

## FREE-RADICAL REPAIR BY A NOVEL PERTHIOL: REVERSIBLE HYDROGEN TRANSFER AND PERTHIYL RADICAL FORMATION

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2-(3-Aminopropyl-amino) ethanepertiol (RSSH, the perthiol analogue of the thiol radioprotector, WR-1065) reacts with the  $\alpha$ -hydroxy alkyl radical  $(\text{CH}_3)_2\text{C}\cdot\text{OH}$  by donating a hydrogen atom as indicated by the characterization of perthiyl radicals ( $\text{RSS}\cdot$ ;  $\lambda_{\text{max}} \approx 374 \text{ nm}$ ,  $\epsilon_{374} \approx 1680 \pm 20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) by pulse radiolysis. The perthiyl radical abstracts a hydrogen from the alcohol to establish a reversible hydrogen-transfer equilibrium. This equilibrium lies predominantly on the side of radical repair since the rate constants for the forward and reverse reactions at pH 4 are:  $k(\text{RSSH} + (\text{CH}_3)_2\text{C}\cdot\text{OH}) = (2.4 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  and  $k(\text{RSS}\cdot + (\text{CH}_3)_2\text{CHOH}) = (3.8 \pm 0.3) \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  respectively. The  $\text{p}K_a$  ( $\text{RSSH} \rightleftharpoons \text{RSS}^- + \text{H}^+$ ) =  $6.2 \pm 0.1$  was determined from the pH dependence of the rate of perthiol repair. Identical experiments have been performed with WR-1065 allowing a direct comparison of free-radical repair reactivity to be made with the parthiyl analogue. At pH  $\approx 7.4$  the reactivities of the thiol and perthiol were similar, both repairing the alcohol radical with a rate constant of  $\sim (2.4 \pm 0.1) \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . However, at pH 5 whilst the hydrogen-donation rate of the thiol was 15–20% higher than at pH 7.4, the perthiol reactivity was over an order of magnitude higher. The thermodynamic driving force for the observed enhanced free-radical repair reactivity of RSSH compared to RSH is attributed to the resonance stabilization energy of  $8.8 \text{ kJ mol}^{-1}$  within the RSS $\cdot$  radical. These results indicate a possible application of RSSH/RSS $^-$  as DNA-targeted antioxidants or chemoprotectors.

**KEY WORDS:** WR-2721, WR-1065, 2-(3-aminopropylamino) ethanepertiol, free-radical repair, reversible hydrogen transfer, perthiyl radicals, pulse radiolysis, antioxidant.

### INTRODUCTION

The drug S-2-(3-aminopropylamino) ethyl phosphorothioic acid (WR-2721) (Figure 1, 1) reduces the damaging effects on DNA of ionizing radiation<sup>1</sup> and anti-tumour alkylating drugs e.g. cisplatin<sup>2</sup> and 5-fluorouracil.<sup>3</sup> The thiophosphate ester is a pro-drug which undergoes biotransformation to the active free thiol, 2-(3-aminopropyl-amino) ethanethiol (WR-1065) (Figure 1, 2) when dephosphorylated by alkaline phosphatase in endothelial cells.<sup>4</sup> The preferential formation and uptake of

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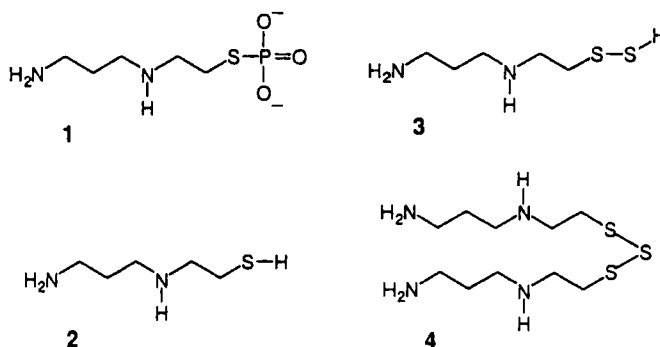


FIGURE 1 Structures of the chemicals referred to in the text. 1, WR-2721; 2, WR-1065; 3, perthiol analogue of WR-1065; 4, symmetrical trisulphide of WR-1065.

WR-1065 into non-tumour tissues has been exploited with the application of WR-2721 in clinical cancer radiotherapy<sup>5</sup> and chemotherapy.<sup>6</sup>

WR-1065 can modify radiation-induced DNA damage by repairing DNA radicals via hydrogen transfer from the SH moiety.<sup>7,8</sup> Under physiological conditions (pH  $\approx$  7.4) a significant proportion ( $\approx$ 20%) of WR-1065 exists as the deprotonated thiolate anion (RS<sup>-</sup>)<sup>9</sup> but the repair of DNA radicals by electron transfer rather than hydrogen transfer is still possible.<sup>10</sup> The ability of WR-1065 to protect DNA is enhanced by the doubly-protonated polyamino side chain of WR-1065 targeting the negatively-charged DNA backbone via an electrostatic counter-ion condensation phenomenon.<sup>11-13</sup>

Chemical repair by thiols results in the formation of thiyl radicals (RS<sup>•</sup>), which have also been shown to abstract hydrogen atoms from some C-H bonds, (i.e. the reverse of the repair reaction) to establish a general equilibrium reaction (1):<sup>14</sup>



Particular attention has been focused on RS<sup>•</sup> radical reactions with alcohols and ethers<sup>15</sup> which constitute simple models for the sugar moieties in DNA nucleotides due to the similarities in the carbon-hydrogen bond dissociation energies.<sup>16</sup> Rate constants for the *repair* ( $k_1$ ) and *reverse repair* ( $k_{-1}$ ) reactions were in the order of  $10^7$ – $10^8$  dm<sup>3</sup> mol<sup>-1</sup> s<sup>-1</sup> and  $10^3$ – $10^4$  dm<sup>3</sup> mol<sup>-1</sup> s<sup>-1</sup> respectively.<sup>14,15</sup> RS<sup>•</sup> radicals have been shown to abstract hydrogen from activated C-H bonds of polyunsaturated fatty acids (major constituents of the lipid bilayer of cellular membranes) and to initiate the chain of lipid peroxidation.<sup>17,18</sup>

We compare here the free-radical repair reactivity of WR-1065 with that of the novel disulphur perthiol analogue (Figure 1, 3) utilizing an  $\alpha$ -hydroxyalkyl radical e.g. (CH<sub>3</sub>)<sub>2</sub>C<sup>•</sup>OH as a model for carbon-centred radicals.

