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ON THE ATTACK OF HYDROXYL RADICALS ON POLYHYDRIC ALCOHOLS AND SUGARS AND THE REDUCTION OF THE SO-FORMED RADICALS BY 1,4-DITHIOTHREITOL*

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ABSTRACT

To study the reactions of OH radicals with polyhydric alcohols, N_2O-saturated aqueous solutions of diols or sugars were exposed to ionizing radiation from a 2.8 MeV electron-accelerator (for kinetic studies) or a ^{60}Co γ-source (for steady-state irradiations).

Cyclohexanediols react rapidly with OH radicals ($k \approx 4.5 \times 10^9$ dm^3.mol^{-1} s^{-1}). With the 1,3- and 1,4-isomers H-abstraction from a position α to an OH group is twice as likely (per carbon-bound H atom) as at any other position. In the case of the 1,2-isomer this ratio is about unity, possibly because of a mutual inactivation of the two α positions, each by its neighbouring hydroxyalkyl group, against attack by electrophilic $^{\cdot}OH$.

Oxygen converts α-hydroxyalkyl radicals into peroxyl radicals, which eliminate $HO_2^{\cdot}/O_2^{\cdot-}$ yielding ketones. This reaction was followed by measuring the change in conductivity due to the formation of H^+ and $O_2^{\cdot-}$ [pK_a($HO_2^{\cdot}$) = 4.7]. The spontaneous $HO_2^{\cdot}$-elimination is slow (40–1750 s^{-1}), but the OH^--induced $O_2^{\cdot-}$-elimination is fast ($k \approx 4 \times 10^9$ dm^3.mol^{-1}s^{-1}), and even in neutral solutions it contributes significantly to the overall reaction rate.

1,2-Dihydroxyalkyl radicals, the dominant primary carbohydrate radicals, undergo water elimination yielding α-oxoalkyl radicals. 1,4-Dithiothreitol restores primary carbohydrate radicals to the educts (or their stereoisomers) by H-donation, but does not react with α-oxoalkyl radicals on the time scale of a pulse radiolysis experiment ($k \leqslant 10^7$ dm^3.mol^{-1}s^{-1}). In alkaline solutions, however, such radicals are readily reduced by thiolate ions *via* electron transfer.

INTRODUCTION

Much of our present knowledge of the free-radical chemistry of carbohydrates in aqueous solution and in the solid state has been gained with the help of radiation techniques (for a review see ref. 1). To a considerable extent the incentive

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*In memoriam Professor Karl Freudenberg on occasion of the centenary of his birth.

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for work in this field was to understand better the principles behind the free-radical chemistry of the sugar moiety of DNA (for a review see ref. 2), since DNA sugar-radicals must be the precursors of DNA strand breaks. One DNA double-strand break that remains unrepaired is lethal for a cell, *i.e.* it can no longer proliferate. There is considerable analogy between the free-radical processes leading to DNA strand breakage by phosphoric ester elimination and the water-elimination reaction from 1,2-dihydroxyalkyl radicals (*cf.* reaction *14*) which dominates the free-radical chemistry of carbohydrates. Thus in many respects carbohydrates have proven to be good model systems for the reactions of DNA sugar-radicals.

Cells contain about 70% water and one line of thought is that water radicals, mainly $^{\cdot}OH$, contribute to cell damage by reacting with the sugar moiety of DNA and thus causing strand breakage. Although OH radicals are very reactive (their reactions often have rate constants that are close to diffusion-controlled[3]), they can be quite selective because of their high electrophilicity[4,5]. It will be shown that a case in point is the reduced reactivity of the H atoms in the position α to an OH group in the 1,2-cyclohexanediols compared to analogous H atoms in the 1,3- and 1,4-isomers.

The repair of DNA sugar-radicals *via* H-donation from the thiol pool is widely considered as one of the cell's defense mechanisms against free radical-induced damage. There are some reports in the literature dealing with carbohydrates as model systems[6–8] for DNA, and it was felt that such systems should be investigated in more detail.

EXPERIMENTAL

The commercially available *cis–trans* mixtures of 1,3-cyclopentanediol (90% *cis*) (EGA), 1,2-cyclohexanediol (48% *cis*) (Merck), 1,3-cyclohexanediol (63% *cis*) (EGA), 1,4-cyclohexanediol (43% *cis*) (Merck), as well as *trans*-1,2-cyclohexanediol (EGA), cyclopentanol, cyclohexanol, 2-deoxy-D-ribose, D-ribose, ethylene glycol, and 1,4-dithiothreitol (Merck), and D-ribose 5-phosphate (Boehringer) were used as supplied. Pure (>98.5%) *cis* and *trans* isomers of 1,3- and 1,4-cyclohexanediol were prepared by trimethylsilylation of their mixtures, distillation of the trimethylsilyl derivatives, and detrimethylsilylation with methanol.

