Absorption Spectra of Isomeric OH Adducts of 1,3,7-Trimethylxanthine[1]

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The reactions of $OH^•$, $O^{•-}$, and $SO_4^{•-}$ with 1,3,7-trimethylxanthine (caffeine) were studied by pulse radiolysis with optical and conductance detection techniques. The absorption spectra of transients formed in $OH^•$ reaction in neutral solutions exhibited peaks at 310 and 335 nm, as well as a broad absorption maximum at 500 nm, which decayed by first-order kinetics. The rate ($k = (4.0 \pm 0.5) \times 10^4$ s^{-1}) of this decay is independent of pH in the range 4−9 and is in agreement with that determined from the conductance detection ($k = 4 \times 10^4$ s^{-1}). The spectrum in acidic solutions has only a broad peak around 330 nm with no absorption in the higher wavelength region. The intermediates formed in reaction of $O^{•-}$ absorb around 310 and at 500 nm, and the first-order decay at the latter wavelength was not seen. The OH radical adds to C-4 (X−4OH$^•$) and C-8 (X−8OH$^•$) positions of caffeine in the ratio 1:2 as determined from the redox titration and conductivity measurements. H abstraction from the methyl group is an additional reaction channel in $O^{•-}$ reaction. Dehydroxylation of the X−4OH$^•$ adduct occurs, whereas the X−8OH$^•$ adduct does not undergo ring opening. The rate constant for addition of O_2 to X−4OH$^•$ is estimated to be $\sim 1 \times 10^9$ M^{-1} s^{-1}, whereas it is unreactive toward X−8OH$^•$. The spectrum obtained for $OH^•$ reaction in oxygenated solutions is similar to that observed in $SO_4^{•-}$ reaction in basic solutions. The radical cation of caffeine formed from its reaction with $SO_4^{•-}$ (λ_{max} = 320−340 nm) is hydrolyzed in basic solutions to yield the X−8OH$^•$ adduct. The molar absorptivities of the X−8OH$^•$ and the X−4OH$^•$ adducts at 300 and 335 nm are 6500 and 5300 M^{-1} cm^{-1}, respectively. The yield of 1,3,7-trimethyluric acid in $OH^•$ reaction in the absence of O_2 (28%) is reduced by more than 50% in its presence. The differences in the mechanism of $OH^•$ reaction with caffeine and its isomer 1,3,9-trimethylxanthine (isocaffeine) are discussed.

Introduction

Radiation chemical studies on the reactions of primary radicals of water (e^-_{aq}, $OH^•$, and $H^•$) and secondary radicals (e.g., $SO_4^{•-}$,$Br_2^{•-}$, and $O^{•-}$) derived from them with pyrimidines and purines are of current interest due to their importance in the understanding of DNA radiation chemistry (see refs 2−4 for recent reviews). Both e^-_{aq} and $OH^•$ show high reactivity ($k = 10^9 - 10^{10}$ M^{-1} s^{-1}) toward these compounds.[4,5] Of particular importance are the studies carried out on the reactions of oxidizing radicals with the derivatives of guanine[6−8] and adenine.[9−11] It has been reported[10] that at least two different isomeric OH adducts are formed in the reaction of $OH^•$ with adenine derivatives. The additives have been identified as the radicals formed by $OH^•$ addition to C-4 (A−4OH$^•$) and C-8 (A−8OH$^•$) positions of these compounds.

In contrast to pyrimidines,[12−14] the A−4OH$^•$ and A−8OH$^•$ radical adducts of purines are shown[9−11] to undergo unimolecular transformation reactions involving dehydration of the former and ring opening of the latter. The opening of the imidazole ring of the A−8OH$^•$ adduct of purines was manifested in the first order increase of absorption around 350 nm, while the decrease in the higher wavelength region was attributed to the elimination of OH^- from the A−4OH$^•$ adduct. In the case of fully alkylated adenines such as N^6,N^6,9-trimethyladenine,[10] the rate constant values for the OH^- elimination and the ring opening reactions are 2.2×10^6 and 2.3×10^5 s^{-1}. Furthermore, the rate of the dehydration process as compared to the ring opening reaction has been found to be strongly influenced by the substituent at C-6.

Xanthine and its methyl derivatives are structurally similar to purines, but the six-membered ring closely resembles that of uracil. Thus, the methylated xanthines form an interesting class of compounds for examining the effect of the nature and position of the substituents on the rates of transformation processes of the adducts formed in the reaction with primary radicals of water radiolysis. We have undertaken a comprehensive study of reactions of these radicals with xanthine derivatives and have previously reported[15] the reactions of e^-_{aq} with di- and trimethylxanthines.

This paper deals with the study of reactions of $OH^•$, $O^{•-}$, and $SO_4^{•-}$ with caffeine (structure 1, Scheme 1). The double bond in the imidazole ring of this compound is between C-8 and N-9, unlike other purine derivatives. This structural difference, combined with the possibility to employ conductance detection in addition to optical absorption techniques to investigate the nature of the transformation reactions as in the case of other fully substituted adenines,[9,10] makes the study interesting. While our study has been in progress, results from the oxidation of methyl derivatives of xanthine were reported.[16,17] In their work on isocaffeine, an isomer of caffeine

