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REVERSIBLE H-ATOM ABSTRACTION FROM ALCOHOLS BY THIYL RADICALS: DETERMINATION OF ABSOLUTE RATE CONSTANTS BY PULSE RADIOLYSIS

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Penicillamine thiyl radicals, $PenS^{\cdot}$, are shown to abstract hydrogen atoms from 2-propanol and to establish an equilibrium

$$PenS^{\cdot} + (CH_3)_2CHOH \rightleftharpoons PenSH + (CH_3)_2\dot{C}OH.$$

The rate constants for the forward and back reaction have been determined to $(1.4 \pm 0.3) \times 10^4$ and $(1.2 \pm 0.3) \times 10^8\,M^{-1}s^{-1}$, respectively, by means of pulse radiolysis. The data have been obtained from various independent methods which include direct measurements and competitive schemes involving irreversible interception of the alcohol radical by electron acceptors (e.g. CCl_4, PNAP) and/or the thiyl radical by antioxidants (e.g. α-tocopherol). The results demonstrate that the reaction of carbon-centered radicals with thiols, in radiation biology commonly known as "repair" reaction, may be reversed and thus imply the possibility of thiyl radical induced biological damage.

KEY WORDS: Thiyl radicals, reversible H-atom abstraction, alcohols, CCl_4, α-tocopherol; pulse radiolysis.

ABBREVIATIONS: PenSH, penicillamine; $PenS^{\cdot}$, penicillamine thiyl radical; PNAP, p-nitroacetophenone; VitE-OH, α-tocopherol.

INTRODUCTION

Thiols are generally known as protecting agents against the damaging effects of free radicals in biological systems.[1,2] Such radicals may be produced via enzymatic metabolism of xenobiotics (e.g. CCl_4)[3-5] or hydroperoxides,[6-7] for example, or whenever a biological sample is subjected to ionizing radiation.[1] One of the most important thiols in this respect is considered to be glutathione, owing to its high abundance in cells ([GSH] $\leqslant$ 0.01 M).[8]

Chemically the protective effect results from an inactivation of a highly reactive, usually carbon-centered radical site via hydrogen atom transfer from the thiol. As a result of this "repair" reaction thiyl radicals are generated which were considered to be generally harmless with respect to any damaging effects. There is, however, increasing evidence that thiyl radicals may not only remove but, in fact, also regenerate other reactive radicals in the reverse of the repair reaction, i.e. to exist in the general equilibrium,

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$$RS^{\cdot} + R'H \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RSH + R'^{\cdot} \qquad (1)$$

This forward reaction of eq. (1) was quantitatively described for formate/thiol[9-11] and phosphite/thiol[12] systems. More recently Akhlaq *et al.*[13] presented evidence for the racemization of optically pure 2,5-dimethyltetrahydrofurane initiated via thiyl radicals and intermediary generation of a generally quite reactive α-alkoxy radical. Examples for H-atom abstraction from various aromatic and aliphatic compounds through $RS^{\cdot}$ were reported by Pryor *et al.*[14] and Miyashita *et al.*[15]

Direct experimental evidence of equilibrium (1) in general is still considered to be difficult[16] if not impossible to obtain, owing to the seemingly large difference in rate constants, with $k_1 \ll k_{-1}$. The "repair" (back reaction of eq. 1) has been found to occur with rate constants k_{-1} typically in the order of 10^7–$10^8\,M^{-1}s^{-1}$.[17,18] For the "reverse repair" (forward reaction) few rate constants reported suggest that these reactions mostly proceed with $k_1 \ll 10^7\,M^{-1}s^{-1}$.[9-15] There is one example, however, where H-atom abstraction by thiyl was observed to occur at a practically diffusion controlled rate, namely from a particularly activated C-H group.[19]

Reversibility of the repair reaction, i.e. the existence of equilibrium (1) would be of potential significance in biological systems since it implies the possibility of thiyl radical-induced damage. It would, in particular, provide the basis for a more quantitative understanding of the role of thiols in radical-mediated processes. It also would explain why the unquestionable protective effect exerted by thiols never appears to be complete.