Solutions were made up in triply distilled water and saturated with O_2-free N_2O or 4:1 v/v N_2O–O_2. The dose rate of the ^{60}Co γ-irradiations was 0.19 $Gy.s^{-1}$. In the pulse-radiolysis experiments 3 Gy pulses of 0.4 μs duration were applied. The pH of the unbuffered solutions was 6.8 if not stated otherwise. The pulse radiolysis setup has been described previously[9].

The ketones were determined by g.l.c. using a 25 m glass capillary column coated with carbowax 20M. In order to avoid destruction of the column by successive injections of aqueous solutions, the film was cross-linked using γ-radiation.

RESULTS AND DISCUSSION

Hydroxyl radicals were generated using radiation techniques. In the radiolysis of water (reaction *1*) OH radicals, solvated electrons (e^-_{aq}) and H atoms are formed as reactive intermediates. Solvated electrons can be converted by nitrous oxide into further OH radicals (reaction *2*).

$$H_2O \xrightarrow{\text{ionizing radiation}} {}^{\cdot}OH,\ e^-_{aq},\ H^{\cdot},\ H^+,\ H_2O_2,\ H_2 \qquad (1)$$

$$e^-_{aq} + N_2O + H_2O \rightarrow {}^{\cdot}OH + N_2 + OH^- \qquad (2)$$

The system then consists of mainly OH radicals (~90%, $G = 0.57\ \mu mol.J^{-1}$) with a small contribution of H atoms (~10%, $G = 0.06\ \mu mol.J^{-1}$).

Rate constant of OH attack. — To determine the rate constant for the reaction of OH radicals with polyhydric alcohols (RH_2; reaction *3*) the KSCN competition method was chosen. In a pulse-radiolysis experiment oxygen-free N_2O-saturated solutions containing KSCN and varying amounts of the carbohydrate were subjected to electron pulses and the yield of $(SCN)_2^{\cdot-}$ ($\varepsilon_{480\,nm} = 7600\ dm^3.mol^{-1}cm^{-1}$) measured. The product $(SCN)_2^{\cdot-}$ is formed according to reactions *4* and *5*. The equilibrium *5* is fully shifted to the right at $[SCN^-] \geqslant 10^{-4}\ mol.dm^{-3}$.

$${}^{\cdot}OH + RH_2 \rightarrow H_2O + {}^{\cdot}RH \qquad (3)$$

$${}^{\cdot}OH + SCN^- \rightarrow OH^- + SCN^{\cdot} \qquad (4)$$

$$SCN^{\cdot} + SCN^- \rightleftarrows (SCN)_2^{\cdot-} \qquad (5)$$

The carbohydrate radicals $^{\cdot}RH$ do not react with SCN^-, at least not on the time scale of such an experiment (*i.e.*, within a few microseconds). Hence, the yield of $(SCN)_2^{\cdot-}$ reflects the number of OH radicals that have reacted with thiocyanate. The rate constant $k_4 = 1.1 \times 10^{10}\ dm^3.mol^{-1}s^{-1}$ is well known[3] and k_3 can be calculated from equation *6*,

$$\frac{[(SCN)_2^{\cdot-}]_0}{[(SCN)_2^{\cdot-}]_{RH_2}} = 1 + \frac{k_3[RH_2]}{k_4[SCN^-]} \qquad (6)$$

where the indexes 0 and RH_2 indicate the yield of $(SCN)_2^{\cdot-}$ in the absence and presence of carbohydrate, respectively. Experiments of this kind can be carried out with great accuracy (*cf.* Fig. 1).

The data thus obtained are compiled in Table I. As expected, the rate constants for the reaction of the OH radical with the compounds studied are close to diffusion-controlled, and hence do not vary very much. However, the site of attack within a given series of polyhydric alcohols was subject to considerable variation.

Site of OH attack. — Attack at the position α to the hydroxyl function (reaction *7*) can be determined as follows.

