Radiation-induced Reduction of Thymine Derivatives in Aqueous Solution. Part 4.[1] Promoted Transformation of Thymine Glycol into Thymine by Aromatic Amines and Low-valent Transition Metal Salts

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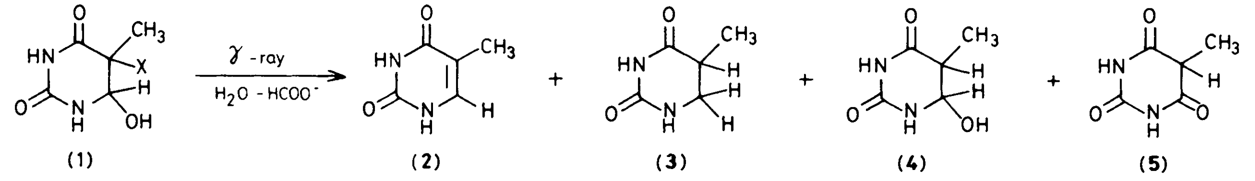
The radiation-induced reduction of thymine glycol [(**1a**), *cis*-5,6-dihydroxy-5,6-dihydrothymine] in aqueous solution containing sodium formate (pH 7.0) has been studied under deaerated and N_2O-saturated conditions at room temperature. For comparison, the radiolysis of (**1a**) has also been performed with alcohols as a scavenger of $^{\bullet}OH$ and $H^{\bullet}$, instead of formate. γ-Irradiation of the deaerated formate solution gave thymine (**2**), 5,6-dihydrothymine (**3**), and 6-hydroxy-5,6-dihydrothymine (**4**) along with a minor product, 5-methylbarbituric acid (**5**). The radiolytic transformation of (**1a**) into these products was inefficient under N_2O, indicating that $CO_2^{-\bullet}$ as a primary active species produced by irradiation has much less ability to reduce (**1a**) compared with e^-_{aq}. The presence of either inorganic salts (**6a—c**) or aromatic amines (**7e—f**) greatly promoted the total formation of (**2**) + (**3**), which depressed those of (**4**) and (**5**). The sum of the *G* values (= molecules/100 eV of absorbed energy) of (**2**) and (**3**) {*G*[(**2**) + (**3**)]} increased and that of (**4**) {*G*[(**4**)]} decreased, respectively, in the sigmoidal forms with decreasing one-electron oxidation potential of the amines. The ratio *G*[(**2**) + (**3**)]/*G*[(**4**)] increased upon increasing the concentration of *o*-phenylenediamine (**7e**). In the absence of added reductants, (**6a—e**) or (**7a—g**), the yields of (**2**) + (**3**) and (**4**) changed either with the formate concentration or with variation of alcohols used as radical scavengers. The mechanism involving consecutive one-electron reductions of (**1a**) and an intermediate 6-hydroxythymin-5-yl radical (**8**) is discussed and compared with the reduction of 5-bromo-6-hydroxy-5,6-dihydrothymine (**1b**).

The highly efficient transformation of 5,6-dihydropyrimidines ($PyrH_2$), *e.g.* 5,6-dihydrouracil and 5,6-dihydrothymine (**3**), into the parent pyrimidines (Pyr) has been observed in the radiolysis of aqueous systems containing high-valent transition metal ions such as Cu^{2+} and $Fe(CN)_6^{3-}$.[2–4] In accord with the pulse radiolysis studies, the proposed mechanism involves two key steps: (i) formation of dihydropyrimidinyl radical ($PyrH^{\bullet}$) *via* hydrogen-atom abstraction from $PyrH_2$ by $^{\bullet}OH$ ($PyrH_2$ + $^{\bullet}OH \longrightarrow PyrH^{\bullet} + H_2O$), and (ii) one-electron oxidation of the $PyrH^{\bullet}$ by the metal ions to produce dihydropyrimidine cation ($PyrH^+$) followed by proton dissociation ($PyrH^+ \longrightarrow Pyr + H^+$).[5,6] With respect to the radiation-induced *reductive* transformation into pyrimidines, however, few investigations have been performed despite having radiation chemical and biological interest equal to that of the *oxidative* process. Recently we have reported in preliminary form that the



		(2)	(3)	(4)	(5)
X = OH (1a) pH 7·0	none	23%		55%	3%
	FeSO₄(6a), 1·0 mM	73%		14%	trace
	TMPD (7f), 0·2 mM	60%		16%	2%
X = Br (1b) pH 3·0	none	8%	trace	19%	15%
	FeSO₄(6a), 1·0 mM	90%	trace	trace	trace

Scheme.

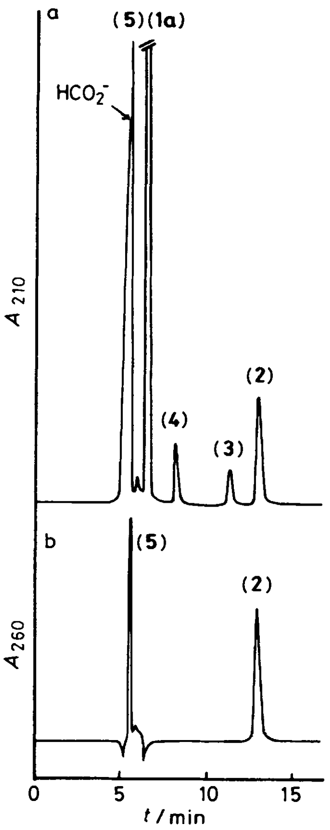


Figure 1. H.p.l.c. charts of irradiated solution of thymine glycol (**1a**) monitored by the u.v. absorbance (A) at (a) 210 and (b) 260 nm: (**2**), thymine; (**3**), 5,6-dihydrothymine; (**4**), 6-hydroxy-5,6-dihydrothymine; (**5**), 5-methylbarbituric acid

reduction of 5-bromo-6-hydroxy-5,6-dihydrothymine (**1b**) to thymine (**2**) in the radiolysis of deaerated aqueous solution (pH 3.0) containing sodium formate is promoted by the addition of low-valent transition metal salts such as $FeSO_4$ (**6a**) [see Scheme where the yields are based on consumed (**1b**)].[7] Concerning the mechanism, we also obtained evidence supporting a one-electron reduction of a possible intermediate 6-hydroxythymin-5-yl radical (**8**) by the metal ions.