In this communication we will report on a method which allows direct proof of equilibrium (1) and in particular the determination of the rate constant k_1 by various independent measurements. The system chosen involves penicillamine as thiol and 2-propanol as H-atom donor R'H, and serves as an example for such studies.

EXPERIMENTAL

The studies were conducted by means of the radiation chemical technique of pulse radiolysis.[20] Radicals were generated by applying short pulses of high energy electrons from a 1.55 MeV Van de Graaff accelerator to the solutions. Dosimetry was based on the oxidation of thiocyanate by $^{\cdot}OH$ radicals in N_2O sat. solutions using $\varepsilon_{(SCN)_2^{-\cdot}} = 7{,}200\,M^{-1}cm^{-1}$ at 500 nm, G = 5.5. (G denotes the yield of changed species per 100 eV absorbed energy; G = 1 corresponds to ca. $0.1\,\mu mol\,J^{-1}$.) The absorbed doses in the investigated systems were corrected for differences in electron density by using specific gravity approximation. Generally pulses of ca. 0.3 μs duration were applied with doses per pulse in the order of 2 Gy.

2-Propanol, t-butanol and acetone were obtained from Merck (p.a.), α-tocopherol (Sigma) and penicillamine (Fluka), all used as received. CCl_4 (Merck, p.a.) was destilled twice prior to use. α-Tocopherol solutions were kept under the minimum of light and prepared by dissolving it in oxygen-free mixtures to prevent oxidation. Experiments were performed at room temperature. Solutions were prepared using deionized water (Millipore filtered). Deaeration was achieved by bubbling with N_2 at ca. 1 h per dm^3 sample. Subsequent saturation with N_2O ensured conversion of most solvated electrons into $^{\cdot}OH$ radicals. Solutions were adjusted to pH 5 with $HClO_4$ to avoid dissociation of PenSH.

RESULTS and DISCUSSION

Generation of thiyl radicals

The pulse radiolysis experiments were performed with N_2O-saturated, pH $\approx$ 5, 2-propanol/water mixtures (usually 50 vol% each) and relatively high concentrations (typically 10^{-2} M) of penicillamine, PenSH, as major components. In such solutions thiyl radicals, $PenS^{\cdot}$, are produced according to reactions (2)–(6).[21,22]

$$H_2O \rightsquigarrow e^-_{aq}, {}^{\cdot}OH, H^{\cdot} \tag{2}$$

$$(CH_3)_2CHOH \rightsquigarrow (CH_3)_2\dot{C}OH, {}^{\cdot}CH_3, {}^{\cdot}CH_2(CH_3)CHOH, e^-_{sol} \tag{3}$$

$$e^-_{aq}, e^-_{sol} + N_2O \xrightarrow{H_2O} {}^{\cdot}OH + OH^- + N_2 \tag{4}$$

$${}^{\cdot}OH/H^{\cdot} + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O/H_2 \tag{5}$$

$$R^{\cdot}[(CH_3)_2\dot{C}OH, {}^{\cdot}CH_3, {}^{\cdot}CH_2(CH_3)CHOH] + PenSH \longrightarrow PenS^{\cdot} + RH \tag{6}$$

All these reactions are completed within the pulse duration, i.e. $PenS^{\cdot}$ is the only radical species present in the system at the end of the pulse.

Optical absorption measurements at 330 nm (λ_{max} for $PenS^{\cdot}$, $\varepsilon = 1{,}200\,M^{-1}\,cm^{-1}$)[23,24] gave $G_{PenS^{\cdot}} = 6.0$ immediately after the pulse. The decay of the $PenS^{\cdot}$ radicals was of pure second order kinetics indicating that their removal was solely due to the recombination process (7)

$$2\ PenS^{\cdot} \rightarrow PenSSPen \tag{7}$$

The rate constant of $k_7 = 1.5 \times 10^9\,M^{-1}\,s^{-1}$ obtained from the appropriate plot of $1/[RS^{\cdot}]$ vs. time at various initial concentrations of the thiyl radical species (2–14 μM) is lower than the published value of $2.85 \times 10^9\,M^{-1}\,s^{-1}$ in aqueous solution containing no alcohol.[24] This difference reflects the effect of solution viscosity on diffusion limited rate constants (relative viscosity at 20°C of water compared with 50 vol% 2-propanol/water mixture increases from 1.00 to 3.73, respectively).[25]