## MATERIALS AND METHODS

### Chemicals

WR1065 (2-(3-aminopropylamino)-ethanthiol, RSH) was donated by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer

Institute (NCI), USA. The perthiol analogue (RSSH) synthesis was based on literature methods<sup>19,20</sup> and involved an alkoxide-induced nucleophilic displacement of the perthiolate anion ( $\text{RSS}^-$ ) from the methoxycarbonyl disulphide ( $\text{RSSCO}_2\text{CH}_3$ ).<sup>20</sup> A full description will be given elsewhere. The symmetrical trisulphide (RSSSR) (Figure 1, 4) was synthesized either by an amine catalysed, RSH-induced fragmentation of the methoxycarbonyl trisulphide<sup>22</sup> ( $\text{RSSSCO}_2\text{CH}_3$ ) or alternatively by the facile reaction of the thiolate anion ( $\text{RS}^-$ ) with sulphur.<sup>23</sup> All compounds were characterized and found to be chromatographically pure by GC/EI/CI/MS and HPLC.

Solutions containing RSH or RSSH were prepared by dissolving weighed aliquots directly into nitrous oxide (BOC) saturated deionized water (Millipore Q) to minimize autoxidation. Solutions contained phosphate buffer adjusted to the appropriate pHs using A.R.  $\text{HClO}_4$  and NaOH (Merck). Methyl viologen (1,1'-dimethyl-4,4'-bipyridinium dichloride) (Sigma) was either used as received or purified by dissolving in methanol and precipitation by acetone, then drying the anhydrous salt in vacuo over silica gel at room temperature. Propan-2-ol (Merck, Aristar) and sodium formate (Merck) were of the highest purity commercially available and utilized without further purification. All experiments were carried out at room temperature ( $20 \pm 2^\circ\text{C}$ ).

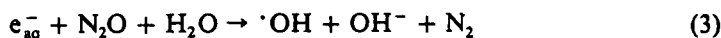
### Pulse Radiolysis

The CRC Gray Laboratory pulse radiolysis facility including the data acquisition and analysis have been described elsewhere.<sup>24</sup> In this study 30 ns pulses ( $\approx 2$  Gy) of 3.5 MeV electrons were used except for the determination of second order rate constants for the decay of radicals, when the pulse width was increased up to  $1 \mu\text{s}$  ( $\approx 12$  Gy). The doses were monitored by a toroidal detector<sup>25</sup> and the absorbed dose per pulse calibrated by the change in absorption at 472 nm for  $\text{N}_2\text{O}$ -saturated solutions of  $10 \text{ mmol dm}^{-3}$  KSCN using the value  $G\varepsilon = 4.81 \times 10^{-4} \text{ m}^2 \text{ J}^{-1}$  for the product of the radiation chemical yield and the extinction coefficient of  $(\text{SCN})_2^-$  at 472 nm.<sup>26</sup>

## RESULTS AND DISCUSSION

### Hydrogen Atom Transfer and Perthiyl Radical Formation

Pulse radiolysis of an  $\text{N}_2\text{O}$ -saturated aqueous solution containing  $0.1 \text{ mol dm}^{-3}$  propan-2-ol and  $0.5 \text{ mmol dm}^{-3}$  RSSH in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 4.6 generated a transient absorption spectrum with  $\lambda_{\text{max}} \approx 374 \text{ nm}$  shown in curve (a) of Figure 2. By comparison with similar transient absorption spectra observed for the one-electron reduction of the symmetrical trisulphides, penicillamine trisulphide<sup>27</sup> and bis(2-hydroxyethyl) trisulphide,<sup>28</sup> the one-electron oxidation of amino acid disulphides<sup>29</sup> and the photolysis of disulphides<sup>30,31</sup> the observed absorption spectrum in this study was tentatively assigned to the perthiyl radical,  $\text{RSS}^\cdot$  generated according to reactions (2)–(5):



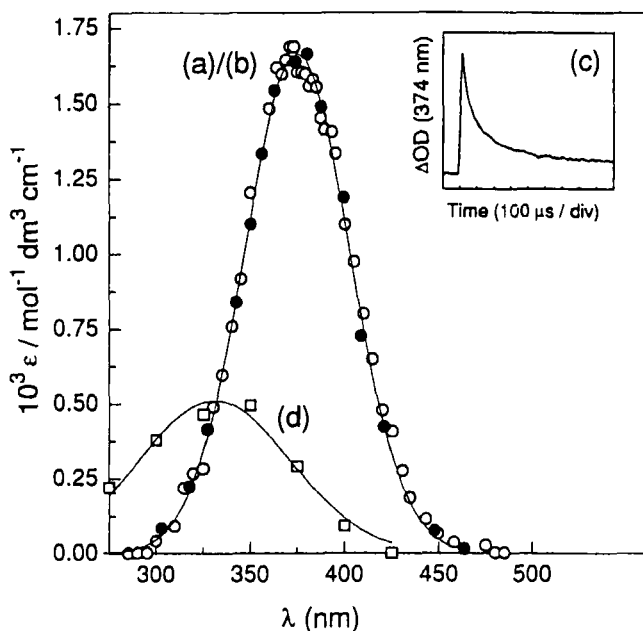
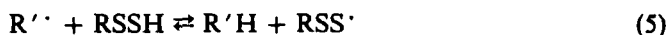
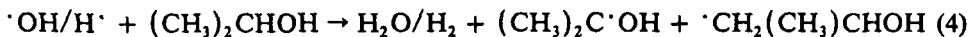
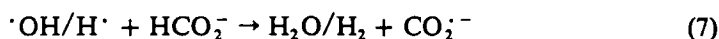