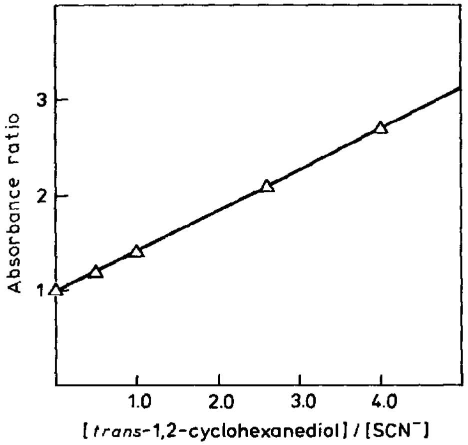
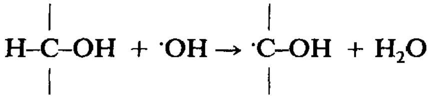
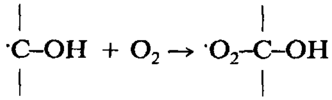


Fig. 1. Pulse radiolysis of N_2O-saturated aqueous solutions containing 10^{-3} mol.dm^{-3} KSCN and varying concentrations of *trans*-1,2-cyclohexanediol. Plot of the ratio of absorbance of $SCN_2^{\bullet-}$ at 480 nm in the absence and presence of the diol *versus* the ratio of the concentrations of the diol and KSCN (see equation *6*).

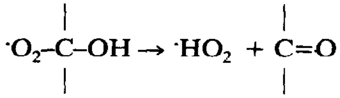


$$\mathrm{H{-}\overset{|}{\underset{|}{C}}{-}OH + {}^{\bullet}OH \rightarrow {}^{\bullet}\overset{|}{\underset{|}{C}}{-}OH + H_2O} \qquad (7)$$

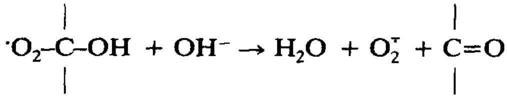
In the presence of oxygen (*i.e.*, when the solutions to be irradiated are saturated with a 4:1 v/v mixture of N_2O and O_2 instead of with N_2O alone) the α-hydroxyalkyl radicals formed in reaction *7* are rapidly converted into the corresponding peroxyl radicals[10] (reaction *8*, k_8 ~2 × 10^9 dm^3.mol^{-1}s^{-1}). α-Hydroxyalkylperoxyl radicals are known (for a review see ref. 11) to eliminate $HO_2^{\bullet}$ (reaction *9*). In alkaline solution this reaction is speeded up by an OH^--induced reaction (reaction *10*, k_{310} ~4 × 10^9 dm^3.mol^{-1}s^{-1}, *cf.* ref. 12). The H atom from reaction *1* is scavenged by oxygen (reaction *11*).



$$\mathrm{{}^{\bullet}\overset{|}{\underset{|}{C}}{-}OH + O_2 \rightarrow {}^{\bullet}O_2{-}\overset{|}{\underset{|}{C}}{-}OH} \qquad (8)$$



$$\mathrm{{}^{\bullet}O_2{-}\overset{|}{\underset{|}{C}}{-}OH \rightarrow {}^{\bullet}HO_2 + \overset{|}{\underset{|}{C}}{=}O} \qquad (9)$$



$$\mathrm{{}^{\bullet}O_2{-}\overset{|}{\underset{|}{C}}{-}OH + OH^- \rightarrow H_2O + O_2^{\bullet-} + \overset{|}{\underset{|}{C}}{=}O} \qquad (10)$$

$$\mathrm{H^{\bullet} + O_2 \rightarrow HO_2^{\bullet}\ (p}K_a\ \mathrm{of\ HO_2^{\bullet} = 4.7)} \qquad (11)$$

The amounts of ketones formed were measured by g.l.c. Their radiation-chemical yields (G values, in units of μmol.J^{-1}) are listed in Table I.

A less direct method of determining the yield of OH attack α to the hydroxyl group (reaction *7*) is to measure the change in conductivity in a pulse radiolysis experiment. At pH $\leq$10 the educts and the peroxyl radicals are uncharged, but in reactions *9* and *10* $HO_2^{\cdot}$ and $O_2^{\cdot-}$ are eliminated. The pK_a of the $HO_2^{\cdot}$ radical is 4.7 (ref. 13). Thus, in neutral and alkaline solutions $HO_2^{\cdot}$ is largely dissociated into H^+ and $O_2^{\cdot-}$. As a result of the formation of these ions, an increase in conductivity is observed in neutral and slightly acidic solutions [$\lambda_0(H^+) = 325$ cm$^2.\Omega^{-1}$equiv^{-1} at 20°]. In basic solutions H^+ is neutralized by OH^- and a net decrease in conductivity is observed due to the replacement of OH^- ($\lambda_0 = 180$ cm$^2.\Omega^{-1}$equiv^{-1}) by $O_2^{\cdot-}$ ($\lambda_0 =$ 60 cm$^2.\Omega^{-1}$equiv^{-1}).