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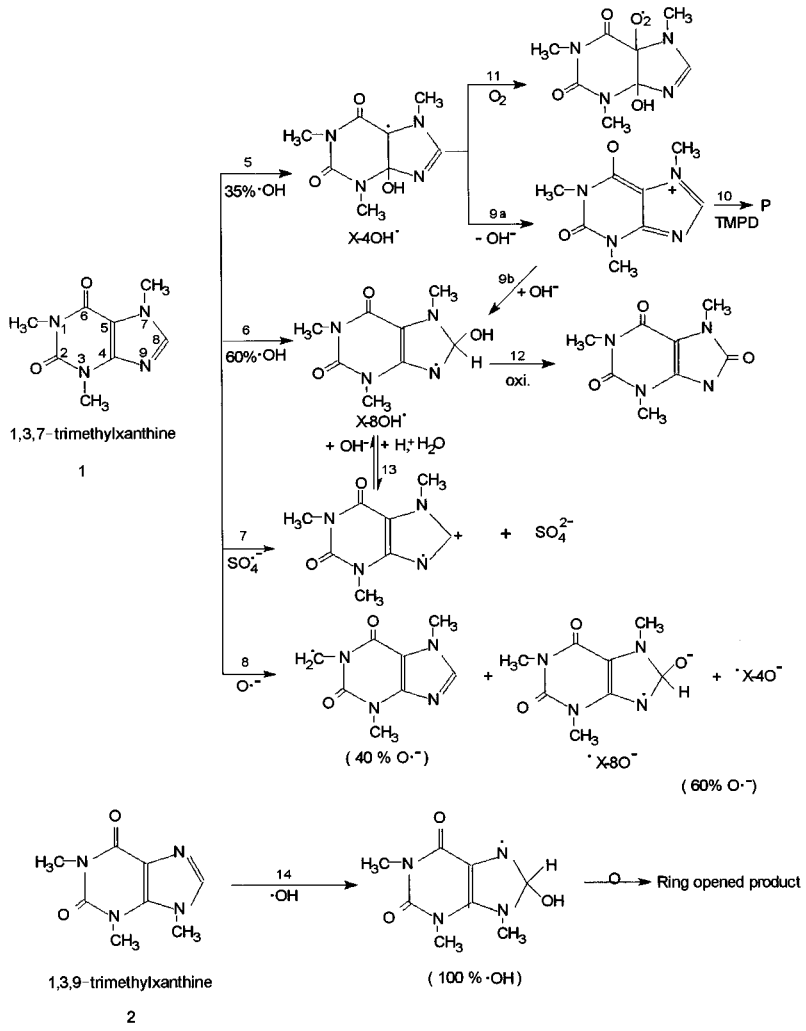
SCHEME 1



(structure 2, Scheme 1), Viera and Steenken[17] have shown that the X−4OH• adduct, even if it is formed, did not undergo dehydroxylation and that the X−8OH• adduct underwent a unimolecular transformation due to the ring opening in agreement with the results obtained with DNA purine derivatives.[9] In contrast, it will be shown that the OH• attack in caffeine leads to the formation of both X−4OH• and X−8OH• adducts and that while OH^- elimination takes place from the former, the latter does not undergo a ring-opening reaction. This study demonstrates the usefulness of radiation chemical methods in obtaining the distinct absorption spectra of these two adducts.

Experimental Section

Caffeine (SD Fine) 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 OH• was studied in N_2O saturated aqueous solutions of caffeine (10^{-3} M) where e^-_{aq} is converted into OH• (reactions 1 and 2),

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

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

The reaction of $O^{\bullet-}$ was studied at pH 13 ($OH^\bullet \rightleftharpoons O^{\bullet-} + H^+$, $pK_a = 11.9$) where >90% OH is converted and thus the radical anion is essentially the reacting species. N_2O:O_2 (4:1 v/v) saturated solutions were used to measure the OH adduct spectra in the presence of O_2.

The reaction of $SO_4^{\bullet-}$ was studied in N_2 saturated aqueous solutions of caffeine (10^{-3} M) containing $K_2S_2O_8$ (1.5×10^{-2} M) and 0.2 M *tert*-butyl alcohol.

The OH radicals are scavenged by *tert*-butyl alcohol, while e^-_{aq} react quantitatively with $S_2O_8^{2-}$ to produce the $SO_4^{\bullet-}$ radical anion (reactions 3 and 4),

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

$$S_2O_8^{2-} + e^-_{aq}\,(H) \rightarrow SO_4^{\bullet-} + SO_4^{2-}\,(HSO_4^-) \qquad (4)$$

Pulse radiolysis experiments for optical absorption measurements were carried out using 7 MeV electron pulses (pulse width 50 ns) from a linear accelerator at the Bhabha Atomic Research Centre, Mumbai. KSCN dosimeter was used in the optical pulse radiolysis. The dose per pulse was between 10−15 Gy. The details of this facility have been described elsewhere.[18] The 16 MeV electron LINAC facility[19,20] (pulse width 4−20 ns) at the Argonne National Laboratory was employed for the pulse conductivity experiments.

Separation of the products formed in the reactions of OH• and $SO_4^{\bullet-}$ with caffeine under steady-state conditions was done by reverse phase HPLC technique (Perkin Elmer Series 10 Liquid Chromatograph, Nucleosil-5-C_{18} column, 40% acetonitrile in water as an eluent under isocratic conditions). A diode array detector was used for optical detection.