Systems containing CCl_4

If various amounts of CCl_4 were added to the solution the half-lives of $PenS^{\cdot}$ radicals decreased and the kinetics changed to pseudo-first order with respect to CCl_4 concentration. Figure 1 shows the plot of the obtained first order rate constants ($\ln 2/t_{1/2} = k_{obs}$) as a function of $[CCl_4]$. The almost linear relationship could lead to the conclusion that thiyl radicals reacted directly with CCl_4 at a rate calculable from the slope of the straight line. However, as shown below, this could be a wrong conclusion.

Using tert-butanol or acetone instead of 2-propanol as co-solvents gave a completely different picture. With these solvent components the $PenS^{\cdot}$ decay was unaffected by CCl_4 and second order kinetics were observed as in the absence of CCl_4. Similarily, in solutions containing only water as solvent CCl_4 addition (possible only up to the ca. 10^{-3} M solubility limit) had no effect. It must therefore be concluded that a direct reaction

$$PenS^{\cdot} + CCl_4 \not\rightarrow \tag{8}$$

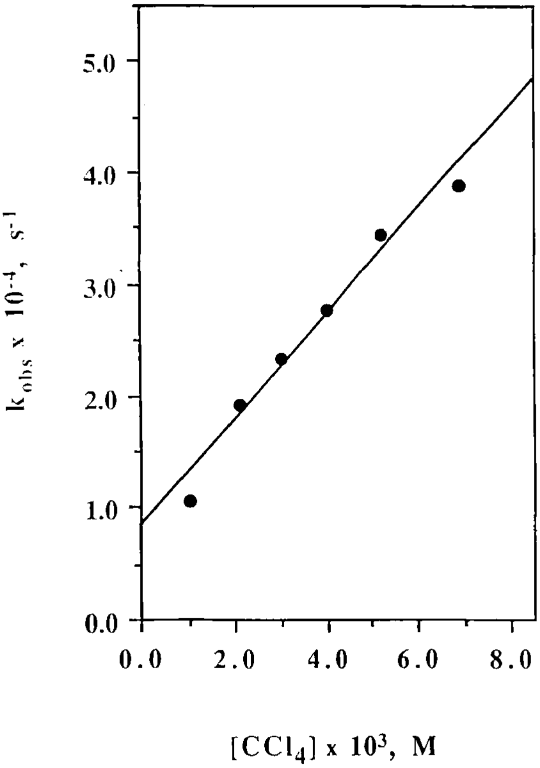


FIGURE 1 First-order rate constant (k_{obs}) for $PenS^{\bullet}$ decay vs. $[CCl_4]$. N_2O-saturated, 50 vol% 2-propanol/water, pH 5, 1×10^{-2} M PenSH and various CCl_4 concentrations.

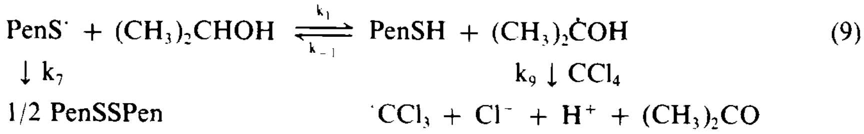
does not occur and that the observed effect of CCl_4 in 2-propanol containing systems is due to a more complex mechanism.