The yield of $H^+ + O_2^{\cdot-}$ from α-hydroxyalkylperoxyl radicals can be determined in alkaline solutions more accurately than in neutral and slightly acidic solutions because $O_2^{\cdot-}$-formation is speeded up by reaction *10*, and this reduces complications from competing reactions (see below). It is found that the G values for $O_2^{\cdot-}$ and ketone formation agree within ±15% if the contribution of $HO_2^{\cdot}$ from reaction *10* [$G(H^{\cdot}) \approx 0.06$ μmol.J^{-1}) is taken into account.

The last column of Table I shows the percentage of H-abstraction by OH from positions α to a hydroxyl group relative to the total number of available carbon-bound hydrogen atoms. As can be seen from this entry, in all systems investigated an H atom α to a hydroxyl group is abstracted about twice as readily by $^{\cdot}$OH as any other carbon-bound H atom, with one exception: the 1,2-cyclohexanediols. Their marked deviation from the rule appears to be significant, and one can suggest two possible reasons for this deviation. One could be an electronic effect: the OH radical is electrophilic and, with respect to a CH_2 group, a β-situated

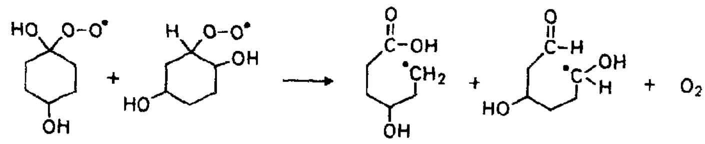
TABLE I

RATE CONSTANTS OF THE REACTION OF OH RADICALS WITH SOME POLYHYDRIC ALCOHOLS AND G VALUES OF KETONE FORMATION IN 4:1 (v/v) NO_2–O_2-SATURATED SOLUTIONS

Alcohol	k ($^{\cdot}OH + RH_2$) (dm^3.mol^{-1}s^{-1})	*Percent α-CH*	G (ketone) (μmol.J^{-1})	*% Attack at α-CH*	*% Attack at α-CH/ % α-CH*
Cyclopentanol		11	0.14	25	2.3
Cyclohexanol		9	0.13	23	2.5
1,2-Cyclohexanediol, *cis*		20	0.15	26	1.3
1,2-Cyclohexanediol, *trans*	4.7×10^9		0.10	18	0.9
1,2-Cyclohexanediol, mixture	5.4×10^9		0.14	25	1.3
1,3-Cyclohexanediol, *cis*		20	0.23	40	2.0
1,3-Cyclohexanediol, *trans*			0.28	49	2.5
1,3-Cyclohexanediol, mixture	4.3×10^9		0.24	42	2.1
1,4-Cyclohexanediol, *trans*		20	0.27	47	2.4
1,4-Cyclohexanediol, mixture	4.5×10^9		0.24	42	2.1
1,3-Cyclopentanediol, mixture		25	0.32	56	2.2

CHOH group will withdraw electron density from the site of reaction. Thus, in the 1,2-cyclohexanediols the two neighbouring hydroxyl groups may mutually reduce the reactivity of their α-situated hydrogens. Another explanation is steric hindrance; that steric effects may play a role is suggested by the difference between *cis-* and *trans-*1,2-cyclohexanediol (*cf.* Table I).

The rate constant for $HO_2^{\cdot}$ elimination from α-hydroxyalkylperoxyl radicals. — The rate constant for the spontaneous elimination of $HO_2^{\cdot}$ from α-hydroxyalkylperoxyl radicals (reaction *9*) is not as readily obtained as the yield of these radicals. Because the OH^--induced reaction is very fast ($k_{10} \approx 4 \times 10^9$ dm^3.mol^{-1}s^{-1}, *cf.* ref. 12 and the comment below where reaction *14* is discussed) its contribution at pH 6.8 ([OH^-] = 6.3×10^{-8} mol.dm^{-3}, $k_{10} \times [OH^-] \approx 250$ s^{-1}) cannot be neglected. Furthermore, there is always some conductivity due to the bimolecular decay of the peroxyl radicals. A recent study on the fate of cyclopentyl- and cyclohexyl-peroxyl radicals shows that such peroxyl radicals are prone to fragmentation reactions[14]. In the present case this path should lead to open-chain carboxylic acids (reaction *12*).