FIGURE 2 Optical absorption spectra of the perthiyl radical formed by pulse radiolysis of: (a) (○)  $\text{N}_2\text{O}$ -saturated aqueous solution containing  $0.2 \text{ mol dm}^{-3}$  RSSH in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 4.5 and  $0.2 \text{ mol dm}^{-3}$  propan-2-ol; (b) (●)  $\text{N}_2$ -saturated aqueous solution containing  $0.1 \text{ mol dm}^{-3}$   $\text{NaHCO}_2$  and  $0.2 \text{ mol dm}^{-3}$  RSSR in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 5.6; (d) (□)  $\text{N}_2\text{O}$ -saturated aqueous solution containing  $0.5 \text{ mmol dm}^{-3}$  RSH in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 5 and  $0.2 \text{ mol dm}^{-3}$  propan-2-ol. Insert (c): Typical trace of absorption (374 nm) vs time obtained under the experimental conditions described in (a) showing the second order decay of the perthiyl radical.



(In reaction (5) the abbreviation ( $\text{R}'\cdot$ ) represents the summation of all the carbon-centred radicals generated in reaction (4); the  $\alpha$ -hydroxy radical predominates). The  $\text{RSS}\cdot$  radicals were formed with a total radiation chemical yield of  $G\epsilon_{374} = 1.09 \times 10^{-4} \text{ m}^2 \text{ J}^{-1}$ . In this system the radiation chemical yield of carbon-centred radicals  $G(\text{R}'\cdot) = G(\text{e}_{\text{aq}}^- + \text{H}\cdot + \cdot\text{OH}) \approx 0.65 \mu\text{mol J}^{-1}$ , yielding an extinction coefficient of  $\epsilon_{374} = 1680 \pm 20 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ , which is in good agreement with those previously reported for other perthiyl radicals (e.g  $1630 \pm 30$ ,  $\approx 1700$  and  $1720 \pm 20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  respectively)<sup>27,28,30</sup> and are typically invariant with the nature of the side group.<sup>32</sup> For comparison and support of the assignment of the 374 nm band to the  $\text{RSS}\cdot$  radical a one-electron reduction of the symmetrical trisulphide,  $\text{RS}_3\text{R}$  (see experimental section) was performed by the hydrated electron ( $E^0(\text{aq}/\text{e}_{\text{aq}}^-) = -2.7 \text{ V}$ )<sup>33</sup> and the carbon dioxide radical anion ( $E^0(\text{CO}_2/\text{CO}_2^-) = -2 \text{ V}$ )<sup>34</sup> as an alternative source of the postulated perthiyl radical.<sup>27,28</sup> Pulse-saturated of an  $\text{N}_2$ -saturated aqueous solution containing  $0.1 \text{ mol dm}^{-3}$   $\text{NaHCO}_2$ ,  $0.25 \text{ mmol dm}^{-3}$   $\text{RS}_3\text{R}$  and  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 6.7 generated a transient absorption spectrum virtually identical to that observed for the perthiyl/propan-2-ol system as indicated by the similarity of curves

(a) and (b) in Figure 2. In this case  $\text{RSS}\cdot$  radicals are formed according to reactions (2) and (6)–(8):



with a radiation chemical yield of  $G\varepsilon_{374} \approx 1.14 \times 10^{-4} \text{ m}^2 \text{ J}^{-1}$ . Taking the total radiation chemical yield as  $G(e_{\text{aq}}^-/\text{CO}_2\cdot^-) \approx 0.68 \mu\text{mol J}^{-1}$  for the reducing radical species<sup>35</sup> gave  $\varepsilon_{374} \approx 1675 \pm 20 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ , a value in excellent agreement with that determined for the RSSH/propan-2-ol system. The trisulphide radical anion  $(\text{RSSSR})\cdot^-$ , a likely intermediate in reaction (6), was not detected and must have a half-life of  $\leq 1 \mu\text{s}$ .

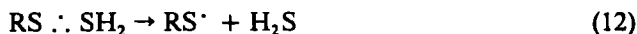
Transients recorded at 374 nm (e.g. insert (c) of Figure 2) show that the  $\text{RSS}\cdot$  radicals are formed in a fast process and then decay by second-order kinetics suggesting that the perthiyl radicals undergo radical-radical recombination via reaction (9) (presumably to the tetrasulphide  $\text{RS}_4\text{R}$  by analogy to the bimolecular decay of thiy radicals to the disulphide).