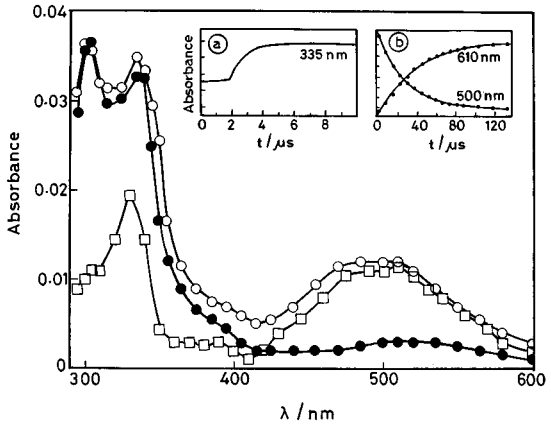


Figure 1. Transient absorption spectra measured in N_2O saturated neutral solutions of caffeine (1×10^{-3} M) at 2 (○) and 20 μs (●) after the pulse. (□) This plot is drawn from the difference in the fully grown spectra recorded in the absence and the presence of O_2 at 7 μs. Dose/pulse = 15 Gy Inset: Traces showing the (a) buildup at 335 nm [caffeine] = 4×10^{-4} M and (b) first-order decay at 500 nm and the corresponding growth of $TMPD^{\bullet+}$ at 610 nm on pulsing N_2O-saturated neutral solutions [caffeine] = 1×10^{-3} M. Dose:pulse was 12 Gy.

TABLE 1: Various Rate Constants and Yields Obtained in Reactions of $OH^\bullet$ and $SO_4^{\bullet-}$ with Caffeine

reaction	optical work	conductance detection
caffeine (X)		
$OH^\bullet + X$	8.5×10^9 [a]	$(X\text{-}4)^{\bullet+} + OH^-$ 3×10^9 [a]
$O^{\bullet-} + X$	1×10^9	
$SO_4^{\bullet-} + X$	4.5×10^9	
$X\text{-}4OH^\bullet + O_2$	1×10^9	$X\text{-}8OH^\bullet + OH^- \rightleftharpoons (X\text{-}8O)^{\bullet-} + H_2O$
$(X\text{-}4)^{\bullet+} + TMPD$	1.5×10^9	
$(^\bullet X(\text{-}H)) + MV^{\bullet+}$	2×10^9	
dimethyladenosine[b] (A)		$k_f = 6.0 \times 10^9$ [a] $k_b = 3.0 \times 10^4$ [c]
$A\text{-}4OH^\bullet + O_2$	4×10^8	
$A\text{-}8OH^\bullet + O_2$	1.6×10^{10}	
trimethyladenine (A)		decay at 500 nm $(4 \pm 0.5) \times 10^4$ [c,d] OH^- elimination 4×10^4 [c,d]
$A\text{-}4OH^\bullet + O_2$	5×10^8	

product yields (%$OH^\bullet$)			
X-4OH$^\bullet$		8-OH-X	
35[f]	33[e]	28[g]	12[h]

[a] k (M^{-1} s^{-1}). [b] Reference 10. [c] k (s^{-1}). [d] pH 4−9. Yields of X−4OH$^\bullet$ from the [e]conductivity measurements and [f]buildup of $TMPD^{\bullet+}$ at 610 nm. Yields of 1,3,7 trimethyluric acid from [g]N_2O and [h]N_2O:O_2 saturated solutions.

Results and Discussion

a. Determination of Rate Constants. The reaction of $OH^\bullet$ with caffeine was studied by measuring the transient absorption from the pulse radiolysis of N_2O-saturated neutral solutions of caffeine (10^{-3} M). The rate of the reaction was monitored by the buildup in the absorption of the transients at 335 nm (inset Figure 1) and the bimolecular rate constant obtained from the plot of k_{obs} *versus* [solute] in the range $(0.2-1) \times 10^{-3}$ M (data not shown) at neutral pH is 8.5×10^9 M^{-1} s^{-1}. This is in reasonable agreement with the value (6.9×10^9 M^{-1} s^{-1}) reported[21] earlier. The rate constants obtained (accurate to within ±10%), for this and other reactions in this work, are listed in Table 1.

The rate of the reaction of OH radical with purines was reported[6−8] to be influenced by the nature and position of the substituents. For example, the second-order rate constants have been shown[10] to vary by more than an order of magnitude with $k = 1.3 \times 10^8$ M^{-1} s^{-1} for N^6,N^6,9-trimethyladeninium to 8.4×10^9 M^{-1} s^{-1} for N^6,N^6,9-trimethyladenine. The second-order rate constant value obtained in the case of caffeine is comparable to that found for N^6,N^6,9-trimethyladenine, and this increase in reactivity can be attributed to larger electron-density due to the electron donating methyl groups. However, the rate of reaction of $O^{\bullet-}$ with caffeine was reduced by nearly an order of magnitude ($k \simeq 1 \times 10^9$ M^{-1} s^{-1}) relative to the $OH^\bullet$ reaction and represents the diffusion-controlled reaction. Such a decrease in the reactivity of $O^{\bullet-}$ with the substrate was also seen in the case of uridine and adenosine systems.[22] For instance, the rate constants of $OH^\bullet$ and $O^{\bullet-}$ with uridine are 5×10^9 and 0.7×10^9 M^{-1} s^{-1}, respectively. Since there is no ionizable H in caffeine, the variation in the reactivity between $OH^\bullet$ and $O^{\bullet-}$ must be attributed to differences in the reaction pathways (section f, below).