This paper describes the further finding that low-valent transition metal salts, *e.g.* (**6a**), and aromatic amines, *e.g.* *NNN'N'*-tetramethyl-*p*-phenylenediamine [(**7f**), TMPD], can be effective reductants promoting the radiolytic transformation of thymine glycol [(**1a**), *cis*-5,6-dihydroxy-5,6-dihydrothymine] into thymine (**2**) in deaerated formate solution at pH 7.0 (Scheme). Although the radiolysis of (**1a**) in deaerated aqueous solution has been previously reported to produce (**2**),[8] the mechanistic details still remain equivocal. In the light of this study for (**1a**) and the previous results for (**1b**), we present herein the generalized mechanism of the radiation-induced reductive transformation of 5-substituted-6-hydroxy-5,6-dihydrothymine into (**2**).

Experimental

Materials.—Thymine glycol [(**1a**), *cis*-5,6-dihydroxy-5,6-dihydrothymine],[9] 6-hydroxy-5,6-dihydrothymine (**4**),[10] and 5-methylbarbituric acid (**5**)[11] were prepared following reported methods. Thymine (**2**) and 5,6-dihydrothymine (**3**) were obtained from Sigma. The aromatic amines (**7a**—**g**) and the other chemicals were of the best available grades and used without further purification. Oxygen-free N_2O gas was supplied by Seitetsu Chemicals. Predistilled water was purified by the successive distillations from acid dichromate, from alkaline permanganate, and finally without additives.

γ-*Irradiation.*—Typically, a solution of (**1a**) (1mM) in distilled water (3 ml) containing sodium formate (100mM) and an additive, inorganic salt [(**6a**—**e**), 1.0mM] or aromatic amine [(**7a**—**g**), 0.2mM], was buffered at pH 7.0 ± 0.1 with sodium phosphate (2mM). In the comparative experiments, solutions of (**1a**) (1mM; pH 7.0 ± 0.1) containing alcohols such as propan-2-ol, ethanol, and 2-methylpropan-2-ol (150mM) were used. The solution in a Pyrex glass ampoule was either deaerated by repeated freeze–pump–thaw cycles on a vacuum line (*ca.* 0.4 Pa) or purged with N_2O or N_2O–O_2 (4:1 v/v) for 20 min, and then sealed off prior to irradiation. Irradiation was performed at room temperature with a ^{60}Co γ-ray source. The dose rate was determined as 280 Gy h^{-1} * using a standard Fricke dosimeter (*G* value † of Fe^{3+} = 15.5). It was confirmed by h.p.l.c. that the dark reaction to produce (**2**) or any other products is negligible under these conditions.

H.p.l.c. Analysis.—The irradiated solution (100 μl) was analysed by h.p.l.c. using a Toyo Soda HLC-803 high-performance liquid chromatograph equipped with a reversed-phase column (Machery Nagel Nucleosil $7C_{18}$, 4 mm × 30 cm). The H_2O–MeOH (95:5 v/v) mixture was delivered as the mobile phase at a flow rate of 0.6 ml min^{-1}. For the analysis of amines (**7a**—**g**), H_2O–MeOH of 30:70 composition was used as an eluant. The column eluates were monitored by u.v. absorbance at 210 or 260 nm using a Toyo Soda UV-8 spectrophotometer.

Results and Discussion

Radiolysis of Thymine Glycol (**1a**) *in Aqueous Formate Solution.*—H.p.l.c. analysis of a formate solution of (**1a**) irradiated under deaerated conditions revealed the formation of (**2**)—(**4**) together with a minor product (**5**) (Figure 1). These products were confirmed by comparison with authentic samples. In the h.p.l.c. chart the elution-peak separation between formate and (**5**) was insufficient when monitored at 210 nm (Figure 1a), but (**5**) could be successfully quantified from the u.v. absorbance at 260 nm because the peak intensity of the formate was negligibly small at this wavelength (Figure 1b). In addition, since (**4**) was very stable with a half-life of *ca.* 8 days at pH 7.0 (24 °C), the formation of (**2**) *via* dehydration of (**4**) is minor under these conditions. This is also the case even at pH 3.0 as shown previously.[7]

Figure 2 shows a dose–conversion plot for (**1a**) and dose–yield plots for products (**2**)—(**5**). The concentration of (**1a**) decreased and those of (**4**) and (**5**) increased linearly with an irradiation dose up to 1.12 kGy. In contrast, the concentration changes of (**2**) and (**3**) showed nonlinear dose dependences. Thus, the prolonged irradiation caused the consumption of initially produced (**2**) with the enhanced formation of (**3**). In the light of the reactivity of (**2**) which is readily reduced to (**3**) in the radiolysis of the deaerated formate system,[12,13] the consecutive reductions of equation (1) seem to proceed. This is, however, not

$$(\mathbf{1a}) \longrightarrow (\mathbf{2}) \longrightarrow (\mathbf{3}) \qquad (1)$$

the case for the radiolysis of (**1b**) at pH 3.0 under similar conditions, in which (**2**) was also produced with a trace amount

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* 1 Gy = 100 rad = 1 J kg^{-1}.

† The *G* value is defined as the number of molecules formed or consumed per 100 eV of energy absorbed by the solution. *G* = 1 corresponds to concentration change by 0.1036μM over an irradiation dose of 1 Gy.

Table 1. Radiolysis of thymine glycol (**1a**) in deaerated, N_2O-, and N_2O–O_2 (4:1 v/v)-saturated aqueous solutions containing 0.1M-sodium formate; dose 0—1.12 kGy

Solution	Reducing species			Product			
	$G(e_{aq}^-)$	$G(CO_2^{-\bullet})$	$G[-(\mathbf{1a})]$	$G[(\mathbf{2}) + (\mathbf{3})]$	$G[(\mathbf{4})]$	$G[(\mathbf{5})]$	G_{total}
Deaerated + formate	2.7	3.25	2.26	0.51	1.25	0.07	1.83
N_2O + formate	0	5.95	0.27	Trace	Trace	Trace	Trace
N_2O–O_2 + formate	0[a]	0[a]	0.00	0.00	0.00	0.00	0.00

[a] e_{aq}^- and $CO_2^{-\bullet}$ are quantitatively converted into unreactive $O_2^{-\bullet}$.