Pulse radiolysis of a  $\text{N}_2\text{O}$ -saturated aqueous solution containing  $0.1 \text{ mol dm}^{-3}$  propan-2-ol and  $0.5 \text{ mmol dm}^{-3}$  RSSH in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 4.6 was used to monitor changes in the second order decay of the 374 nm absorption upon variation of the dose per pulse between 2 and 12 Gy. The rate of decay (reciprocal of the first half life =  $2k \times$  initial concentration) determined by non-linear least squares fit of the hyperbolic decay curves was found to depend linearly on the absorbed dose (not shown here). From the slope of the linear dependence and using the known value for the radiation chemical yield of perthiyl radicals ( $= G(\text{RSS}\cdot) = 0.61 \mu\text{mol J}^{-1}$ ) the second order rate constant for the decay of the perthiyl radical was determined as  $2k_9 = (1.1 \pm 0.1) \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ . Similar rate constants of  $(1.9 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  and  $(1.4 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  have been obtained for the second-order decay of the penicillamine and bis-(2-hydroxyethyl) perthiyl radicals respectively.<sup>27,28</sup>

The characterisation of the perthiyl radical represents unequivocal identification of reaction (5) and therefore perthiol *hydrogen transfer* to carbon-centred radicals. In this respect reaction (5) clearly parallels the repair reaction of thiols.<sup>7,8,14,15,36</sup> However, in a previous study,  $\alpha$ -hydroxyalkyl radicals e.g.  $(\text{CH}_3)_2\text{C}\cdot\text{OH}$  have been shown to reduce disulphides such as oxidised dithiothreitol.<sup>37</sup> The reaction has been shown not to proceed by a simple electron transfer but via an alcohol radical-RSSR adduct which then decomposes via disulphide radical anion  $(\text{RS} \cdot \cdot \text{SR})^-$ . This has a sulphur-sulphur two-centre-three-electron bond which is  $(2\sigma/1\sigma^*)$  in electronic nature.<sup>38</sup> Rate constants for such alcohol/disulphide reactions are very slow ( $< 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ) and significant rates were only observed when the neutral alcohol radical was ionized at high pH ( $\text{p}K_a((\text{CH}_3)_2\text{C}\cdot\text{OH} \rightleftharpoons (\text{CH}_3)_2\text{CO}\cdot^-) \approx 12.1$ ).<sup>39</sup> An analogous mechanism might be envisaged for reduction of the perthiol in reactions (10)–(12), which represent a potential source of  $\text{RS}\cdot$  radicals.





However, no experimental evidence was obtained in this study for the formation of  $\text{RS} \cdot$  radicals following the reaction of alcohol radicals with RSSH. Pulse radiolysis of a  $\text{N}_2\text{O}$ -saturated aqueous solution containing  $0.1 \text{ mol dm}^{-3}$  2-propanol and  $0.5 \text{ mmol dm}^{-3}$  RSH (instead of RSSH) in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at  $\text{pH} \approx 4.5$  generated a transient absorption (Figure 2 curve (d)) with  $\lambda_{\text{max}} = 340 \text{ nm}$ , clearly distinguishable from that of  $\text{RSS} \cdot$  and attributable to the thiyl radical produced according to reactions (2)–(4) and (13):<sup>40</sup>

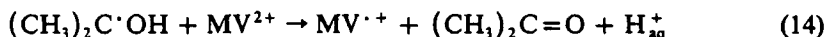


The radiation-chemical yield of thiyl radicals in this system was  $G\epsilon \approx 0.305 \times 10^{-4} \text{ m}^2 \text{ J}^{-1}$ . Again taking  $G(\text{R}' \cdot) = 0.61$  gave  $\epsilon_{340 \text{ nm}} \approx 500 \pm 20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ , a value constant with most thiyl radicals<sup>41</sup> which exhibit  $\epsilon \approx 330 - 1200 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ . The observed optical characteristics of  $\text{RSS} \cdot$  ( $\lambda_{\text{max}} = 374 \text{ nm}$ ,  $\epsilon_{374} = 1680 \pm 20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) and  $\text{RS} \cdot$  ( $\lambda_{\text{max}} = 340 \text{ nm}$ ,  $\epsilon_{500} = 500 \pm 20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) facilitate distinguishing between thiyl and perthiyl radicals. The complete absence of any shoulder on the  $\text{RSS} \cdot$  radical absorption spectrum in Figure 2 (particularly at  $340 \text{ nm}$ ) indicated that no  $\text{RS} \cdot$  radicals were formed in the reaction of RSSH with the alcohol radicals. No evidence was therefore obtained for reduction of the perthiol disulphide bond.

*The Rate of RSSH Repair Determined by Direct Measurements of Perthiyl Radical Formation and by Indirect Competition with Methyl Viologen*

A series of pulse radiolysis experiments (doses  $\approx 2 \text{ Gy}$ ) were performed on  $\text{N}_2\text{O}$ -saturated aqueous solutions containing  $0.1 \text{ mol dm}^{-3}$  propan-2-ol and varying concentrations of RSSH ( $0.25\text{--}2.0 \text{ mmol dm}^{-3}$ ) in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at  $\text{pH} 4.5$ . Direct measurements of the rate constant  $k_3$  were obtained by observing the buildup of the perthiyl radical at  $374 \text{ nm}$  (insert b of Figure 3). The kinetics of perthiyl radical formation followed an exponential rate law with the half-life proportional to the perthiol concentration. The rate constant  $k_3 = (2.5 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  determined from the slope of the linear plot of the observed first order rate constant  $k_{\text{obs}}$  vs.  $[\text{RSSH}]$  (shown in Figure 3) is an order of magnitude faster than the corresponding rate constant  $k_{13} = 2 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  for RSH.<sup>40</sup>

Independent measurements of  $k_3$  were also made by applying competition kinetics to the RSSH/propan-2-ol system. Methyl viologen ( $\text{MV}^{2+}$ ) generates a strongly absorbing, long lived radical cation ( $\text{MV}^{\cdot+}$ ,  $\lambda_{\text{max}} = 600 \text{ nm}$ ,  $\epsilon_{600} = 1.37 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) in reaction (14).<sup>35</sup> The competition between the carbon-centred radicals reducing  $\text{MV}^{2+}$  in reaction (14) and being repaired by the perthiol in reaction (5) was characterised by analysis of the  $\text{MV}^{\cdot+}$  chromophore at  $600 \text{ nm}$ .<sup>42</sup>



