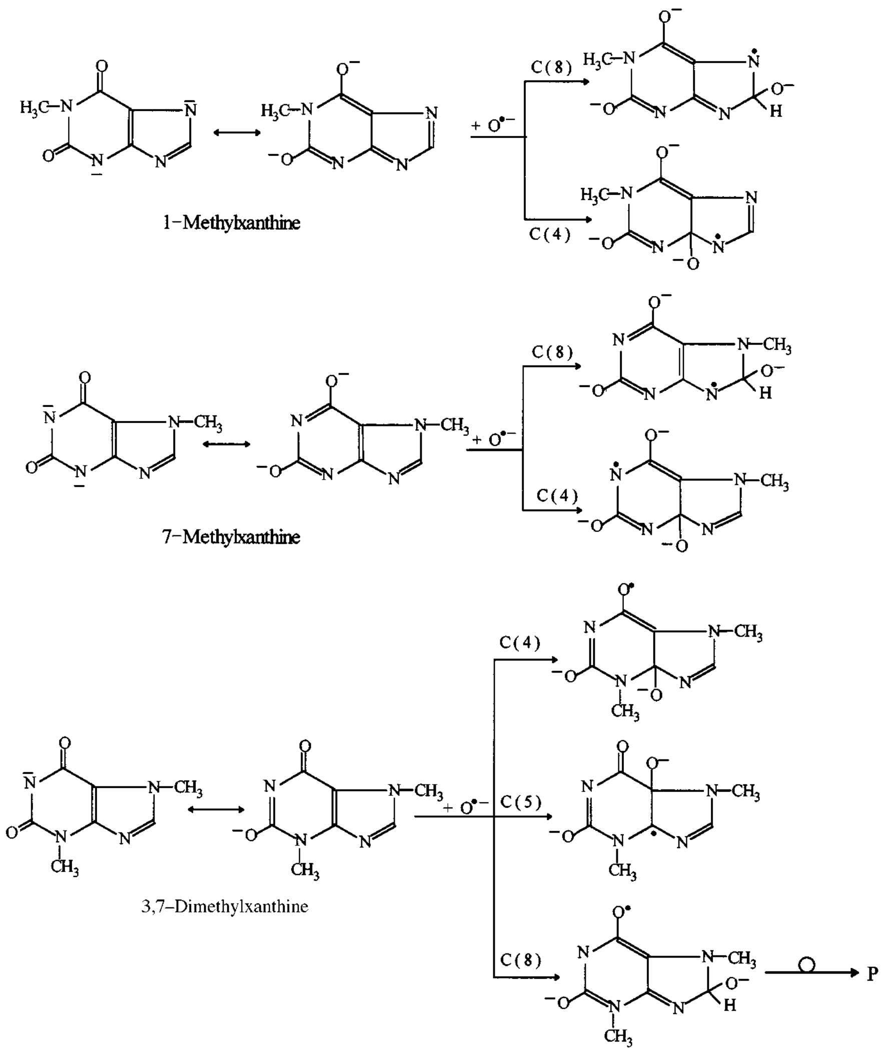
While O_2 addition predominates over the ring opening in 1-MX, 3-MX and 1,3-DMX, the X–8OH• adducts of 7-MX and 3,7-DMX are relatively unreactive. This may be due to the inductive effect of the methyl group at N(7). The X–8OH• adducts of 3,7-DMX, and 1,3,7-TMX,[17a] are relatively more stable which is evident from their similar spectral nature with maxima at 305 nm. The spectrum obtained with 7-MX at 20 µs can be attributed to its ring opened product, though the reason for the enhancement of the ring opening in the presence of O_2 is not clear.

$O^{\bullet -}$ reaction

Mono- and dimethylxanthines exist in di- and monoanionic forms at the pH employed in the $O^{\bullet -}$ reaction. In addition to the usual H-abstraction from the methyl group by $O^{\bullet -}$, its addition to the double bonds seems to occur as is evident from the spectral behaviour and redox titration experiments. The reducing radicals consisting of ~35% $O^{\bullet -}$ corresponding to $MV^{\bullet +}$ yield are assigned to the H-abstraction from the methyl group. The observed delayed increase at 370 nm in the $O^{\bullet -}$ spectra due to ring opening of $(X\text{–}8O^\bullet)^-$ is consistant with the addition mechanism. Furthermore, such an addition of $O^{\bullet -}$ to halotoluenes besides H-abstraction from the $-CH_3$ group was reported[21] by us earlier.