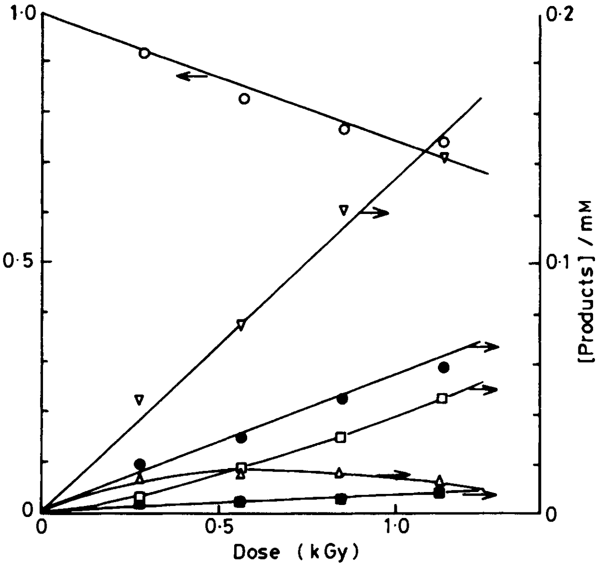

Figure 2. Dose–conversion plot (○) for thymine glycol (**1a**) and dose–yield plots for products, (△) thymine (**2**), (□) 5,6-dihydrothymine (**3**), (▽) 6-hydroxy-5,6-dihydrothymine (**4**), (■) 5-methylbarbituric acid (**5**), (●) (**2**) + (**3**), in the radiolysis of deaerated aqueous solution containing 1.0mM-(**1a**) and 100mM-sodium formate

of (**3**).[7] It is suggested that the reduction potential of (**2**) as a common product for these systems is more positive than that of (**1a**), and more negative than that of (**1b**). In accord with reaction (1), on the other hand, the concentration of (**2**) plus (**3**) was found to increase linearly with the irradiation dose as plotted in Figure 2. From the slopes of the straight lines in Figure 2, the G values (molecules/100 eV of absorbed energy) for the conversion of (**1a**) $\{G[-(\mathbf{1a})]\}$ and for the formation of the products, the sum of (**2**) and (**3**) $\{G[(\mathbf{2}) + (\mathbf{3})]\}$, (**4**) $\{G(\mathbf{4})]\}$, and (**5**) $\{G[(\mathbf{5})]\}$, were obtained and are listed in Table 1. It is noted in Table 1 that 81% of the consumed (**1a**) accounts for products (**2**)—(**5**) in deaerated formate solution (see also Scheme).

In a dilute aqueous solution system, under deaerated conditions, the radiolysis of water first occurs to produce the primary active species in equation (2). The presence of excess

$$H_2O \rightsquigarrow {}^{\bullet}OH\ (G = 2.7),\ H^{\bullet}\ (0.55),\ e_{aq}^-\ (2.7) \qquad (2)$$

of formate, as in the present systems, converts $^{\bullet}OH$ and $H^{\bullet}$ into a reducing species $CO_2^{-\bullet}$ almost quantitatively. Thus, the

$$^{\bullet}OH(H^{\bullet}) + HCO_2^- \longrightarrow H_2O(H_2) + CO_2^{-\bullet} \qquad (3)$$

hydrated electron (e_{aq}^-) and $CO_2^{-\bullet}$ are potentially active species for the reduction of (**1a**). In order to compare the individual reducing abilities of e_{aq}^- and $CO_2^{-\bullet}$ toward (**1a**), an aqueous solution (pH 7.0) of (**1a**) (1mM) containing sodium formate (100mM) was also irradiated under N_2O-saturated conditions. In contrast to the deaerated formate solution, radiolysis under N_2O is expected to produce a single active species $CO_2^{-\bullet}$ (G = 5.95) for the reaction of (**1a**), because e_{aq}^- will be converted into $CO_2^{-\bullet}$ *via* reactions (4) and (3).

$$e_{aq}^- + N_2O \longrightarrow {}^{\bullet}OH + OH^- + N_2 \qquad (4)$$

As shown in Table 1, N_2O saturation of the formate solution lowered $G[-(\mathbf{1a})]$ to *ca.* 1/10 compared with the value under deaerated conditions. The ratio of $G[-(\mathbf{1a})]$ (= 0.27) to $G(CO_2^{-\bullet})$ (=5.95) provides a measure of the efficiency of $CO_2^{-\bullet}$ for the reduction of (**1a**) as 0.05, *i.e.* only 5% of the total $CO_2^{-\bullet}$ generated is effective for the conversion of (**1a**). A possible fate of the residual $CO_2^{-\bullet}$ (95%) is bimolecular disappearance to produce oxalic acid [reaction (5)].[13] Support for the reduction of (**1a**) with $CO_2^{-\bullet}$ was obtained by

$$2\,CO_2^{-\bullet} + 2H^+ \longrightarrow (CO_2H)_2 \qquad (5)$$

irradiation of an N_2O–O_2-saturated formate solution; the conversion of (**1a**) was completely inhibited, probably because of preferential electron transfer from $CO_2^{-\bullet}$ to O_2 with formation of unreactive $O_2^{-\bullet}$. Using the efficiency of $CO_2^{-\bullet}$ described above, the $G[-(\mathbf{1a})]$ originated from the reduction by e_{aq}^- in deaerated formate solution [$G(e_{aq}^-)$ = 2.7 and $G(CO_2^{-\bullet})$ = 3.25] is estimated as 2.10 (= 2.26 − 0.05 × 3.25). Thus, the efficiency of e_{aq}^- for the reductive conversion of (**1a**) is 0.78. These results clearly show that $CO_2^{-\bullet}$ makes quite a small contribution (*ca.* 7%), compared with e_{aq}^-, to the reduction of (**1a**). This is in contrast to the reductions of (**2**),[12] thymidine,[1] and (**1b**),[7] in which the $CO_2^{-\bullet}$ had potent reducing ability comparable with that of e_{aq}^-. It is likely that the reduction potentials of these compounds are more positive than that of (**1a**).*

Effect of Inorganic Salts (**6a**—**e**).—As for (**1b**),[7] addition of a low-valent transition metal salt (**6a**) (1.0mM) promoted the radiolytic reduction of (**1a**) to (**2**) or (**3**) with a decreased yield of (**4**) in deaerated formate solution (Scheme). Among the inorganic salts studied, $K_4Fe(CN)_6$ (**6b**) and $Na_2S_2O_3$ (**6c**) were

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* One-electron reduction potential will increase in the following order; $E[(\mathbf{1a})/(\mathbf{1a}^{-\bullet})] < E(CO_2/CO_2^{-\bullet})$ (−1.3 V[14] and < −0.64 V[15] *versus* s.c.e. in water, respectively) $< E[(\mathbf{1b})/(\mathbf{1b}^{-\bullet})]$ (−0.47 V[7] *versus* s.c.e. in acetonitrile).