The rate constant  $k_3 = (2.6 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  was determined by analysis of the first-order build-up of the  $\text{MV}^{\cdot+}$  radical cation absorption at  $600 \text{ nm}$  after pulse radiolysis ( $\approx 2 \text{ Gy}$ ) of  $\text{N}_2\text{O}$ -saturated aqueous solutions containing  $0.1 \text{ mol dm}^{-3}$  2-propanol and  $\text{MV}^{2+}$  ( $8\text{--}20 \mu\text{mol dm}^{-3}$ ). Inserts (b) and (c) in Figure 4 show the effects of thiol and perthiol, respectively, on the build-up of  $\text{MV}^{\cdot+}$  produced from  $(\text{CH}_3)_2\text{C} \cdot \text{OH}$  compared to the formation (a) in the absence of thiol or perthiol. In solution (a)  $\text{MV}^{\cdot+}$  radical cations are produced with a total yield of

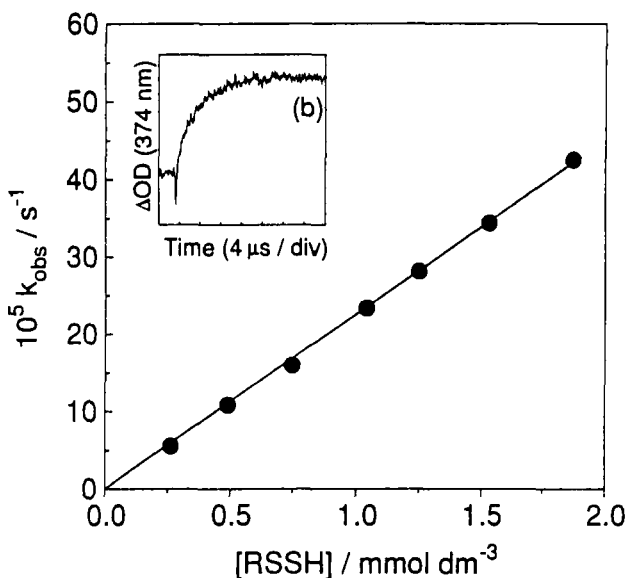


FIGURE 3 Determination of the rate of reaction of  $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$  with RSSH by pulse radiolysis of  $\text{N}_2\text{O}$ -saturated aqueous solutions containing various amounts of RSSH in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 4.5 and  $0.2 \text{ mol dm}^{-3}$  2-propanol. Observed first-order rate constant  $k_{\text{obs}}$  of the build-up of the perthiyl radical (measured at 374 nm) as a function of [RSSH].

$G(\text{MV}^{\bullet+}) = G(\text{R}^{\bullet'}) = 0.61 \mu\text{mol J}^{-1}$  corresponding to the full yield of carbon-centred radicals. The addition of either thiol or perthiol did not affect the overall yield of the radical cation but its formation was divided into two distinctive processes characterised by a prompt and then slower delayed formation of  $\text{MV}^{\bullet+}$  radical cation. The fast, initial build-up of the radical cation was attributed to reaction (14) and was lowered by increased concentrations of either perthiol and thiol due to the repair of carbon-centred radicals in reactions (5) or (13) respectively. The slower delayed step was due to the corresponding reverse repair reactions i.e. generation of reducing  $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$  radicals through  $\text{RS}^\bullet$  and  $\text{RSS}^\bullet$  radical reactions with the alcohol.<sup>15</sup>

By applying standard competition kinetics<sup>43</sup> in equations (Ia) and (Ib) it was possible to derive the rate constants  $k_5 = (2.3 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  and  $k_{13} = (2.5 \pm 0.1) \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  from the individual slopes of the linear plots (in Figure 4) of the initial yield of  $\text{MV}^{\bullet+}$  radical cation ( $A_0/A$ ) measured for different [RSSH or RSH]/[ $\text{MV}^{2+}$ ] ratios, where  $A_0$  is the yield in the absence of RSSH or RSH.

$$A_0/A = 1 + k_5[\text{RSSH}]/k_{14}[\text{MV}^{2+}] \quad (\text{Ia})$$

$$A_0/A = 1 + k_{13}[\text{RSH}]/k_{14}[\text{MV}^{2+}] \quad (\text{Ib})$$

The average value  $k_5(\text{RSSH} + \text{R}^{\bullet'}) = (2.5 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  determined by both direct and indirect competition methods is close to the diffusion-controlled limit and reflects the efficient hydrogen atom donor properties of RSSH. The perthiol exhibits an order of magnitude higher repair rate compared with the corresponding thiol (at least at low pH when both are fully protonated, see below).