One possibility, namely, the reduction of CCl_4 by the disulfide radical anion or its conjugate acid via

$$\begin{array}{ccc} PenS^{\bullet} + PenS^{-} \rightleftharpoons & PenS\therefore SPen^{-} & \\ & \updownarrow H^{+} & + CCl_4 \not\rightarrow \\ & PenS\therefore S(H)Pen & \end{array}$$

can immediately be eliminated (electronically the disulfide radical anion and its conjugate protonated form are characterized by a $2\sigma/1\sigma^*$ three-electron bond).[26] Such a reaction should be independent of the solvent composition. Furthermore, direct observation of the $(PenS\therefore SPen)^{-}$ anion (λ_{max} 450 nm) at high pH did also not reveal any measurable reactivity towards CCl_4. The protonated form of the disulfide radical anion is an even less reducing species.[27]

The effect of CCl_4 in 2-propanol/water systems is quantitatively explained, however, by invoking an equilibrium between $PenS^{\bullet}$ and $(CH_3)_2\dot{C}OH$ radicals and consideration of the well-known reduction of CCl_4 by the α-hydroxyl radical.[28]



$$\begin{array}{lcl} \mathrm{PenS^{\cdot} + (CH_3)_2CHOH} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & \mathrm{PenSH + (CH_3)_2\dot{C}OH} \\ \downarrow k_7 & & k_9 \downarrow \mathrm{CCl_4} \\ \mathrm{1/2\ PenSSPen} & & \mathrm{{}^{\cdot}CCl_3 + Cl^- + H^+ + (CH_3)_2CO} \end{array} \qquad (9)$$

Kinetic analysis

The kinetics for the thiyl radical disappearance in this scheme is described by

$$-\frac{d[\mathrm{PenS^{\cdot}}]}{dt} = k_7[\mathrm{PenS^{\cdot}}]^2 + k_1[\mathrm{PenS^{\cdot}}][\mathrm{(CH_3)_2CHOH}] - k_{-1}[\mathrm{(CH_3)_2\dot{C}OH}][\mathrm{PenSH}] \qquad \text{(I)}$$

Applying steady state approximation for the concentration of $(CH_3)_2\dot{C}OH$ and considering that the concentrations of the molecular compounds PenSH, 2-propanol and CCl_4 remain essentially constant in the course of the reactions (radical concentration ca. 10^{-6} M) eq. (II) is derived as solution of equ. I.[29]

$$k_{obs} = \frac{k_9[\mathrm{CCl_4}] \times k_1[\mathrm{(CH_3)_2CHOH}]}{k_{-1}[\mathrm{PenSH}] + k_9[\mathrm{CCl_4}]} \qquad \text{(II)}$$

i.e. an expression which relates the measurable first order rate constant with all other rate constants in the scheme and the various concentrations of all the compounds. Accordingly, k_{obs} is linearly related to the CCl_4 concentration only if $k_{-1}[\mathrm{PenSH}] \gg k_9[\mathrm{CCl_4}]$. With $k_{-1} \approx 1.2 \times 10^8\,M^{-1}s^{-1}$ (see below) and $k_9 = 1.2 \times 10^8\,M^{-1}s^{-1}$ this does not apply any more if the CCl_4 concentration becomes similar to that of PenSH. This may be the reason why the point in Figure 1 relating to the highest $[CCl_4]$ falls below the straight line.

Rearrangement of II leads to III

$$\frac{[\mathrm{(CH_3)_2CHOH}]}{k_{obs}} = \frac{1}{k_1} + \frac{1}{k_1} \times \frac{k_{-1}[\mathrm{PenSH}]}{k_9} \times \frac{1}{[\mathrm{CCl_4}]} \qquad \text{(III)}$$

A corresponding plot of $[(CH_3)_2CHOH]/k_{obs}$ vs. $1/[CCl_4]$ of the experimental data in Figure 2 now exhibits an excellent linear relationship. The intercept yields the rate constant $k_1 = 1.4 \times 10^4\,M^{-1}s^{-1}$ for the "reverse repair" reaction. Using this value the rate constant for the "repair" reaction is calculated from the slope to be $k_{-1} = 1.2 \times 10^8\,M^{-1}s^{-1}$.