Table 2. Effect of inorganic reductants (**6a**—**e**) on the radiolysis of thymine glycol (**1a**)[a]

		product	
Additive	$G[-(\mathbf{1a})]$	$G[(\mathbf{2}) + (\mathbf{3})]$	$G[(\mathbf{4})]$
(**6a**) $FeSO_4$	1.87	1.36	0.26
(**6b**) $K_4Fe(CN)_6$	2.13	1.60	0.35
(**6c**) $Na_2S_2O_3$	2.01	1.26	0.69
(**6d**) $SnCl_2$	2.53	0.69	0.93
(**6e**) Na_2SO_3	2.13	0.44	1.07
None[b]	2.26	0.51	1.25

[a] In deaerated aqueous solution containing 0.1M-sodium formate, dose 0.84 kGy, [(**1a**)] = [(**6a**—**e**)] = 1.0mM, pH 7.0. [b] Dose 0—1.12 kGy.

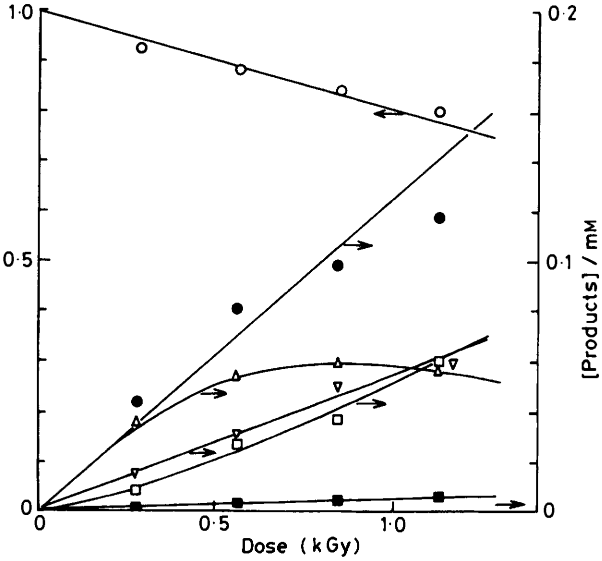

Figure 3. Dose–conversion plot for thymine glycol (**1a**) and dose–yield plots for products (**2**)–(**5**) in the radiolysis of deaerated aqueous solution containing 1.0mM-(**1a**), 100mM-sodium formate, and 0.2mM-*o*-phenylenediamine (**7e**). The symbols designate the same compounds as in Figure 2

also effective for the enhancement of $G[(\mathbf{2}) + (\mathbf{3})]$, while the relatively less reducing $SnCl_2$ (**6d**) and Na_2SO_3 (**6e**) were virtually ineffective (Table 2). These results strongly suggest a mechanistic similarity between the promoted reductions of (**1a**) and (**1b**) to produce (**2**). It seems most likely that radiolysis of not only (**1b**) but also (**1a**) in deaerated formate solution leads to the formation of 6-hydroxythymin-5-yl radical (**8**). The one-electron reduction of (**8**) by certain inorganic reductants such as (**6a**—**c**) accounts for the promoted transformation of (**1a**) to (**2**).

Effect of Aromatic Amines (**7a**—**g**).—Recently, Fujita and Steenken have suggested from a pulse radiolysis study that (**8**) undergoes one-electron reduction by TMPD (**7f**) with formation of $TMPD^{+\cdot}$.[16] For comparison, a further attempt was made to use several aromatic amines (**7a**—**g**) including TMPD as the electron donors for promoting the radiolytic transformation of (**1a**) into (**2**). As shown in the Scheme, the promotion effect of (**7f**) (0.2mM) turned out to be comparable with that of (**6a**) (1.0mM).

Figure 3 shows the representative dose–yield plots for (**2**)—(**5**) produced in the radiolysis of (**1a**) with *o*-phenylenediamine (**7e**) (0.2mM). Obviously, the dose dependence of the formation of these products is similar to that observed without (**7e**) (Figure 2), although the yield of (**2**) plus (**3**) is enhanced and that of (**4**) is reduced significantly. From the dose–yield plots as in Figure 3, the values of $G[(\mathbf{2}) + (\mathbf{3})]$, $G[(\mathbf{4})]$, and $G[(\mathbf{5})]$ in the presence of a series of aromatic amines (**7a**—**g**) were evaluated (Table 3). It is worth noting that the G values of the major products (**2**)—(**4**) depend on the half-wave one-electron oxidation potential ($E_{1/2}^{Ox}$)[17] of the amines measured against Ag–0.1M-Ag^+ in acetonitrile. Thus, the $G[(\mathbf{2}) + (\mathbf{3})]$ increased whereas the $G(\mathbf{4})$ decreased, in the sigmoidal forms, with decreasing oxidation potential $E_{1/2}^{Ox}$ (Figure 4). The maximum $G[(\mathbf{2}) + (\mathbf{3})]$ value of *ca.* 1.05 could be ultimately attained by the use of amines with negative $E_{1/2}^{Ox}$ values. The result in Figure 4 suggests the redox-related mode of action of aromatic amines for promoting the radiolytic transformation of (**1a**) into (**2**).

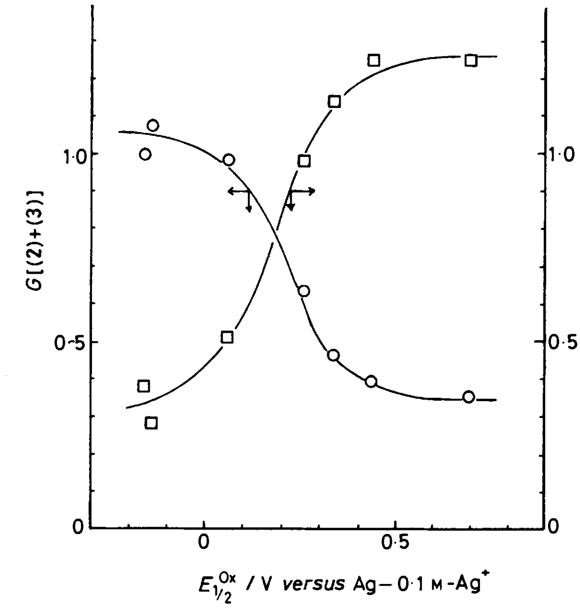

Figure 4. Variation of $G[(\mathbf{2}) + (\mathbf{3})]$ and $G[(\mathbf{4})]$ as a function of the half-wave oxidation potential ($E_{1/2}^{Ox}$ *versus* Ag–0.1M-Ag^+) of aromatic amines. The data points are based on the values in Table 3