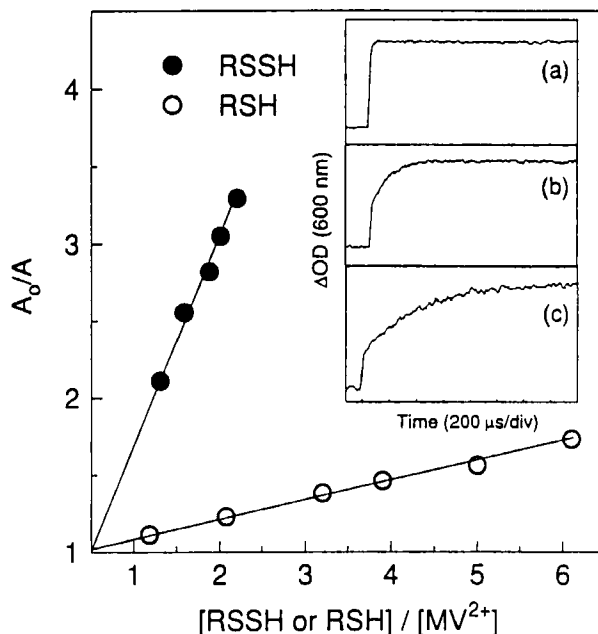


FIGURE 4 Determination of the rate constants for the reaction of  $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$  with RSSH and RSH by competition with methyl viologen. Plots of  $A_0/A$  versus  $[\text{MV}^{2+}]/[\text{RSSH or RSH}]$  obtained from the pulse radiolysis of  $\text{N}_2\text{O}$ -saturated aqueous solutions containing  $0.1 \text{ mmol dm}^{-3} \text{ MV}^{2+}$  and various concentrations of either RSSH or RSH, in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 4.5 and  $0.2 \text{ mol dm}^{-3}$  2-propanol. Inserts: Absorption vs time traces at 600 nm in pulse-irradiated  $\text{N}_2\text{O}$ -saturated 2-propanol/water solutions, in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 4.5 containing  $0.1 \text{ mmol dm}^{-3} \text{ MV}^{2+}$  with (a) neither RSSH nor RSH, (b)  $1 \text{ mmol dm}^{-3}$  RSH, or (c)  $0.25 \text{ mmol dm}^{-3}$  RSSH respectively. In the presence of either RSSH or RSH the traces are characterized by a prompt and delayed formation of the radical cation.

#### *Reversible Repair by RSSH and RSH: Hydrogen Abstraction by $\text{RSS}^\bullet$ and $\text{RS}^\bullet$ Radicals*

The competition method with  $\text{MV}^{2+}$  was also employed to compare rate constants for the reverse repair reactions (–5) and (–13) corresponding to hydrogen abstraction from propan-2-ol by  $\text{RSS}^\bullet$  and  $\text{RS}^\bullet$  radicals, respectively. A kinetic analysis of the slow formation of the  $\text{MV}^{\bullet+}$  radical cation was carried out based on the equation (II):<sup>15</sup>

$$[(\text{CH}_3)_2\text{CHOH}]/k_{\text{obs}} = 1/k_{-5} + 1/k_{-5} \times k_3[\text{RSSH}]/k_{14}[\text{MV}^{2+}] \quad (\text{II})$$

The polarity of the solvent at the high alcohol concentrations used for these experiments, i.e. (50% v/v) 2-propanol/water mixtures had a negligible effect on  $k_3$  and also  $k_{-5}$  but did influence the rate of reduction of  $\text{MV}^{2+}$  by  $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$ . The actual rate constant for the reduction of  $\text{MV}^{2+}$  was found to decrease from  $k_{14} = (2.6 \pm 0.1) \times 10^9$  to  $(2.1 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  in (5% v/v) and (50% v/v) 2-propanol/water mixtures, respectively, and the appropriate correction was made in equation (II). A linear plot (Figure 5) of  $[(\text{CH}_3)_2\text{CHOH}]/k_{\text{obs}}$  vs  $[\text{RSSH}]/[\text{MV}^{2+}]$  ratios gave an intercept from which  $k_{-5} = (3.8 \pm 0.3) \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  was obtained. Kinetic data obtained from  $\text{RS}^\bullet$  radical mediated build-up of the radical cation were also analysed by equation (II) (see Figure 5). The corresponding



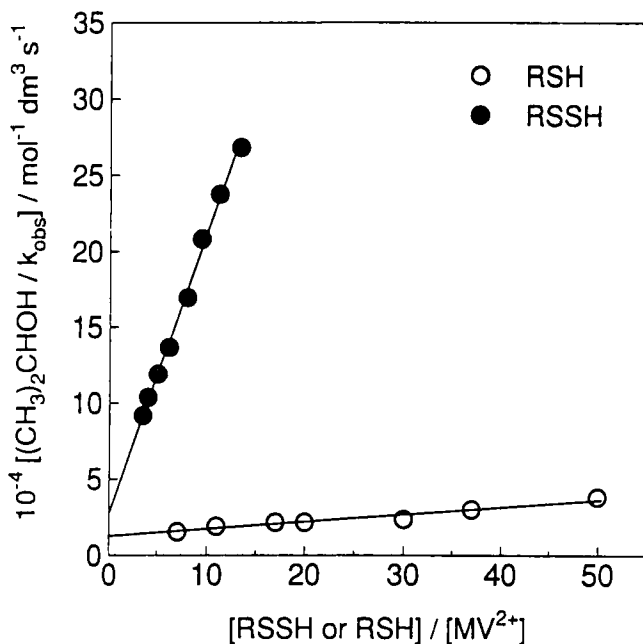
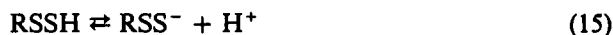


FIGURE 5 Plots of  $[(\text{CH}_3)_2\text{CHOH}]/k_{\text{obs}}$  for delayed formation of the methyl viologen radical cation (600 nm) versus  $[\text{RSSH or RSH}]/[\text{MV}^{2+}]$  concentration ratios obtained in the pulse-irradiated  $\text{N}_2\text{O}$ -saturated 2-propanol/water solutions, in  $1 \text{ mmol dm}^{-3}$  phosphate buffer at pH 4.5 containing various concentrations of either RSSH or RSH.

rate constant  $k_{-13} = (1.4 \pm 0.1) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  obtained was in excellent agreement with other thiyl radical hydrogen atom abstraction reactions e.g. penicillamine thiyl ( $\text{PenS}^\cdot + (\text{CH}_3)_2\text{CHOH} = (1.4 \pm 0.2) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ , cysteine thiyl ( $\text{CysS}^\cdot + (\text{CH}_3)_2\text{CHOH} = (2.0 \pm 0.2) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ , and glutathione thiyl ( $\text{GS}^\cdot + (\text{CH}_3)_2\text{CHOH} = (1.2 \pm 0.2) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  radicals, respectively).<sup>14,15</sup> The  $\text{RSS}^\cdot$  radical reacts approximately an order of magnitude slower than the  $\text{RS}^\cdot$  radical.