Systems containing p-nitroacetophenone (PNAP)

The mechanism and kinetic scheme outlined for solutions containing CCl_4 applies in principle to any system with a different electron acceptor which is reduced by the alcohol radical. A suitable compound from this point of view is p-nitroacetophenone (PNAP), particularly since it has the advantage to form an optically absorbing and long-lived, i.e. easily detectable transient radical anion ($\lambda_{max} = 360$ nm, $\varepsilon = 17{,}600\,M^{-1}cm^{-1}$).[30]

$$\mathrm{(CH_3)_2\dot{C}OH + PNAP \rightarrow (PNAP)^{-\cdot} + H^+ + (CH_3)_2CO} \qquad (10)$$

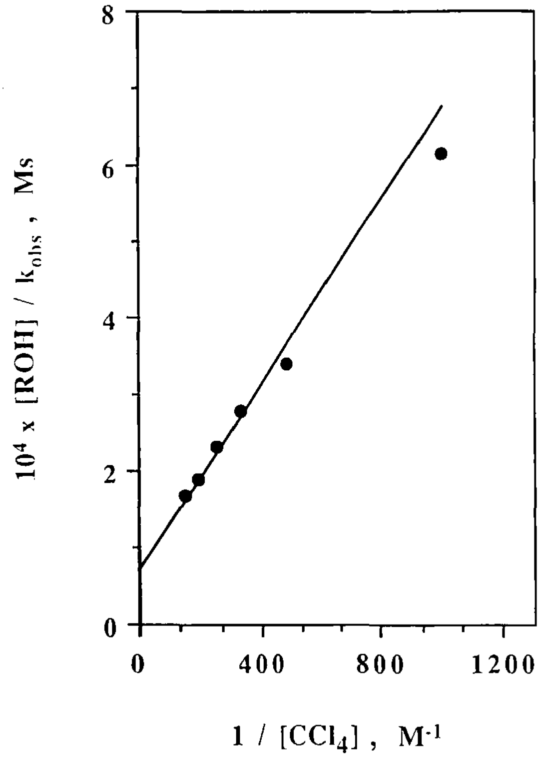


FIGURE 2 $[(CH_3)_2CHOH]/k_{obs}$ plotted vs. $1/[CCl_4]$ (data from Figure 1).

Figure 3 shows the individual time-dependent absorption signals taken at 360 nm in pulse-irradiated N_2O-saturated, 50 vol% 2-propanol/water solutions containing a) 4.0×10^{-4} M PNAP and no PenSH; b–e) $(7.7\text{–}1.2) \times 10^{-4}$ M PNAP and 5.1×10^{-3} M PenSH. In solution a) the $PNAP^{\cdot -}$ radical anion is seen to be produced during the duration of the pulse via reaction (10) with the yield of G = 6.2.

Addition of 5.1×10^{-3} M PenSH to the above solution did not change the final yield of $PNAP^{\cdot -}$ but its formation is clearly divided into two processes (Figure 3c). The first, fast build-up is due to reaction (10). It is lower in yield by a factor of ca. 2 compared with 3a); this is attributed to the reaction of $(CH_3)_2\dot{C}OH$ with PenSH which competes with reaction (10). From the initial $PNAP^{\cdot -}$ yields measured at different [PenSH]/[PNAP] ratios and applying standard competition kinetics[29] the rate constant for the H-atom abstraction from PenSH by $(CH_3)_2\dot{C}OH$ is derived as $k_{-1} = 1.5 \times 10^8 M^{-1}s^{-1}$, in good agreement with k_{-1} evaluated from the CCl_4 system. The calculation is based on $k_{10} = 1.5 \times 10^9$ which was determined separately in PenSH-free, 50 vol% 2-propanol in water (see below).

Since the total yield of $(PNAP)^{\cdot -}$ from both processes remains constant, the slow formation must be associated with the alcohol radicals reacting initially with PenSH to yield $PenS^{\cdot}$ radicals. These are not able to reduce PNAP directly. Consequently, the