It is also interesting that the amines (**7a**—**f**), except *p*-phenylenediamine (**7g**), in the initial concentration range of 0.01 to 1.00mM are not consumed during the irradiation.* This indicates the catalytic nature of these amines in the promoted formation of (**2**), presumably originating from the reversible redox process between the amine and the corresponding cation-radical intermediate $[(\mathbf{7}) \underset{+e}{\overset{-e}{\rightleftharpoons}} (\mathbf{7})^{+\cdot}]$. The similar catalytic nature of $FeSO_4$ (**6a**) was also indicated by the failure to observe the appearance of a u.v. absorption band centred at 304 nm, which is characteristic of Fe^{3+} (ε 2 201 l mol^{-1} cm^{-1} in 0.4M-H_2SO_4),[18] during the irradiation of a deaerated formate solution of (**1a**). This is in contrast to the behaviour of the (**6a**) in the radiolysis of (**1b**) under similar conditions; *i.e.* one-electron oxidation of Fe^{2+} to Fe^{3+} proceeded irreversibly.[7] As discussed below, the characteristic reactivity of $CO_2^{-\cdot}$ is possible to determine the action of amines (**7a**—**f**) and metal salt (**6a**) whether catalytic as in the (**1a**) system or non-catalytic as in the (**1b**) system.

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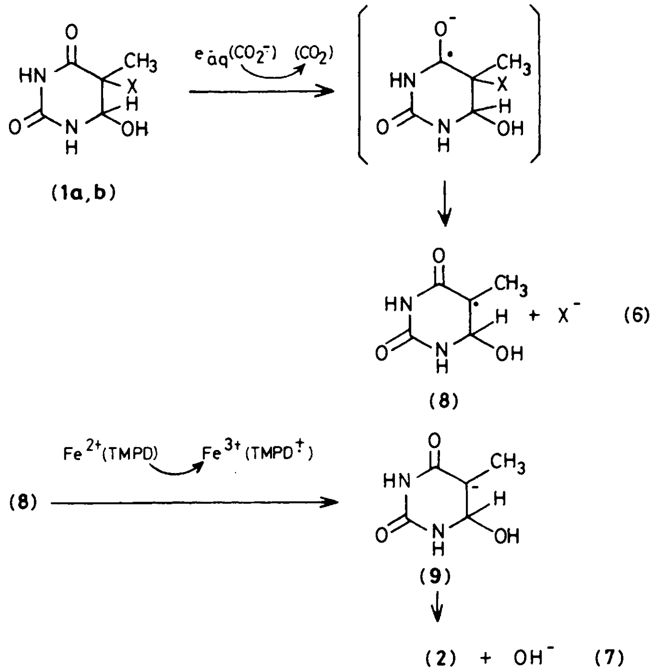
* This was confirmed by h.p.l.c. analysis, in which the amines (**7a**—**g**) were eluted with much larger retention volumes than (**1a**) and radiolysis products.

Table 3. G Values for the conversion of thymine glycol $\{G[-(\mathbf{1a})]\}$ and the formation of radiolysis products $\{G[(\mathbf{2}) + (\mathbf{3})], G[(\mathbf{4})], G[(\mathbf{5})]\}$ in th presence of aromatic amines (**7a**—**g**)[a]

			Product				
Amine	$E^{Ox}_{1/2}$/V[b]	$G[-(\mathbf{1a})]$	$G[(\mathbf{2}) + (\mathbf{3})]$	$G[(\mathbf{4})]$	$G[(\mathbf{5})]$	G_{total}	(%)[c]
Aniline (**7a**)	0.70	2.15	0.35	1.25	0.05	1.60	(74)
p-Toluidine (**7b**)	0.44	2.01	0.39	1.25	0.05	1.64	(82)
o-Anisidine (**7c**)	0.34	2.15	0.46	1.14	0.05	1.60	(74)
p-Anisidine (**7d**)	0.26	1.79	0.63	0.98	0.05	1.61	(90)
o-Phenylenediamine (**7e**)	0.06	1.67	0.98	0.51	0.04	1.49	(89)
TMPD (**7f**)	−0.14	1.79	1.07	0.28	0.03	1.35	(77)
p-Phenylenediamine (**7g**)	−0.16	2.15	0.99	0.38	0.00	1.37	(64)
None	—	2.26	0.51	1.25	0.07	1.83	(81)

[a] In deaerated aqueous solution containing 0.1M-sodium formate, pH 7.0, [(**1a**)] = 1.0mM, [(**7a**—**g**)] = 0.2mM, dose 0—1.12 kGy. [b] Half-wave oxidation potential *versus* Ag–0.1M-Ag$^+$ couple.[17] [c] $(\{G[(\mathbf{2}) + (\mathbf{3})] + G[(\mathbf{4})] + G[(\mathbf{5})]/G[-(\mathbf{1a})]\} \times 100)$.



Mechanism of the Radiolytic Transformation of (**1a,b**) *into* (**2**) *in the Presence of Aromatic Amine or Inorganic Reductant.*—For the radiolyses of (**1a**) and (**1b**) to produce (**2**) in deaerated formate solution with potent reductants, *e.g.* $FeSO_4$ (**6a**) and TMPD (**7f**), the generalized reaction pathway is proposed as follows. The primary active species involved in these radiolysis systems are possibly e^-_{aq} and $CO_2^{-\cdot}$ [see reactions (2) and (3)]. In the initial step, (**1a**) undergoes reduction almost exclusively by e^-_{aq} while (**1b**) by both e^-_{aq} and $CO_2^{-\cdot}$, producing a common intermediate of 6-hydroxythymin-5-yl radical (**8**) *via* elimination of hydroxide or bromide ion (X^-) from the anion radicals of (**1a,b**). Previously Steenken demonstrated that ionized 1,2-dihydroxyalkyl radicals $[R^1\dot{C}(O^-)C(OH)\dot{C}R^3R^2]$, as is the case for the anion radical of (**1a**) [see reaction (6)], undergo OH^- elimination (*k ca.* 3×10^{-6} s^{-1}) to give 2-oxoalkanyl radicals $(R^1CO\dot{C}R^2R^3)$.[19] The key step leading to (**2**) in the presence of *e.g.* Fe^{2+} (**6a**) or TMPD (**7f**) would be one-electron reduction of the radical (**8**) to the anion intermediate (**9**) and subsequent OH^- elimination as in reactions (6) and (7). Reaction (7) is also in accord with the characteristic dependence of the promoted formation of (**2**) [and (**3**) in the case of (**1a**)] on the reducing ability of the additives and with the reported reactivity of (**8**).[16] Since none of the amines and metal salts could reduce (**1a,b**) directly, the one-electron reduction potential of (**8**) is considered to lie sufficiently above that of the (**1a,b**). Furthermore, the resulting $TMPD^{+\cdot}$ or Fe^{3+} in reaction (7) should undergo one-electron reduction by e^-_{aq} and more probably by $CO_2^{-\cdot}$ to regenerate TMPD or Fe^{2+}, as indicated by the catalytic behaviour.* It is at least likely that such a turnover reaction with $CO_2^{-\cdot}$ competes with the bimolecular disappearance of the $CO_2^{-\cdot}$ [reaction (5)], particularly in the radiolysis system of (**1a**).