(12)

The products of this reaction also give rise to conducting species, but their formation follows second-order kinetics while the $HO_2^{\cdot}$-elimination reactions *9* and *10* are kinetically first order with respect to peroxyl radicals. Thus, a plot of the

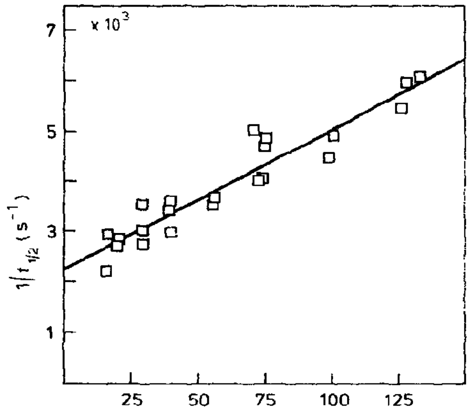


Fig. 2. Pulse radiolysis of 4:1 (v/v) N_2O–O_2-saturated aqueous solutions of 1,3-cyclopentanediol (10^{-2} mol.dm^{-3}). Plot of the reciprocal of the first half-life of conductivity increase as a function of dose rate (100 relative units ≈ 14 Gy; 1 Gy = 1 J.kg^{-1}).

reciprocal of the first half-life of the conductivity increase *versus* the dose rate (Fig. 2) yields a straight line whose *y*-intercept represents the half-life of the conductivity increase due to the sum of the two first-order reactions *9* and *10*. From the slope, the rate constant of the bimolecular decay can be calculated since the total yield of peroxyl radicals per unit dose is known. In Table II $k_{obs} = k_9 + k_{10} \times [OH^-]$ and $2k_{12}$ are given. A correction for $k_{10} \times [OH^-]$ permits the calculation of k_9. As can be seen from the scatter of the data shown in Fig. 2, this value is subject to considerable uncertainty.

From the data in Table II it can be seen that the spontaneous $HO_2^{\cdot}$ elimination of the compounds investigated is not very fast, and that the OH^--induced reaction contributes appreciably in neutral solution despite the low OH^- concentration. The sum of these two reactions is, however, sufficiently fast that at the low dose rates of the ^{60}Co γ-radiolysis experiments (0.16 Gy.s^{-1}), where the peroxyl radicals attain steady-state concentrations of only about 10^{-8} mol.dm^{-3}, all the α-hydroxyalkyl-peroxyl radicals decay by $HO_2^{\cdot}$ elimination and their bimolecular decay as depicted by reaction *12* can be neglected under these conditions. Thus the ketone yields given in Table I indeed reflect $^{\cdot}OH$ attack α to a hydroxyl group.

It may be noted that the peroxyl radicals derived from the cyclopentyl system eliminate $HO_2^{\cdot}$ faster than the corresponding peroxyl radicals of the cyclohexyl system. In view of our earlier study on the fate of peroxyl radicals derived from a series of polyhydric alcohols and carbohydrates, where similarly high rate constants were found for straight-chain and cyclohexyl systems[15], we feel that this observation cannot be adequately interpreted at present.

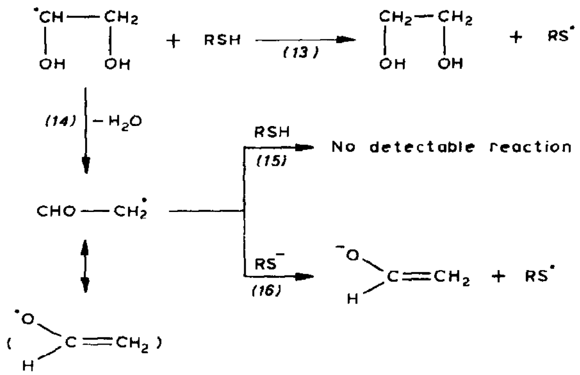
Hydrogen atom and electron transfer from 1,4-dithiothreitol and its anion to carbohydrate-derived radicals. — It is well known (for a review see ref. 16) that thiols can readily transfer an H-atom to some kinds of radicals (*e.g.* $k > 10^7$ dm^3.mol^{-1}s^{-1} for alkyl and α-hydroxyalkyl radicals), but that other radicals (*e.g.* some purine and pyrimidine $^{\cdot}OH$-adduct radicals) react much more slowly (if at all) with thiols, although they may be reduced *via* electron transfer from thiolate ions[17].