The rate of reaction of $SO_4^{\bullet-}$ with caffeine was measured by following the decay of the $SO_4^{\bullet-}$ absorption at 460 nm, and the bimolecular rate constant from this decay was found to be 4.5×10^9 M^{-1} s^{-1} at neutral pH. A value of 4.7×10^9 M^{-1} s^{-1} was obtained from the measurement of the growth of absorption of the intermediates at 320 nm. The rates for $SO_4^{\bullet-}$ reaction are found to be same in both acidic (pH 3) and basic (pH 10) solutions.

b. Absorption Spectra of OH Adducts. *i. Deoxygenated and Oxygenated Solutions.* The transient absorption spectra for the reaction of $OH^\bullet$ with caffeine in neutral solutions (pH ∼ 7) were recorded in the range 280−650 nm. The spectrum exhibited peaks at 310 and 335 nm with a broad maximum around 500 nm. Figure 1 shows the fully grown absorption spectrum at 2 μs after the pulse. The time-resolved spectrum recorded at 20 μs revealed a significant decrease in absorbance around 500 nm. The rate of this decay was found to be first order, $k_{obs} = (4.0 \pm 0.5) \times 10^4$ s^{-1}, and nearly constant in the pH range 4−9.

The observed behavior is in contrast to that observed[11] with its isomer isocaffeine where only the bimolecular decay of absorption in the higher wavelength region (400−450 nm) and a first-order increase in absorbance at 330 nm with $k = 1.7 \times 10^4$ s^{-1} were seen. A typical trace depicting the rate of the decay at 500 nm in neutral solutions of caffeine is shown in the inset of Figure 1. However, this rate was found to decrease above pH = 9 and this variation is given in Figure 2A. For example, k_{obs} values are $\leq 1 \times 10^4$ s^{-1} beyond pH 10. Our k value for the decay of absorption at 500 nm is much lower than those observed[9] for methyl derivatives of adenine (10^5-10^6 s^{-1}); but it is comparable to that found[9] for hypoxanthine where the electron-withdrawing carbonyl group is at the C-6 position. Thus, this is in accord with the observed[10] influence of the C-6 substituent on the rate of this reaction.

The absorption spectrum obtained for $OH^\bullet$ reaction with caffeine in N_2O:O_2 (4:1 v/v) saturated solutions is different from that obtained in deoxygenated solutions. Nor is it similar to that obtained in $SO_4^{\bullet-}$ reaction in neutral solutions of caffeine. The fully grown spectrum recorded in the presence of O_2 at 7 μs after the pulse exhibited a single peak at 300 nm, while the absorption above 335 nm was scavenged to an extent of more than 70% by O_2 compared to that observed in deoxygenated solutions (Figures 1 and 3). This spectrum remained unchanged even up to 50 μs after the pulse. This behavior is in contrast to that observed with its isomer (isocaffeine) where the inhibition of the delayed increase in absorbance at 335 nm due to the ring opening of the $X-8OH^\bullet$ adduct in the presence of O_2, was noticed.[17] Thus, the oxidation of the $X-8OH^\bullet$ adduct predominates over the ring opening reaction.

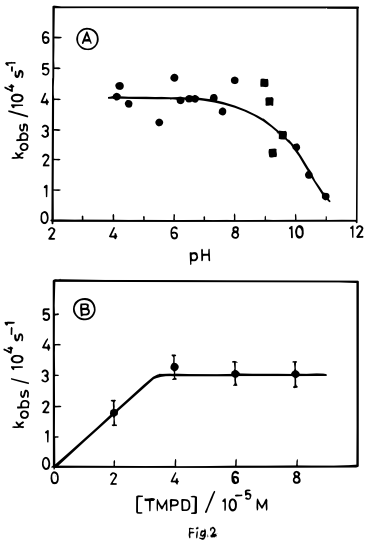


Figure 2. (A) Dependence of the rate constants for the decay at 500 nm (●) and OH^- elimination (■) from conductance measurements on pH. (B) Dependence of the rate (k_{obs}) of increase in absorption at 610 nm (●) on [TMPD]. For both (A) and (B), [caffeine] = 1×10^{-3} M.

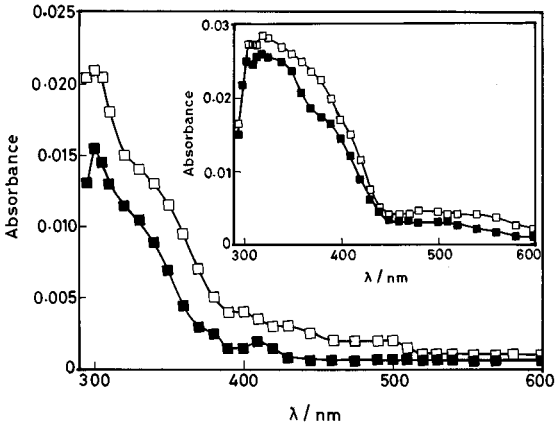


Figure 3. Transient absorption spectra recorded in N_2-saturated solutions (pH = 10) of caffiene (1×10^{-3} M) containing $K_2S_2O_8$ (1.5×10^{-2} M) and 0.2 M *tert*-butyl alcohol at 18 μs (□) after the pulse and in N_2O-O_2 (4:1 v/v) saturated neutral solutions at 7 μs (■) after the pulse. This spectrum is normalized to a G value of 3.3. Inset: Transient absorption spectra recorded for the reaction of $SO_4^{\bullet-}$ at neutral pH at 2 (□) and 18 μs (■) after the pulse. Dose:pulse = 15 Gy.