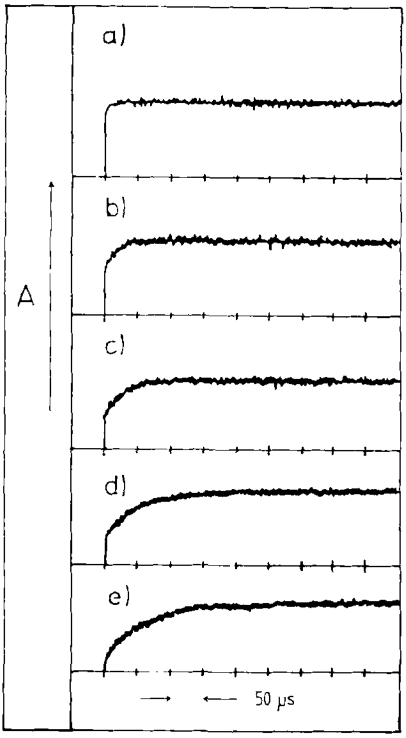


FIGURE 3 Oscillograms showing PNAP$^{\cdot -}$ radical anion formation at 360 nm; N_2O-saturated 50 vol% 2-propanol/water, pH 5 solutions containing a) 4.0×10^{-4} M PNAP and no PenSH, or 5.1×10^{-3} M PenSH and b) 7.7×10^{-4} M; c) 4.0×10^{-4} M; d) 2.5×10^{-4} M; e) 1.2×10^{-4} M PNAP, respectively. Ordinate: optical absorption, arbitrary units. Abscissa: 50 μs per division.

delayed formation of $(PNAP)^{\cdot -}$ implies regeneration of $(CH_3)_2\dot{C}OH$ via $PenS^{\cdot}$ in the "reverse repair" reaction and serves as direct experimental proof of it.

The kinetics of the slow build-up exhibit exponential behaviour, and the half-lives decrease with increasing PNAP concentration. A quantitative kinetic analysis based on the same scheme as in the CCl_4 systems leads to eq. (IV) with CCl_4 being substituted by PNAP:

$$\frac{[(CH_3)_2CHOH]}{k_{obs}} = \frac{1}{k_1} + \frac{1}{k_1} \times \frac{k_{-1}}{k_{10}} \times \frac{[PenSH]}{[PNAP]} \qquad \text{(IV)}$$

Here k_{obs} refers to the slow, i.e. $PenS^{\cdot}$ mediated $(PNAP)^{\cdot -}$ build-up. A corresponding plot of $[(CH_3)_2CHOH]/k_{obs}$ *vs.* the [PenSH]/[PNAP] concentration ratio is shown in Figure 4 and again a linear relationship is observed. The plot includes not only data at different PNAP but also PenSH concentrations (5, 10 and 20 mM).

The rate constant $k_1 = 1.1 \times 10^4\,M^{-1}s^{-1}$ derived from the intercept confirms the result from the CCl_4 system. A similarly good agreement is obtained for k_{-1} ($1.0 \times 10^8\,M^{-1}s^{-1}$) derived from the slope taking $k_{10} = 1.5 \times 10^9\,M^{-1}s^{-1}$ in 50 vol% 2-propanol solutions.

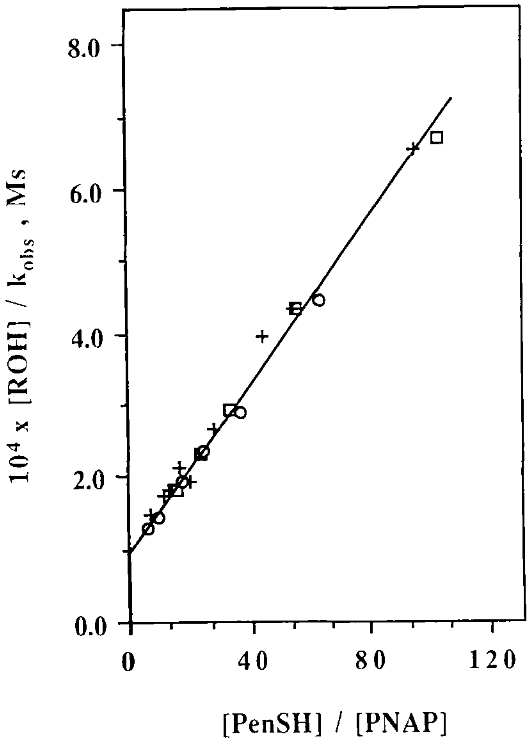


FIGURE 4 $[(CH_3)_2CHOH]/(k_{obs})$ for $PNAP^{\cdot -}$ formation vs. [PenSH]/[PNAP] concentration ratio, obtained in pulse-irradiated N_2O-saturated 50 vol% 2-propanol/water, pH 5. + 5 × 10^{-3} M; ○ 1 × 10^{-2} M; □ 2 × 10^{-2} M PenSH, respectively.