In the carbohydrate series the primary radicals formed by H-abstraction are

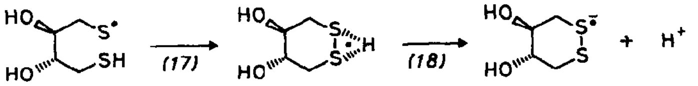
TABLE II

RATE CONSTANTS FOR THE $HO_2^{\cdot}$-ELIMINATION AND FOR THE BIMOLECULAR DECAY AT pH 6.8 OF THE PEROXYL RADICALS FORMED BY OH-RADICAL ATTACK ON SOME ALCOHOLS AND DIOLS IN OXYGENATED AQUEOUS SOLUTION

Alcohol	$k_9 + k_{10} \times [OH^-]$ *(s^{-1})*	k_9 *(s^{-1})*	$2k_{12}$ *(dm^3.mol^{-1}s^{-1})*
Cyclopentanol	2000	1750	2×10^8
Cyclohexanol	290	40	7×10^7
1,2-Cyclohexanediol	560	310	2×10^8
1,3-Cyclohexanediol	650	400	2×10^8
1,4-Cyclohexanediol	430	180	3×10^8
1,3-Cyclopentanediol	1580	1330	3×10^8



mainly α-hydroxyalkyl radicals. This type of radical can be reduced by thiols (reaction 13), the most convenient of which is 1,4-dithiothreitol. It has the advantage over other thiols that it is not as readily oxidized by air and hence very dilute solutions can be made up without an unacceptably rapid loss of reducing capacity[18]. In addition its thiyl radical rapidly complexes to form a six-membered ring[19] (reaction *17*, $k_{17} = 1.6 \times 10^6$ s^{-1}). This ring-closed complex is in equilibrium with its anion, DTT$^{\cdot-}$ (reaction *18*, p$K_a \approx 5.2$) (refs. 18–22), which has a strong absorption at 390 nm ($\varepsilon_{390} = 5900 \pm 500$ dm^3.mol^{-1}cm^{-1}) (ref. 19). Hence the kinetics of H-donation can be followed by the build-up of the absorption at 390 nm, because reactions *17* and *18* are usually fast: a typical example is shown in Fig. 3.



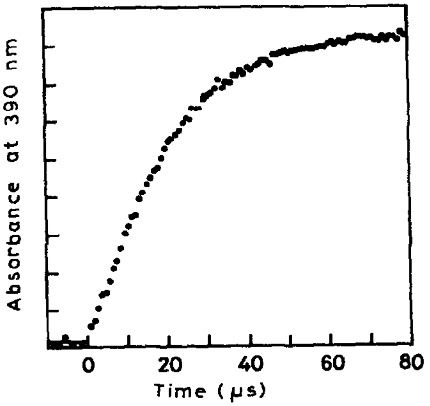


Fig. 3. Pulse radiolysis (3 Gy per pulse) of N_2O-saturated aqueous solution containing deoxyribose (10^{-2} mol.dm^{-3}) and 1,4-dithiothreitol (2×10^{-4} mol.dm^{-3}), pH 7.4. Trace shows the buildup of the *trans*-4,5-dihydroxy-1,2-dithiane radical anion (DTT$^{\cdot-}$) measured at 390 nm.

If α-hydroxyalkyl radicals contain a second hydroxyl group in a β-position they undergo water elimination (reaction *14*). The α-oxoalkyl radicals thus formed are not reduced by thiols on the time scale of pulse radiolysis experiments ($k_{15} \ll 10^7$ dm^3.mol^{-1}s^{-1}) (ref. 7). The water elimination (reaction *14*) may occur spontaneously, but the reaction is usually slow[23]. At neutral pH the rate is mainly governed by an OH$^-$-induced reaction consisting of two steps: deprotonation of the radical, and subsequent loss of OH$^-$. The OH$^-$-induced deprotonation of 1,2-dihydroxyalkyl radicals is fast ($k_{14a} \approx 10^{10}$ dm^3.mol^{-1}s^{-1}) as is the subsequent loss of OH$^-$ ($k_{14b} \approx 3 \times 10^6$ s^{-1}) (ref. 23). Because the pK_a values of the 1,2-dihydroxyalkyl radicals are expected to be $\geqslant$10.7 (*cf.* $^{\cdot}CH_2OH$, ref. 24), reprotonation of the radical anion by water has about the same rate as loss of OH$^-$, and hence at pH $\leqq$10.5 the overall rate is very close to that of the first step. Thus, OH$^-$-induced water elimination plays a role even at pH 7, where $k_{14a} \times [OH^-] \approx k_{14} \approx 10^3$ s^{-1}.