ii. Acidic and Basic Solutions. The transient absorption spectrum recorded in the reaction of $OH^\bullet$ with caffeine in acidic solutions showed a broad maximum around 335 nm with no absorption in the higher wavelength region. This spectrum is similar to that measured in the $SO_4^{\bullet-}$ reaction at neutral pH. The nature of transient absorption spectrum obtained in the reaction of $O^{\bullet-}$ with caffeine was similar to that observed in the $OH^\bullet$ reaction with maxima at 300, 340, and 500 nm, though the intensities of absorption were reduced by nearly 40% compared to those observed for $OH^\bullet$ reaction. Furthermore, the first-order decay of absorption at 500 nm as observed in neutral solutions was not seen (Figure 4).

c. Conductivity Measurements. Pulse conductivity experiments were carried out in both basic and acidic media. As shown in Figure 5A, the conductivity traces clearly indicate the expulsion of OH^- in solutions at pH, near neutral. In acid solutions, there is a clear loss of conductivity arising from the reaction of the ejected OH^- with H^+ in the bulk of the solution while there is an increase in conductivity in basic solutions. The rate of this increase was evaluated to be approximately 4×10^4 s^{-1}. Further, this rate was found to be independent of

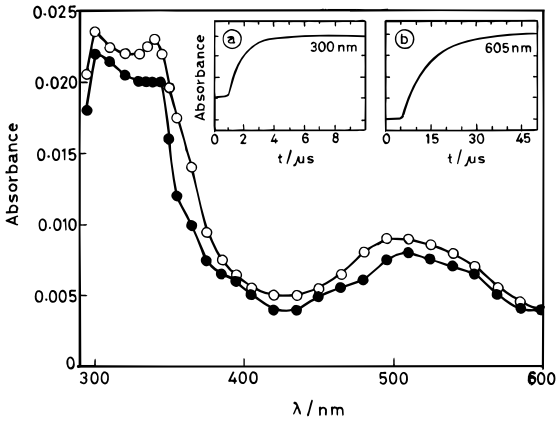


Figure 4. Absorption spectra for $O^{\bullet-}$ reaction recorded in N_2O-saturated basic solutions (pH ≥ 13) of caffeine (1×10^{-3} M) at 2 (○) and 18 μs (●) after the pulse. Dose:pulse = 15 Gy. Inset: (a) Buildup at 300 nm and (b) the rate of formation of $MV^{\bullet+}$ at 605 nm. Dose: pulse = 12 Gy.

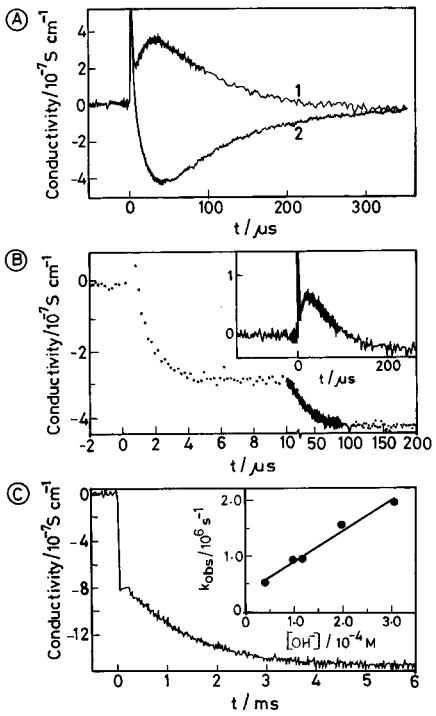


Figure 5. Conductance changes recorded in N_2O-saturated solutions of caffeine (1×10^{-3} M) at (A) 1 = pH 8.9 and 2 = pH 4.9, (B) pH 9.6, and (C) pH 10.3. The figures are all normalized to a dose of ~1 Gy. Inset (B): pH 9.3. Inset (C): Dependence of k_{obs} on [OH^-].

[OH^-] between pH 4 and 9; but decreases at higher pH. The yield of OH^- was estimated to be about $^1/_3$ OH from the growth of conductivity signal at pH 8.9.

d. Reaction of $SO_4^{\bullet-}$. The transient absorption spectrum recorded for reaction of $SO_4^{\bullet-}$ with caffeine at neutral pH exhibited a broad peak with maximum around 320−330 nm (inset of Figure 3). This spectrum is different from that obtained with isocaffeine where the peak was centered at 370 nm. This blue shift indicates that the radical cation of the former is relatively more stable and does not get hydrolyzed in neutral solutions. Though the initial absorption spectrum measured in basic solutions (pH ≃10) immediately after the pulse is similar to that recorded at neutral pH, it was finally transformed to a spectrum having a peak at 300 nm (Figure 3). However, the spectral nature of the transients formed in acidic solutions (pH ≃ 3) is similar to that observed in neutral solutions as well as

in $OH^•$ reaction in acidic medium. This finding suggests that the same transient species was formed under both these condition.

e. Redox Reactions of Transient Adducts. The oxidant methylviologen (MV^{2+}) and the reductant *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) were used to differentiate between the reducing and oxidizing radicals produced in reactions of $OH^•$ and $O^{•-}$ with caffeine. The reduced form ($MV^{•+}$) of the oxidant has two well-defined peaks at 395 and 605 nm. The oxidized form of TMPD ($TMPD^{•+}$) has the absorption maxima at 335, 565, and 610 nm. However, the rate for the oxidation of the transient adducts was monitored at 605 nm ($\epsilon = 12\,800$ M^{-1} cm^{-1}) in the case of MV^{2+} and at 610 nm ($\epsilon = 12\,000$ M^{-1} cm^{-1}) for TMPD as the other maxima are not suitable owing to the absorption of other transients formed at these wavelengths.