The above scheme [reaction (7)] implies that the yield of (**2**) is dependent on the efficiency of the formation of (**9**). One may expect that the conversion of (**8**) into (**9**) becomes more favourable upon increasing the reducing ability of the added reductants, thereby increasing the exothermicity ($-\Delta G$) of electron transfer. The observation by Rehm and Weller[21] and subsequent studies[22] have shown that the rate of exothermic electron-transfer reaction reaches a constant at diffusion-controlled limit. This seems to be the case for the use of aromatic amines (Figure 4). Interestingly, however, with metal salts decreases of the yield of (**2**) after reaching a maximum have been noted at larger exothermicities,[7] which agrees with the prediction of the Marcus theory.[23,24]

Mechanism of the Radiolytic Transformation of (**1a**) *into* (**2**) *and* (**4**) *in the Absence of Added Reductants.*—The mechanism of the radiolytic transformation of (**1a,b**) into (**2**) in the absence of action of the added reductants is also of basic interest. To approach this problem, it should be noted that the yield of (**2**) [plus (**3**) as a related product] based on consumed (**1a**) at pH 7.0 is *ca.* 3 times as large as that in the case of (**1b**) at pH 3.0 (see

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* The deaerated aqueous solution of (**1a**) (1mM) containing TMPD (0.2mM) and 2-methylpropan-2-ol (150mM) was irradiated. Under these conditions where e^-_{aq} and $(CH_3)_2(\dot{C}H_2)COH$ are major primary active species, values of $G[-(\mathbf{1a})] = 2.08$, $G[(\mathbf{2}) + (\mathbf{3})] = 1.49$, $G(\mathbf{4}) = 0.34$, and $G(-\mathrm{TMPD}) = 2.77$ were obtained in the dose range up to 0.92 kGy. The formation of $TMPD^{+\cdot}$ was not observed by u.v. spectroscopy ($TMPD^{+\cdot}$, $\lambda_{max.}$ 325, 565, and 610 nm).[20] In view of the fact that 2-methylpropan-2-ol radical $[(CH_3)_2(\dot{C}H_2)COH]$ has less reducing ability compared with $CO_2^{-\cdot}$ [$E^{Ox}_{1/2} = 0.1$—0.27 V *versus* s.c.e. for $(CH_3)_2(\dot{C}H_2)COH$ and -1.3 or < -0.65 V *versus* s.c.e. for $CO_2^{-\cdot}$],[14,15] it is presumable that $(CH_3)_2(\dot{C}H_2)COH$ reacts with $TMPD^{+\cdot}$, but not by a one-electron reduction mechanism, and thereby prevents the regeneration of TMPD. The decomposition product due to the reaction with $(CH_3)_2(\dot{C}H_2)COH$ was eluted with a retention volume slightly larger than that of TMPD in the h.p.l.c. analysis. The identical product was also produced in the radiolysis of a deaerated aqueous solution containing TMPD (0.2mM) and 2-methylpropan-2-ol (150mM).

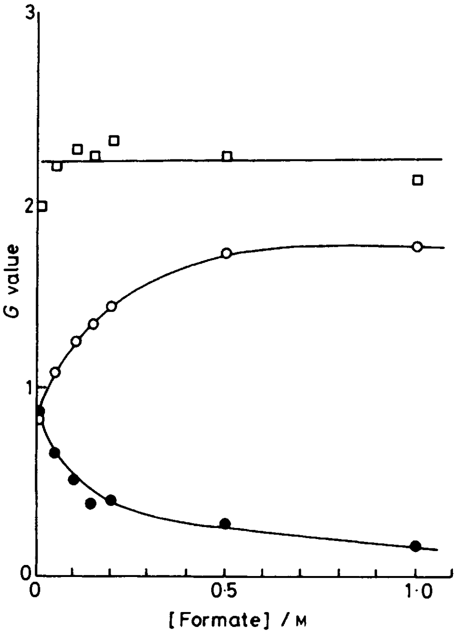


Figure 5. Variations of (○) $G[(\mathbf{2}) + (\mathbf{3})]$, (●) $G[(\mathbf{4})]$, and (□) $G[-(\mathbf{1a})]$ as a function of formate concentration

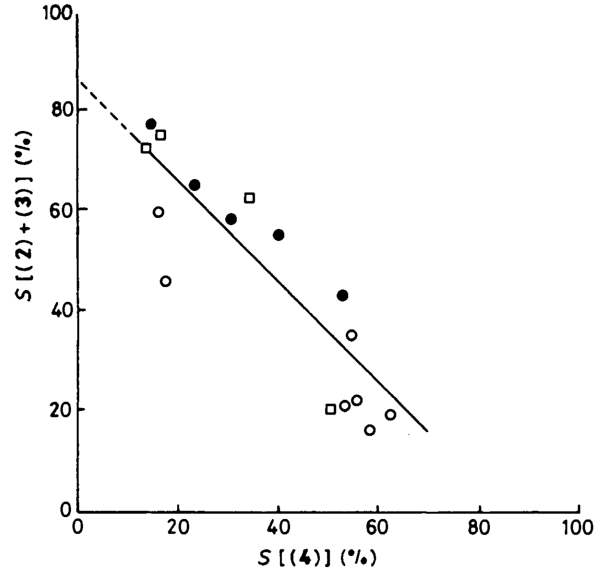
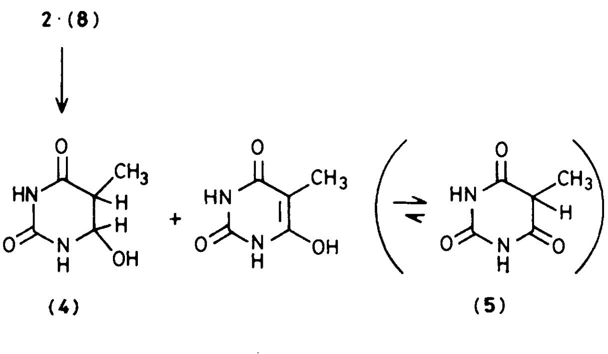