According to equation *19* the kinetics of the build-up of the $DTT^{\cdot}$ formed in reaction *13* are pseudo first order, because the radical concentrations are usually low compared to the thiol concentration.

$$\frac{d[DTT^{\cdot}]}{dt} = k_{13}\,[^{\cdot}R] \times [RSH] \tag{19}$$

A plot of k_{obs} *versus* [RSH] should then give a straight line through the origin, the slope yielding the bimolecular rate constant k_{13}. In the present system the unimolecular decay (water elimination) of the carbohydrate radicals into α-oxoalkyl radicals (reaction *14*), which are no longer capable of reacting with the thiol, competes with the H-donation reaction *13*. This competition causes an intercept on the k_{obs} axis. In addition, the bimolecular decay of the radicals (reactions *20–22*) also competes with reaction *13*.

$$^{\cdot}RH + {}^{\cdot}RH \rightarrow \text{products} \tag{20}$$

$$^{\cdot}RH + RS^{\cdot} \rightarrow \text{products} \tag{21}$$

$$RS^{\cdot} + RS^{\cdot} \rightarrow \text{products} \tag{22}$$

The bimolecular radical-loss also causes an intercept, negligible only if the first half-life of the bimolecular decay of the radicals is long compared to the first half-life of reaction *13*. The total intercept is $k_0 = k_{14} + k_{20-22} \times [R^{\cdot}]$, the latter component being treated as if it were a first order reaction ($k = 0.7/t_{1/2}$, where $t_{1/2}$ is the first half-life of the bimolecular decay of the radicals). If $2k_{20-22} = 3 \times 10^9$ dm^3.mol^{-1}s^{-1} and a radical concentration of 2×10^{-6} mol.dm^{-3} are assumed, $t_{1/2}$ (bimolecular decay) is 1.7×10^{-4} s, a value that would lead to an intercept at 6×10^3 s^{-1}. The conversion of the thiol-reactive radical into a non-reactive radical (reaction *14*) is base-catalysed and occurs at pH 7.4 with $k_{14a} \times [OH^-] = 2.5 \times 10^3$

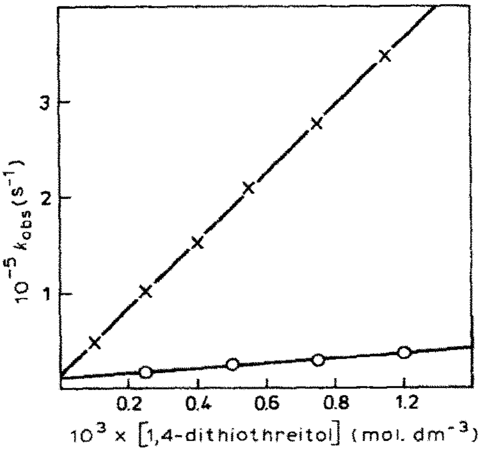


Fig. 4. Pulse radiolysis (3 Gy per pulse) of N_2O-saturated aqueous solutions containing ethylene glycol (10^{-1} mol.dm^{-3}) and 1,4-dithiothreitol at pH 7.4 (○) and 11.1 (×). Plot of the observed first-order rate constant for the formation of *trans*-4,5-dihydroxy-1,2-dithiane radical anions as a function of the 1,4-dithiothreitol concentration.

s^{-1}. The sum of these values leads to $k_0 = 0.85 \times 10^4\ s^{-1}$, in reasonable agreement with the observed intercept of $10^4\ s^{-1}$ (Fig. 4).

In ref. 7 the intercept is considerably higher. This is due not only to the fact that the pH was 8.4 instead of 7.4, but also, and more importantly, to the fact that in order to maintain this pH borate had been added at 5×10^{-3} mol.dm^{-3}. Phosphate and borate ions are usually two to three orders of magnitude less efficient than the hydroxide ion in deprotonation reactions[11,19]. Using a rate constant of 2×10^7 dm^3.mol^{-1}s^{-1} for the buffer-induced water elimination, the intercept is raised to as much as $1 \times 10^5\ s^{-1}$, in agreement with the mentioned[7] results. Thus such intercepts are now well understood.

There still remains the question of whether thiols are capable of repairing all carbohydrate radicals, or whether some of them cannot be repaired, as is suggested by the results presented in ref. 8. The rate constants of H-donation to carbohydrate radicals are not very large (Table III). Thus there is some radical loss during the monitoring period due to the reactions that cause the intercept in Fig. 4. Owing to the contribution of the bimolecular decay of the radicals the complete kinetic analysis of this situation is difficult, but as a first approximation the first-order transformation (reaction *14*) may be considered and the radical loss treated as if it were first order kinetically (see above). According to equations *23* and *24* a plot of $G(R^{\cdot})/G(DTT^{\cdot-})$ *versus* 1/[DTT] should give a straight line with an intercept of unity if all carbohydrate radicals can in principle be repaired.

$$G(DTT^{\cdot-}) = \frac{k_{13} \times [DTT]}{k_{13} \times [DTT] + k_0} \times G(R^{\cdot}) \qquad (23)$$

$$\frac{G(\mathrm{R}^{\cdot})}{G(\mathrm{DTT}^{\cdot})} = 1 + \frac{k_0}{k_{13} \times [\mathrm{DTT}]} \qquad (24)$$

Fig. 5 shows such a plot for the case of D-ribose. It can be seen that the intercept is very close to unity, hence we conclude that all the primary carbohydrate radicals were repaired, and that the reported value of only 70% reflects an incompleteness of the repair reaction under the given experimental conditions. A similar plot for 2-deoxy-D-*erythro*-pentose (deoxyribose) shows the same behaviour. This indicates that the radical at C-2 must also be reduced by the thiol, which conforms to our expectation in view of the observed reduction of the *tert*-butyl alcohol-derived radical (*cf.* Table III).