The probable sites for $O^{\bullet -}$ addition are C(4) and C(8) positions of methylxanthines. The attack at C(5), except in the case of 3,7-DMX, is ruled out based on the stability considerations of the ensuing addition product. The two reaction channels for the addition of $O^{\bullet -}$ to mono- and dimethylxanthines are shown in Scheme 4 (H-abstraction not shown). The redox titration experiments have shown that the yield of oxidising radicals was at a maximum (80% $O^{\bullet -}$) with 1-MX and a minimum (5% $O^{\bullet -}$) with 1,3,7-TMX. An intermediate yield of about 55% $O^{\bullet -}$ was formed with the rest of the derivatives (3-MX, 7-MX and 1,3-DMX). The maximum yield of oxidising radicals observed in the $O^{\bullet -}$ with 1-MX can be explained by the formation of the heteroatom-centered C(4) and C(8) adducts. The extent of the nucleophilic $O^{\bullet -}$ addition to the C(8) position of 7-MX is expected to be relatively lower because of the adjacent N(7)-CH_3. On the other hand, the yields of C(8) addition products in both 3-MX and 1,3-DMX are likely to be higher as the CH_3 substituent at the N(3) makes the C(4) addition less favoured.

The reduction in the yield (20% $O^{\bullet -}$) of oxidising radicals observed in 3,7-DMX is due to the formation of less favoured



Scheme 4 Pattern of $O^{\bullet -}$ addition to some methylxanthines.

C(4) or C(8) adducts in this system. Instead, the formation of reducing carbon centered radicals from the C(5) addition is predominant. The reducing radicals consisting of about 40% $O^{\bullet -}$ are ascribed to the H-abstraction from the two -CH_3 groups and C(5) addition. The remaining (55%) $O^{\bullet -}$ is attributed to the unreactive C(8) ring opened product which is reducing in nature. This fact is supported by the enhanced rate of ring opening ($k = 1.8 \times 10^5$ s^{-1}) which can effectively compete with the oxidation reaction ($k = 1.4 \times 10^5$ s^{-1}).

Pathways in the $SO_4^{\bullet -}$ reaction

The spectral nature found in the $SO_4^{\bullet -}$ reaction with a single broad peak at 330–350 nm with negligible absorption in the higher wavelength region in all the derivatives except 1-MX is straightforward suggesting the formation of neutral N-centered radicals on deprotonation of the initially formed radical cations. This is in accord with the work reported[7] earlier as well as with results obtained by us[17d] on adenine derivatives. The N-centered radicals must be identical with those formed after dehydration of the OH adducts. As reflected in the similar spectral nature observed for $O^{\bullet -}$ and $SO_4^{\bullet -}$ reactions, H-abstraction from the methyl groups by $SO_4^{\bullet -}$ is an additional reaction channel. The absorption spectra obtained in the $SO_4^{\bullet -}$ reactions with 1-MX, 7-MX and 3,7-DMX are shown in Fig. 6.

Conclusions

The second order rate constants for the $^{\bullet}OH$ reaction with methylxanthines range from 8.5×10^9 dm^3 mol^{-1} s^{-1} for 1,3,7-trimethylxanthine to 2.4×10^9 dm^3 mol^{-1} s^{-1} for 1-methylxanthine and these rates are reduced by more than 5 times for the $O^{\bullet -}$ reaction. The order of the degree of activation for the $^{\bullet}OH$ reaction among the methylxanthines is 1,3,7-TMX > 1,3-DMX ≈ 3,7-DMX ≈ 7-MX > 3-MX > 1-MX. As in the case of adenines, it has been demonstrated that the OH radical reacts mainly by addition to the C(4) and C(8) positions of methylxanthines. The C(4) and C(8) $^{\bullet}OH$ adducts of the derivatives also undergo dehydration and ring opening respectively, these rates being structure dependent. The observed rates of dehydration (k = 1-4 × 10^4 s^{-1}) are lower by at least an order of magnitude than those found for the corresponding methyladenines. However, such a significant difference in the rates of ring opening was not seen.

Since both the xanthine and guanine systems have oxygen attached to C(6), the rates of transformation of the guanine OH adducts should be similar to those determined by us for the xanthine derivatives. For example, Candeias and Steenken[22a] have reported that the C(8) $^{\bullet}OH$ adduct of 2′-deoxyguanosine undergoes a fast ring opening ($k = 2 \times 10^5$ s^{-1}) while the C(4) $^{\bullet}OH$ adduct dehydrates at a slower rate ($k = 5 \times 10^3$ s^{-1}). However, the corresponding values measured[22b] recently are 6×10^4 and 4.5×10^4 s^{-1} for the same system. This study demonstrates the usefulness of radiation chemical techniques in obtaining information on the kinetics, spectral and redox behaviour of OH adducts of non-DNA purine derivatives.

Acknowledgements

The authors thank Professor M. S. Wadia and Dr M. G.

Kulkarni, Department of Chemistry, University of Pune, for their valuable suggestions and Dr R. Das, Tata Institute of Fundamental Research, Mumbai for his help in carrying out the laser flash photolysis experiments. M. S. V. thanks the Council for Scientific and Industrial Research, New Delhi, for the award of senior research fellowship. The financial support from the Department of Science and Technology, New Delhi is acknowledged.

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Paper 8/07437B