#### pH Dependence of Hydrogen Atom Transfer

The rate of hydrogen atom transfer to the  $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$  radical by the perthiol was found to decrease with increasing pH, as expected if the S-H hydrogen was involved in the reaction (see Figure 6). This is consistent with the deprotonation of the perthiol to form the perthiolate anion ( $\text{RSS}^-$ ) according to the acid/base equilibrium (15):



The pH-dependence of  $k_t$  was analysed using the appropriate function assuming no reactivity of  $\text{RSS}^-$  and gave  $\text{p}K_a(\text{RSS-H}) = 6.2 \pm 0.1$ . Analysis of the corresponding data ( $k_{13}$ ) for the thiol (Figure 6) gave  $\text{p}K_a(\text{RS-H}) = 7.6 \pm 0.1$ , a value in excellent agreement with the literature value of 7.69 obtained by proton NMR spectroscopy.<sup>9</sup> At physiological pH a greater fraction of the perthiol is ionized to the  $\text{RSS}^-$  anion in relation to the amount of thiol present in the deprotonated form,  $\text{RS}^-$ . Consequently, at  $\text{pH} \approx 7$  the reactivities of the perthiol and RSH are similar,

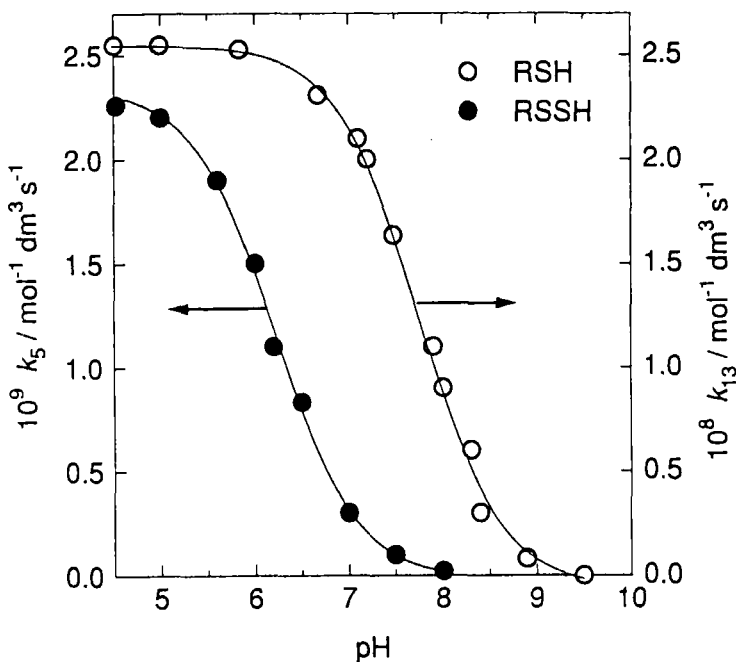
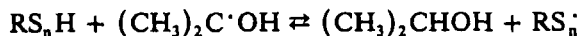


FIGURE 6 Variations with pH of the rate constant for reaction  $(\text{CH}_3)_2\text{C}\cdot\text{OH}$  with RSSH or RSH.

both repairing the 2-propanol radical with a rate constant of  $\approx 2.4 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . However, the rate constant for the RSSH analogue increased with increasing pH, e.g. at pH 4.5 to  $2.4 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ , i.e. by almost an order of magnitude, whereas the corresponding rate constant for RSH had increased by only  $\sim 15\text{--}20\%$  above its pH 7 value.

## CONCLUSIONS

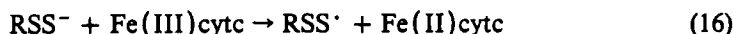
Repair of an  $\alpha$ -hydroxyalkyl radical, i.e.  $(\text{CH}_3)_2\text{C}\cdot\text{OH}$  by the perthiol analogue of WR-1065 results in the formation of perthiyl radicals ( $\text{RSS}\cdot$ ;  $\lambda_{\text{max}} = 374 \text{ nm}$ ,  $\epsilon_{374 \text{ nm}} = 1680 \pm 20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ). No evidence was obtained for the existence of the corresponding thiyl radical ( $\text{RS}\cdot$ ;  $\lambda_{\text{max}} = 340 \text{ nm}$ ,  $\epsilon_{340 \text{ nm}} = 500 \pm 20 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) suggesting that a possible  $(\text{CH}_3)_2\text{C}\cdot\text{OH}$ -induced reduction of the perthiol disulphide bond is unable to compete effectively with hydrogen transfer. An order of magnitude difference in the absolute rate constants  $k_5(\text{RSSH} + (\text{CH}_3)_2\text{C}\cdot\text{OH}) = (2.4 \pm 0.1) \times 10^9$  and  $k_{13}(\text{RSH} + (\text{CH}_3)_2\text{C}\cdot\text{OH}) = (2.5 \pm 0.1) \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$  reflect the superior free-radical repair capability of the perthiol. Both  $\text{RS}\cdot$  and  $\text{RSS}\cdot$  abstract hydrogen from 2-propanol to establish overall reversible equilibrium reactions (5/–5) and (13/–13) respectively.