The 1,2-dihydroxyethyl radical derived from ethylene glycol is reduced by 1,4-dithiothreitol with a rate constant of $k_{13} = 2.6 \times 10^7$ dm^3.mol^{-1}s^{-1}. At pH 8.4 reduction of the formylmethyl radical produced from the 1,2-dihydroxyethyl radical by water elimination is very slow[7] ($k \leqslant 10^7$ dm^3.mol^{-1}s^{-1}), whereas at high pH the water-elimination reaction (*14*) becomes very fast[23] ($k_{14a} \times [\mathrm{OH}^-] \approx 10^6$ s^{-1} at pH 10). Thus only formylmethyl radicals are present to react with 1,4-dithiothreitol

TABLE III

OBSERVED RATE CONSTANTS FOR H-TRANSFER FROM 1,4-DITHIOTHREITOL TO RADICALS GENERATED BY OH-RADICAL ATTACK ON SOME ALCOHOLS AND CARBOHYDRATES

Reactant	*Rate constant (dm^3.mol^{-1}s^{-1})*	*Ref.*
Methanol	6.5×10^7	This work
	6.8×10^7	22
	8.0×10^7	8
Ethanol	9.7×10^7	This work
2-Propanol	2.0×10^8	This work
	2.1×10^8	22
t-Butyl alcohol	4.7×10^7	This work
	6.8×10^7	22
Ethylene glycol	2.6×10^7	This work
	1.2×10^8 [a]	This work
	3.5×10^8 [b]	This work
1.2-Cyclohexanediol	1.4×10^8	This work
1,3-Cyclohexanediol	3.4×10^8	This work
1,4-Cyclohexanediol	3.4×10^8	This work
D-Ribose	9.0×10^7	This work
	1.2×10^7	8
Deoxyribose	1.8×10^8	This work
	2.4×10^7	8
D-Ribose 5-phosphate	$\approx 5 \times 10^7$	This work
	0.5×10^7	8
3,3-Dimethyl-2-hydroxy-1,4-butyrolactone	6.0×10^7	This work

[a] At pH 10 (electron transfer to formylmethyl radicals). [b] At pH 11.1 (electron transfer to formylmethyl radicals).

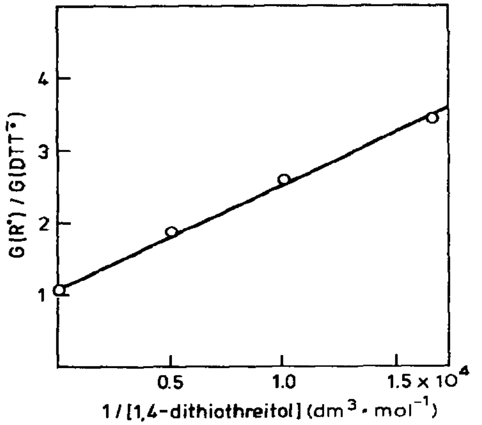


Fig. 5. Pulse radiolysis (3 Gy per pulse) of N_2O-saturated aqueous solutions containing D-ribose (10^{-2} mol.dm^{-3}) and 1,4-dithiothreitol at pH 7.4. Plot of $G(\mathrm{R}^\cdot)/G(\mathrm{DTT}^\cdot)$ *versus* 1/[DTT] (see equation *24*).

(*cf.* Fig. 4). 1,4-Dithiothreitol has pK_a values of 9.12 and 10.15 (ref. 25), and the fast reaction of the formylmethyl radicals under these conditions ($k_{16} = 1.2 \times 10^8$ dm^3.mol^{-1}s^{-1} at pH 10 and $k_{16} = 3.5 \times 10^8$ dm^3.mol^{-1}s^{-1} at pH 11.1, *cf.* Table III) is due to their reduction by the thiolate ion. The higher rate constant at pH 11.1 results from the presence of the more reactive 1,4-dithiothreitol dianion. This reaction is no longer an H-transfer reaction, but occurs *via* electron transfer. Formylmethyl radicals are fairly strongly oxidizing species, and electron transfer reactions of such species are well documented[23].

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