Figure 6. Relationship between product selectivities $S[(\mathbf{2}) + (\mathbf{3})]$ $(= \{G[(\mathbf{2}) + (\mathbf{3})]/G[-(\mathbf{1a})]\} \times 100)$ and $S[(\mathbf{4})](=\{G[(\mathbf{4})]/G[-(\mathbf{1a})]\} \times 100)$: (○) without additive and with aromatic amines (**7a**—**f**) (0.2mM), (□) with inorganic salts (**6a**—**c**, **e**) (1.0mM), (●) with varying concentrations of *o*-phenylenediamine (**7e**) (0.01—1.0mM)

Scheme). Unless the pH effect is important, this difference seems to be closely associated with the reactivity of $CO_2^{-\bullet}$ that could reduce (**1a**) less efficiently than did (**1b**). Thus, a part of the $CO_2^{-\bullet}$ failing to reduce (**1a,b**) may react with the radical (**8**) in competition with the bimolecular disappearance [reaction (5)]. Similar to the process in reaction (7), this reaction leads to (**2**) *via* anion intermediate (**9**). This process is expected to be more important in the (**1a**) system than in the (**1b**) system.

$$(\mathbf{8}) \xrightarrow{CO_2^{-\bullet}\ \ CO_2} (\mathbf{9}) \longrightarrow (\mathbf{2}) + OH^- \qquad (8)$$

As discussed above, the formation of (**3**) characteristic of the (**1a**) system is accounted for by the secondary reaction of (**2**) with reducing species e_{aq}^- and $CO_2^{-\bullet}$.[1,12,13]



(9)

(**4**) (**5**)

Concerning the formation of (**4**) and (**5**) in the radiolysis of (**1b**) at pH 3.0, on the other hand, we previously proposed the disproportionation reaction of the radicals (**8**) with each other [reaction (9)].[7] Evidence supporting reaction (9) is that the yields of (**4**) and (**5**) were approximately equal. In contrast, the radiolysis of (**1a**) at pH 7.0 led to (**4**) alike but only minor amounts of (**5**), suggesting that disproportionation as in reaction (9) becomes minor upon raising the pH. In view of the pK_a $(=3.75)$ of HCO_2H,[25] the formate contained in these systems will be in an ionized form (HCO_2^-) at pH 7.0 whereas it will be in an undissociated form (HCO_2H) at pH 3.0. Furthermore, it is important to note that the reactivity of the ionized form HCO_2^- for the hydrogen abstraction of $^{\bullet}OH$ is one order of magnitude greater than that of HCO_2H.[26,27] We therefore concluded that the hydrogen abstraction by (**8**) from HCO_2^- accounts for the formation of (**4**) as a major product at pH 7.0.

$$(\mathbf{8}) + HCO_2^- \longrightarrow (\mathbf{4}) + CO_2^{-\bullet} \qquad (10)$$

According to the proposed mechanism, the G values of (**2**) and (**4**) derived from a common intermediate (**8**) [reactions (8) and (10)] should be dependent on the formate concentration. This was indeed demonstrated in the radiolysis of (**1a**) with varying concentrations of formate (Figure 5); the G value of (**4**) increased and that of (**2**) [and (**3**) as a related product] decreased on increasing the formate concentration. The value of $G[-(\mathbf{1a})]$ was virtually invariant under these conditions.

The foregoing fate of (**8**) as an important intermediate in the radiolysis of (**1a**) at pH 7.0 shows that the increment of the product selectivity of (**2**) plus (**3**), defined by $S[(\mathbf{2}) + (\mathbf{3})]$ $(=\{G[(\mathbf{2}) + (\mathbf{3})]/G[-(\mathbf{1a})]\} \times 100)$, due to the action of aromatic amines and inorganic salts is equal to the decrement of $S[(\mathbf{4})]$ $(=\{G[(\mathbf{4})]/G[-(\mathbf{1a})]\} \times 100)$. Indeed, we obtained a linear relationship given by $S[(\mathbf{2}) + (\mathbf{3})] = -S[(\mathbf{4})] + 86$ (Figure 6). This relationship also indicates that the total yield of the major products (**2**)—(**4**) accounts for on average 86% of consumed (**1a**), regardless of the presence of aromatic amines and inorganic salts.

Another possibility that protonation of the anion intermediate (**9**) occurs to produce (**4**), in competition with the OH^- elimination to (**2**), is ruled out by the following observation. In the presence of reductants the yield of (**2**) increased, presumably as a result of the enhanced formation of (**9**), whereas that of (**4**) decreased (see also Figure 4). This suggests that the intermediate species leading to (**4**) is not the anion (**9**) but the precursor radical (**8**).

Radiolysis of (**1a**) *in the Presence of Alcohol.*—We also carried out the radiolysis of (**1a**) in deaerated aqueous solution (pH 7.0) containing propan-2-ol, ethanol, or 2-methylpropan-2-ol

Table 4. Radiolysis of thymine glycol (**1a**) in deaerated aqueous solution containing 0.15M-alcohol; dose 0—0.92 kGy

	G(active species)[a]			product	
Alcohol	e_{aq}^-	major alcohol radical	G[−(**1a**)]	G[(**2**) + (**3**)]	G[(**4**)]
Propan-2-ol	2.7	3.25 [$(CH_3)_2\dot{C}OH$, < −0.65[b]]	1.58	0.78	0.73
Ethanol	2.7	3.25 [$CH_3\dot{C}HOH$, < −0.65[b]]	1.65	0.37	0.40
2-Methyl-propan-2-ol	2.7	2.7[c] [$(CH_3)_2(\dot{C}H_2)COH$, 0.1—0.27[b]]	2.07	0.12	0.20

[a] Ref. 6. [b] Half-wave oxidation potential measured *versus* s.c.e. in water.[15] [c] Hydrogen atoms are unreactive toward 2-methylpropan-2-ol.[6]