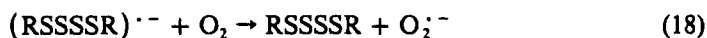
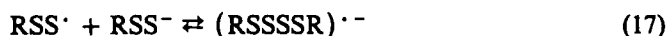
The rate constant for hydrogen abstraction by the  $\text{RSS}^\cdot$  radical  $k_{-5}(\text{RSS}^\cdot + (\text{CH}_3)_2\text{CHOH}) = (3.8 \pm 0.3) \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ , is almost an order of magnitude slower than that for the  $\text{RS}^\cdot$  radical,  $k_{13}(\text{RS}^\cdot + (\text{CH}_3)_2\text{CHOH}) = (1.4 \pm 0.2) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . A comparison of the equilibrium constants  $K_5 = (k_5/k_{-5}) = (6.3 \pm 0.1) \times 10^5$  and  $K_{13} = (k_{13}/k_{-13}) = (1.8 \pm 0.1) \times 10^4$  indicate that the reversible repair reaction (5/–5) is shifted further toward repair than the corresponding reversible repair reaction (13/–13) of WR-1065.

The disulphide bond in RSSH enhances the rate of repair by facilitating faster hydrogen transfer. Thermodynamic calculations have predicted that the S-H bond in higher sulphanes, i.e.  $\text{H}_2\text{S}_{n+1}$ , might be as much as  $90 \text{ kJ mol}^{-1}$  lower than the S-H bonds in aliphatic thiols.<sup>44,45</sup> This S-H bond-weakening effect has been accredited to the stability of  $\text{RSS}^\cdot$  radicals. Molecular orbital calculations have predicted that polysulphide radicals of the type  $\text{RS}_n^\cdot$  ( $n > 1$ ) are highly stabilized compared to simple  $\text{RS}^\cdot$  radicals.<sup>46</sup> The stability of  $\text{RSS}^\cdot$  radicals compared to  $\text{RS}^\cdot$  is paralleled by a significantly lower reactivity toward biological antioxidants and molecular oxygen.<sup>27,47</sup> While the sulphur non-bonding  $3p^2$  atomic orbitals (AOs) in a thiyl radical remain degenerate, the overlapping  $3p^2$  AOs in the sulphur-sulphur bond of perthiyl radicals establish  $\pi$ -bonding molecular orbitals which accommodate the  $\text{RSS}^\cdot$  radical electron spin.<sup>48</sup> The electronic nature of the perthiyl radical can be interpreted as a resonance hybrid of a normal sulphur-sulphur sigma bonded state ( $2\sigma$ ) and a five-electron bonded state ( $2\sigma/2\pi/1\pi^*$ ).<sup>38,46</sup> In this study the resonance stabilization energy of  $\text{RSS}^\cdot$  radical over the unstabilized  $\text{RS}^\cdot$  radical is  $8.8 \text{ kJ mol}^{-1}$ , calculated from the difference in Gibbs free energies for the reversible repair reactions (5/–5) and (13/–13) i.e.  $(\Delta G_{(5/-5)} - \Delta G_{(13/-13)}) = (-RT \ln K_5 + RT \ln K_{13})$ . This is in good agreement with the previously published value of  $\approx 8 \text{ kJ mol}^{-1}$  obtained from the thermal decomposition of tetrasulphides.<sup>49,50</sup> The inherent stability of the perthiyl radical therefore provides the thermodynamic driving force for the repair reaction (5).

A lower S-H bond energy also influences the acid/base properties of perthiol compared to thiol, with a reduction of the  $\text{p}K_a(\text{RS-H}) = 7.6 \pm 0.1$  to  $\text{p}K_a(\text{RSS-H}) = 6.2 \pm 0.1$ . Consequently, at physiological pH, while the thiol WR-1065 has 20% in the  $\text{RS}^-$  form,<sup>9</sup> its perthiol analogue has 95% in the  $\text{RSS}^-$  form. Any advantage of utilizing this particular perthiol analogue for DNA repair would probably be nullified at physiological pH, at least with respect to hydrogen-donation. However,  $\text{RS}^-$  anions undergo rapid electron-transfer reactions with, for example, peroxy radicals ( $\text{ROO}^\cdot$ )<sup>51</sup> and some DNA base radicals.<sup>10</sup> Perthiols may be much better electron donors at physiological pH where the thermodynamic stability of the  $\text{RSS}^\cdot$  might also increase the thermodynamic driving force for electron transfer from the  $\text{RSS}^-$  anion. It is noteworthy in this respect that  $\text{RSS}^\cdot$  radicals are formed in the reduction of Fe(III)-cytochrome-c by  $\text{RSS}^-$  in reaction (16):<sup>52</sup>



By analogy to reduction of molecular oxygen to the superoxide radical anion ( $\text{O}_2^{\cdot-}$ ) by the disulphide radical anion<sup>8</sup> reactions (17) and (18) may also represent a possible pathway by which perthiols might induce cellular oxidative stress:



However, during this study the variation of pH provided no evidence of a stable tetrasulphide radical anion (RSSSSR)<sup>•-</sup> followed by reaction (18). The central S-S bond in tetrasulphides is very weak<sup>48</sup>  $D(\text{RSS-SSR}) = 147.6 \text{ kJ mol}^{-1}$ . Thus, accommodating an extra electron in the antibonding orbital of this already weak bond would have a highly destabilizing effect, assuming that this species has a localized three-electron bond which is  $(2\sigma/1\sigma^*)$  in electronic nature, i.e.  $(\text{RSS} \cdot \text{SSR})^{\cdot-}$ .

Since  $pK_a$  values for dissociation are readily varied by substituents, perthiols and perthiolates merit further evaluation as antioxidants or alternatively as chemoprotective agents.

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