Variation of alcohol concentrations

According to eq. (II) k_{obs} should be directly proportional to the 2-propanol concentration. This was proven with systems containing different amounts of 2-propanol in water (10–70 vol%) N_2O-saturated, pH 5, and constant (5 × 10^{-3} M) PenSH and (2.3 × 10^{-4} M) PNAP. Equation (II) is accordingly rearranged to

$$k_{obs} = k_1 \times \frac{k_{10}[PNAP]}{k_{-1}[PenSH] + k_{10}[PNAP]} \times [(CH_3)_2CHOH] \qquad (V)$$

A linear relationship between k_{obs} and $[(CH_3)_2CHOH]$ cannot be expected, however, since redox processes like reaction (10) are affected by the polarity of the solvent, and this becomes significant at the high alcohol concentrations used in our experiments. The actual rate constants for the reduction of PNAP were determined to be $k_{10} = 3.0 \times 10^9$, 2.0×10^9, 1.5×10^9 and $1.2 \times 10^9\,M^{-1}s^{-1}$ at 10, 30, 50 and 70 vol% 2-propanol solutions ([PNAP] = (0.1–1.5) × 10^4 M, no PenSH). Our values are in accordance with the literature values, of 3.8 × 10^9[31] and 1.8 × $10^9\,M^{-1}s^{-1}$[30] at ca. 1.5 and 50 vol% 2-propanol, respectively. An alternative consideration for the observed effect on the basis of a reaction between α-hydroxyalkyl radicals and nitrobenzenes which does not proceed by outer-sphere electron transfer but by an (inner sphere) addition/elimination process[32] is probably not relevant. Decomposition of a possible $(CH_3)_2\dot{C}OH$/PNAP adduct in aqueous solution occurs within $\leqslant 1\ \mu s$.[32]

This solvent-dependence of k_{10} can be taken into consideration by calculating the factor

$$f = \frac{k_{10}[\mathrm{PNAP}]}{k_{-1}[\mathrm{PenSH}] + k_{10}[\mathrm{PNAP}]}$$

for each individual experiment and plotting k_{obs} *vs.* $f \times [(CH_3)_2CHOH]$ according to the simplified equation

$$k_{obs} = k_1 \times f \times [(CH_3)_2CHOH] \qquad \text{(V-a)}$$

The result is shown in Figure 5 which shows indeed a straight line through the origin as required from eq. (V-a). The rate constant $k_1 = 1.6 \times 10^4\ M^{-1}\ s^{-1}$ derived from the slope is the same as that obtained before. This shows that this rate constant is independent of the water/2-propanol ratio in contrast to the redox reaction between $(CH_3)_2\dot{C}OH$ and PNAP.[32]