The redox experiments, were done over the range $(2 - 8) \times 10^{-5}$ M [TMPD] with solutions of 1×10^{-3} M caffeine saturated with N_2O, where all the OH radicals will essentially react with caffeine ($k = 8.5 \times 10^9$ M^{-1} s^{-1}). The dose per pulse was kept at 12 Gy to minimize the effect of bimolecular radical recombination reactions. The formation of $TMPD^{•+}$ was observed showing that oxidizing radicals were indeed formed.

The dependence of the rate of formation of $TMPD^{•+}$ on [TMPD] is shown in Figure 2B. The k_{obs} reaches a plateau value of 3×10^4 s^{-1} at 4×10^{-5} M TMPD, which matches reasonably well with that obtained for the rate of decay at 500 nm. This behavior indicates that the radical cation formed by OH^- elimination from the X−4OH$^•$ is only responsible for the oxidation and that the neutral radical itself is unable to oxidize TMPD. If it were so, the k_{obs} *versus* [TMPD] plot should have yielded two lines with different slopes corresponding to the rates of these two reactions. The bimolecular rate constant estimated from the linear part of the plot of k_{obs} *versus* [TMPD] is $\sim 1.5 \times 10^9$ M^{-1} s^{-1}. Similar behavior with $k_{plateau} \sim 1.2 \times 10^5$ s^{-1} was noticed in the case of adenine,[10b] which was explained on the basis of the reaction of TMPD with the neutral radical formed from dehydration of A−4OH$^•$ and its reactivity with A−4OH$^•$ adduct itself was seen to be low. However, the larger oxidizing strength observed for the A−4OH$^•$ adduct of adenine-5′-triphosphate and 2′−deoxyadenosine was attributed[10] to the electron-withdrawing ribose phosphate group at N-9.

The electron-donating methyl groups in caffeine make it relatively less reactive toward the reductant. The yield of the oxidizing radicals calculated from the maximum absorbance at 610 nm was about 35% OH$^•$. Complementary experiments using MV^{2+} as a scavenger were done to estimate the yield of the reducing radicals. The yield of $MV^{•+}$ was marginal ($\leq$ 5% OH$^•$) indicating that the formation of the reducing adduct radicals is negligible. In contrast, a reverse trend was observed in the case of $O^{•-}$ reaction, where the yields of $MV^{•+}$ and $TMPD^{•+}$ were about 40% and $\sim$5% OH$^•$, respectively.

f. Reaction Mechanism. In caffeine, the three possible sites of OH$^•$ addition are at C-4 (reaction 5, Scheme 1), C-5, and C-8 positions (reaction 6). The attack at C-5 position is not likely since the X−5OH$^•$ adduct will be structuraly unstable. The unimolecular decay observed at 500 nm in neutral solutions is due to the OH^- elimination from the X−4OH$^•$ adduct (reaction 9a) leading to the formation of the radical cation in analogy with the behavior reported[9,10] in adenine derivatives. Confirming this are the conductivity data (the yield of OH^- and its rate of formation, Table 1), which are in excellent agreement with the corresponding values obtained in our optical absorption measurements supporting the proposed OH^- elimination from X−4OH$^•$.

The radical cation, $(X-4)^{•+}$, is stabilized by the +I effect of the methyl group that is expected to be oxidizing in nature. Thus, about 35% OH$^•$ corresponding to the yields of $TMPD^{•+}$ in optical and OH^- in conductivity experiments (reaction 10) seems to attack at C-4 and the remainder adds to the C-8 position of caffeine. The yield of the X−8OH adduct is higher than that of the X−4OH$^•$ adduct owing to the electrophilic nature of the OH radical. Furthermore, being a nitrogen-centered radical, it is relatively more stable and does not undergo ring opening as is observed with other purine derivatives and with its isomer isocaffeine.[17] The lack of attack at C-4 In the case of isocaffeine is possibly due to the steric hindrance from the adjacent methyl groups at the N-3 and N-9 positions.

The OH adduct spectrum recorded in the presence of O_2 at 7 μs after the electron pulse (Figure 3) matches very well with the absorption spectrum measured in $SO_4^{•-}$ reaction in basic solutions (pH 10) at 18 μs after the pulse (*i.e.*, after the reaction of OH^- with the radical cation is complete). The radical cation formed after SO_4^{2-} elimination (reaction 7) gets converted much faster in basic solutions to give the more stable X−8OH$^•$ adduct (reaction 13).