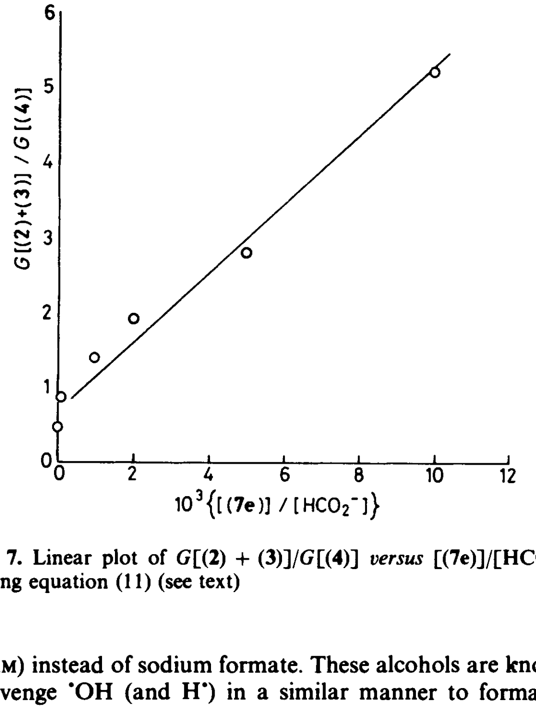


Figure 7. Linear plot of G[(**2**) + (**3**)]/G[(**4**)] *versus* [(**7e**)]/[HCO_2^-] following equation (11) (see text)

(150mM) instead of sodium formate. These alcohols are known to scavenge ˙OH (and H˙) in a similar manner to formate.[6] Thus, major active species under these conditions are e_{aq}^- and alcohol radicals. The observed values of G[(**4**)] 0.73 (propan-2-ol), 0.40 (ethanol), and 0.20 (2-methylpropan-2-ol), were considerably smaller than that (1.25) in formate solution (Tables 1 and 4). Since only *ca.* 5% of $CO_2^{-\bullet}$ (G 3.25) could contribute to one-electron reduction of (**1a**) to (**8**) in formate solution, the decreased G[(**4**)] with the use of alcohols is not attributable to the decrease in the yield of (**8**). In view of the order of the rate constant for hydrogen abstraction by ˙OH,[26,28] 2.45 × 10⁹ (HCO_2^-) > 1.74 × 10⁹ (propan-2-ol) > 7.2 × 10⁸ (ethanol) > 2.8 × 10⁸ l mol⁻¹ s⁻¹ (2-methylpropan-2-ol), it is deduced that the yield of (**4**) increases with facilitating hydrogen abstraction from alcohols as in reaction (10).

It is also interesting that the G[(**2**) + (**3**)] value (0.78) with propan-2-ol producing a potent reducing radical {$E_{1/2}^{ox}[(CH_3)_2\overset{+}{C}OH/(CH_3)_2\dot{C}OH]$ < −0.65 V *versus* s.c.e.}[15] was significantly greater than that (0.12) with 2-methylpropan-2-ol producing a less reducing radical {$E_{1/2}^{ox}[(CH_3)_2(\overset{+}{C}H_2)COH/(CH_3)_2(\dot{C}H_2)COH]$ = 0.1—0.27 V *versus* s.c.e.}[15] (Table 4). With methanol the G[(**2**) + (**3**)] value was intermediate between those of propan-2-ol and 2-methylpropan-2-ol. These results suggest that the reducing ability of the alcohol radicals determines the yield of (**2**) [and (**3**) as a related product], as is the case for the systems containing aromatic amines or inorganic reductants.

The G[(**4**)] increased whereas the G[(**2**) + (**3**)] decreased upon increasing the concentration of added propan-2-ol in the range of 0.02—1M. This is similar to the observation in the formate solution (Figure 5) and is consistent with a competition between hydrogen-atom abstraction by intermediate (**8**) from propan-2-ol and one-electron reduction of (**8**) by $(CH_3)_2\dot{C}OH$ [see also reactions (8) and (10)].

Estimation of the Rate Constant for One-electron Reduction of (**8**) *by* (**7e**).—Finally, an attempt was made to estimate the rate constant for the one-electron reduction of (**8**) by *o*-phenylenediamine (**7e**). Assuming that the radiolytic formation of (**8**) is independent of (**7e**) and that the competition among reactions (7), (8), and (10) is dominant, relationship (11) is obtained, where k_7, k_8, and k_{10} are rate constants for individual reactions. Figure 7 shows a linear plot for equation (11) with varying concentrations of (**7e**), the slope and intercept of which provide estimates of $k_7[(\mathbf{7e})/(\mathbf{7e}^{+\bullet})]/k_{10} = 4.4 \times 10^2$ and $k_8[CO_2^{-\bullet}]/k_{10}[HCO_2^-] = 7.5 \times 10^{-1}$, respectively. By reference to this linear relationship [equation (11)] with the use

$$\frac{G[(\mathbf{2}) + (\mathbf{3})]}{G(\mathbf{4})} = \frac{k_7}{k_{10}}\frac{[(\mathbf{7e})]}{[HCO_2^-]} + \frac{k_8}{k_{10}}\frac{[CO_2^{-\bullet}]}{[HCO_2^-]} \qquad (11)$$

of the estimate of amine-independent term $k_8[CO_2^{-\bullet}]/k_{10}[HCO_2^-]$, the value of $k_7[(\mathbf{7f})/(\mathbf{7f}^{+\bullet})]/k_{10} = 1.5 \times 10^3$ for TMPD (**7f**) is also obtained from the data in Table 3. Furthermore, substituting $k_7[(\mathbf{7f})/(\mathbf{7f}^{+\bullet})] = 1.3 \times 10^9$ l mol⁻¹ determined by means of pulse radiolysis[16] leads to k_{10} = 8.7 × 10⁵ l mol⁻¹ for the hydrogen abstraction from HCO_2^- by the radical (**8**) [reaction (10)], which is 2 800-fold smaller than $k(^{\bullet}OH + HCO_2^- \rightarrow H_2O + CO_2^{-\bullet}) = 2.45 \times 10^9$ l mol⁻¹. From the values of the slope in Figure 7 and the k_{10} value, we also obtain $k_7[(\mathbf{7e})/(\mathbf{7e}^{+\bullet})] = 3.8 \times 10^8$ l mol⁻¹. Comparison of $k_7[(\mathbf{7e})/(\mathbf{7e}^{+\bullet})]$ with $k_7[(\mathbf{7f})/(\mathbf{7f}^{+\bullet})]$ indicates that the reducing ability of (**7e**) is less than that of (**7f**), as expected from their one-electron oxidation potentials.

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