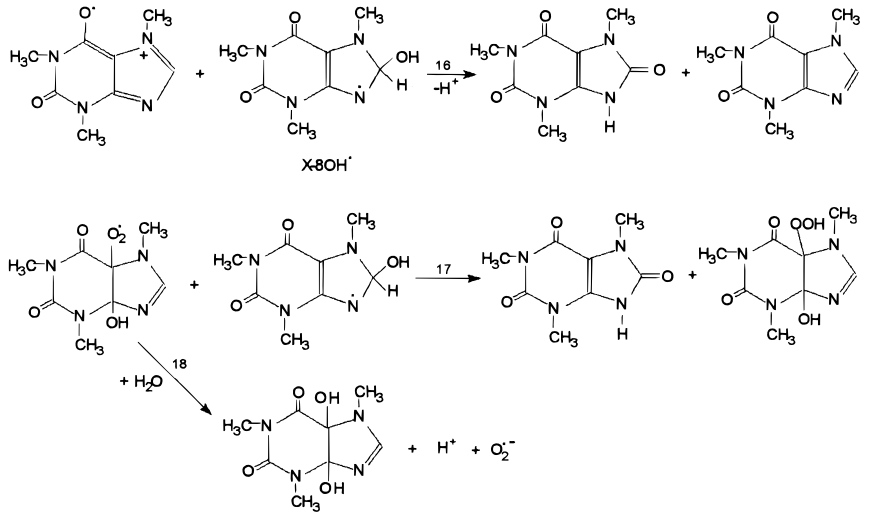
The spectrum obtained in $SO_4^{•-}$ reaction in basic solutions is assigned to this adduct. Since the absorption at 500 nm is completely scavanged by O_2 in N_2O:O_2 (4:1 v/v) ([O_2] = 0.25 mM) solutions within 7 μs, the bimolecular rate constant for the reaction of O_2 with X−4OH$^•$ is estimated to be about 1×10^9 M^{-1} $s^{-1.}$ This rate constant is slightly higher than those observed for the corresponding adducts of dimethyladenosine and trimethyladenine $(4 - 5) \times 10^8$ M^{-1} s^{-1}).[10] However, a reverse trend was seen in the reactivity of O_2 with C-8OH$^•$ adducts of these compounds. For example, the k value for O_2 reaction with A−8OH$^•$ adduct of dimethyladenosine was found to be 1.6×10^{10} M^{-1} s^{-1} (Table 1). The value for isocaffeine is at least $\geq 7 \times 10^7$ M^{-1} s^{-1} based on the observed inhibition of the ring-opening reaction ($k = 1.7 \times 10^4$ s^{-1}) in N_2O:O_2 (4:1) solutions. The corresponding rates in the case of caffeine must be much lower. This is due to the delocalization of the unpaired spin density more on a heteroatom in the X−8OH$^•$ than in X−4OH$^•$ adduct of caffeine. Similar structures of A−4OH$^•$ adducts of methyl derivatives of adenine[10] were shown to be responsible for their low reactivity with O_2. The resulting spectrum obtained from the difference between the OH adduct spectra recorded in deoxygenated and oxygenated solutions must then represent that of X−4OH$^•$.

The molar absorptivities of X−8OH$^•$ and X−4OH$^•$ adducts are found to be 6500 and 5300 M^{-1} cm^{-1}, respectively. These are estimated at 300 and 335 nm from the corresponding yields of OH$^•$ attack (60% and 35% OH$^•$). The X−8OH$^•$ adduct spectrum of caffeine matches reasonably well with the spectra measured for the C-8H$^•$ adduct spectra ($\lambda_{max} = 305$ nm) of caffeine[15] and guanosine[23] obtained in the reaction of e^-_{aq} with these compounds.

In acidic solutions, the X−8OH$^•$ adduct undergoes acid catalyzed water elimination resulting in the formation of its radical cation (reverse of reaction 13). This is in accord with the observed similarity of the absorption spectra induced by OH$^•$ in acidic solution and $SO_4^{•-}$ at neutral pH.

$O^{•-}$ reacts by H-abstraction, besides, addition to the double bonds and abstraction from the CH_3 group at N-1 is more likely because of the electron-withdrawing nature of the neighboring −C═O. This carbon-centered radical ($^•$X(−H)) formed after H-abstraction (reaction 8) will be reducing in nature and reacts with the oxidant methylviologen, as can be seen from the trace recorded ([MV^{2+}] = 5×10^{-5} M) for the absorption of $MV^{•+}$ (inset Figure 2) at 605 nm. The second-order rate constant and

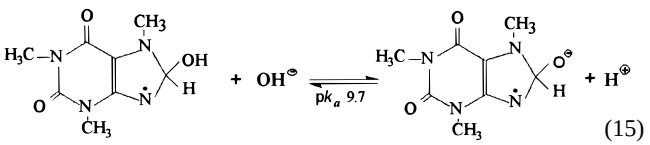
SCHEME 2



the yield of $MV^{\bullet+}$ formed were estimated from this trace are 2×10^9 M^{-1} s^{-1} and 40% $O^{\bullet-}$, respectively. The remaining 60% $O^{\bullet-}$ is assigned to the formation of the radical anion from the addition of $O^{\bullet-}$ at C-4 ($X^{\bullet}-4-O^{-}$) and C-8 ($X^{\bullet}-8-O^{-}$) positions. Such an addition of $O^{\bullet-}$ to halotoluenes besides H-abstraction from the CH_3 was reported[24] by us.

The conductivity data obtained in basic solutions ($8.9 \leq$ pH ≤ 10.5) are shown in Figures 5A−C. In mildly basic solutions (pH ≤ 9), the initial growth of conductivity corresponding to OH^- formation decayed to the base line by a second-order kinetics ($k = (3.0 \pm 0.2) \times 10^9$ M^{-1} s^{-1}). This may be due to the reaction of OH^- either with the radical cation formed from the X−4OH$^{\bullet}$ adduct to form a new product. The other possibility of OH^- reaction with the X−8OH$^{\bullet}$ adduct is not likely as its yield is about 70% OH$^{\bullet}$, and one would expect a net loss of OH^- in that case. It is suggested that the radical cation $(X-4)^{\bullet+}$ formed (reaction 9a, Scheme 1) reacts with OH^- to give X−8OH$^{\bullet}$ (reaction 9b, Scheme 1).

The results in more basic solutions ($9.5 \leq$ pH ≤ 10.5) show a net loss of OH^- immediately after the pulse, suggesting a new reaction that consumes OH^- compared to the growth followed by decay in conductivity observed in less basic solutions. The limiting value for the latter process is pH 9, as the trace recorded at pH 9.3 (inset Figure 5B) showed a mixed behavior with the signal eventually decaying to a negative value. The rate for the former reaction was seen to depend on the [OH^-] (inset Figure 5C) and the second-order rate constant for this reaction was evaluated to be 6×10^9 M^{-1} s^{-1} and an intercept of 3×10^5 s^{-1}. We interpret this as an equilibrium between X−8OH$^{\bullet}$ and OH^- (reaction 15). If the reaction is an approach to equillibrium, one obtains a pK_a value of approximately 9.7. This pK_a value is consistent with the magnitude of the conductivity signal as a function of [OH^-].



(15)

($k_f = 6 \times 10^9$ M^{-1} s^{-1}, $k_b = 3 \times 10^5$ s^{-1})

It seems unlikely that it is the reaction with X−4OH$^{\bullet}$ adduct because of the kinetic shape of the conductivity traces obtained below pH 9.4 Also, if the equillibrium were with X−4OH$^{\bullet}$, the adduct would eventually react *via* OH^- elimination mechanism. Finally, there is a slower reaction ocurring on a millisecond scale at pH 10.6 (Figure 5C) that decreases the conductivity and the increased size of this total signal suggests that both X−4OH$^{\bullet}$ and X−8OH$^{\bullet}$ adducts are reacting and destroying OH^-. The mechanism for this reaction is not clear at present

g. Product Studies. Aqueous solutions containing 1 mM caffeine were ^{60}Co γ-irradiated to obtain the product distribution in reactions of OH$^{\bullet}$ with and without O_2 and in $SO_4^{\bullet-}$reaction. The doses were such that the conversion was below 15% assumming $G(OH^{\bullet}) = 6$ (G is the yield/100 eV energy absorbed). The HPLC analysis has shown the formation of only one major product with a retention time of 3.6 min in all of the cases except in the $SO_4^{\bullet-}$ reaction in basic solutions (pH∼10). Recently, it has been shown[25] that the Fenton type oxidation of caffeine leads to the formation of products arising from hydroxylation, demethylation, and C(8)−N(9) bond scission. The product formed in caffeine is attributed to 1,3,7-trimethyluric acid (8−OH−X) in accord with the published work[10] on the formation of 8−hydroxyadenines from the oxidation of the corresponding A−8OH$^{\bullet}$ adduct and based on the similarity of the chromatogram obtained in our work to that reported[12] with isocaffeine.

The yields of 8−OH−X in reaction of OH$^{\bullet}$ with and without O_2 are 12% and 28% OH$^{\bullet}$, respectively (Table 1). The formation of the oxidized product even in the absence O_2 must be due to the reaction of the radical cation formed after the elimination of OH^- from X−4OH$^{\bullet}$ (reaction 9a, Scheme 1) with X−8OH$^{\bullet}$ (reaction 16, Scheme 2). Such an explanation was also given for the formation of the oxidized product in TMA.[10] The observed yield of the oxidized product (28% OH$^{\bullet}$) is in reasonable agreement with that of $(X-4)^{\bullet+}$ (30−35% OH$^{\bullet}$).

The formation of the oxidized product in the presence of O_2 can be interpreted in terms of H-abstraction from X−8OH$^{\bullet}$ (reaction 17, Scheme 2), and by the peroxyl radical formed from the addition of O_2 to X−4OH$^{\bullet}$ (reaction 11, Scheme 1). Since the yield of the oxidized product is reduced nearly by 50%, the competing hydrolysis reaction (reaction 18, Scheme 2) leading to the elimination of $O_2^{\bullet-}$ must be equally effective. No formation of the oxidized product of trimethyladenine was noticed[10] in the presence of O_2, which was attributed to the complete elimination of $O_2^{\bullet-}$ from the peroxyl radical. The H-abstraction from A−8OH$^{\bullet}$ is not likely to occur due to its ring-opening because the competing ring opening process is too fast.

The formation of the oxidized product of caffeine in reaction of $SO_4^{\bullet-}$ in neutral solutions must be the result of the formation

of X−8OH• (and its subsequent oxidation by $S_2O_8^{2-}$) from the hydrolysis of the radical cation (occurring under steady-state conditions) formed from the initial attack of $SO_4^{\bullet-}$. The lack of formation of 1,3,7-trimethyluric acid in basic solutions is because of the deprotonation of the X−8OH• adduct and its subsequent ring opening. The estimated pK_a value (9.7) for X−8OH• from the conductivity is in support of this observation.

Conclusions

Reaction of OH radicals with caffeine yields the OH adducts at C-4 and C-8 positions, which absorb between 300−330 nm. Ring opening of the X−8OH• has not been observed; but the X−4OH• adduct undergoes OH^- elimination. The yield of the latter adduct estimated from both optical and conductivity measurements is 35% OH•, and the rest is assigned to X−8OH•. The position of the CH_3 group at N-9 or N-7 of the imidazole ring does seem to affect not only the yields of the isomeric OH adducts but also their transformation rates. The resolved spectra of X−8OH• and X−4OH• have λ_{max} at 300 and 335 nm, respectively, and the corresponding ϵ values are 6500 and 5300 M^{-1} cm^{-1}.

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References and Notes

(1) Dedicated to Professor M. S. Wadia on his 60th birthday. This work forms a part of the Ph.D. thesis of M.V. to be submited to the University of Pune.

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