PNAP as reaction partner was also used to determine the rate constant for the

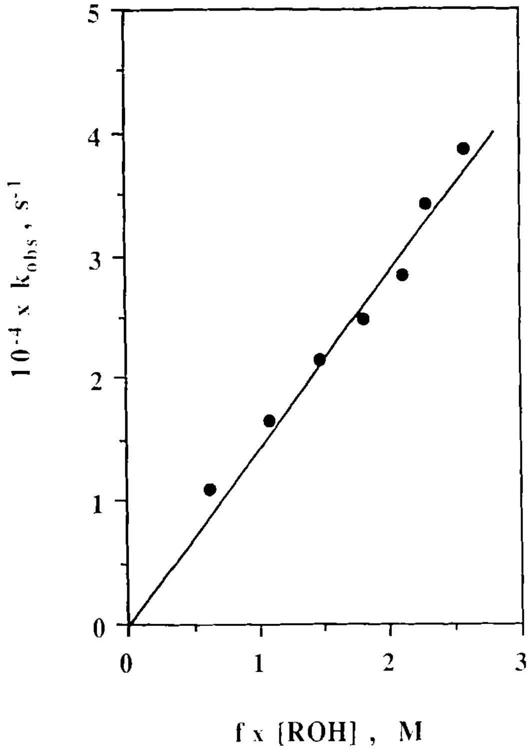


FIGURE 5 First-order rate constant (k_{obs}) for PNAP^{-} formation versus f x $[(CH_3)_2CHOH]$ (for "f" see text) obtained in pulse-irradiated N_2O-saturated 10–70 vol% 2-propanol/water mixtures, pH 5, containing 5.0×10^{-3} M PenSH and 2.3×10^{-4} M PNAP.

reduction of CCl_4 by $(CH_3)_2\dot{C}OH$ radicals (k_9) in 50 vol% 2-propanol in water. Solutions were N_2O-saturated, pH 5, and contained 1.0×10^{-4} M PNAP and various CCl_4 concentrations $(0.1\text{–}1.6) \times 10^{-3}$ M). From the competition plot of the optical absorption of $PNAP^{\cdot -}$ at different $[CCl_4]/[PNAP]$ ratios and taking $k_{10} = 1.5 \times 10^9\,M^{-1}s^{-1}$, $k_9 = 1.2 \times 10^8\,M^{-1}s^{-1}$ was obtained. This value was used for the k_1 calculations from Figures 2 and 6 (see below).

Competition method

The rate constant for the forward reaction of equilibrium (1), i.e. k_1, was also determined with a competition method involving the thiyl radicals. These are known to be moderatley good oxidants[17,27] and should be able to oxidize α-tocopherol (VitE-OH)

$$PenS^{\cdot} + VitE\text{-}OH \rightarrow PenSH + VitE\text{-}O^{\cdot} \qquad (11)$$

The radical product of reaction (11) can be observed by its optical absorption at 425 nm. In 50 vol% 2-propanol/water, N_2O-saturated solution, pH 5, containing 1×10^{-2} M PenSH and various α-tocopherol concentrations ($(1\text{–}5) \times 10^{-3}$ M) the ($VitE\text{-}O^{\cdot}$) radical was formed with a rate of $k_{11} = 4.4 \times 10^5\,M^{-1}s^{-1}$.

Solubility of α-tocopherol in 50 vol% 2-propanol/water mixtures limited the concentration used in the competition experiments and combination of $PenS^{\cdot}$ radicals may not completely be suppressed. Using equation (VI) it is, in fact, calculated that only 60% of the thiyl radicals oxidize α-tocopherol (at 4×10^{-3} M) whereas the rest disappears via disulfide formation according to reaction (7).[33]

$$[VitE\text{-}O^{\cdot}] = \frac{k_{11}\,[VitE\text{-}OH]}{k_7} \ln\left\{1 + \frac{k_7}{k_{11}\,[VitE\text{-}OH]}[RS^{\cdot}]_o\right\} \qquad (VI)$$

Addition of CCl_4 as electron acceptor from the alcohol radical leads to lower $VitE\text{-}O^{\cdot}$ (solutions containing 4.1×10^{-3} M α-tocopherol, $(1.5\text{–}10) \times 10^{-4}$ M CCl_4). Comparing the absorptions of $VitE\text{-}O^{\cdot}$ measured in systems in the absence (A_0) and in the presence (A) fo CCl_4 results in a dependence shown by eq. (VII)

$$\frac{A_0}{A} = 1 + \frac{k_9\,[CCl_4]}{k_{11}\,[VitE\text{-}OH]} \times \frac{k_1\,[(CH_3)_2CHOH]}{k_{-1}\,[PenSH] + k_9\,[CCl_4]} \qquad (VII)$$

which relates equilibrium (1) and both the reduction of CCl_4 by $(CH_3)_2\dot{C}OH$ (reaction (9)) and the oxidation of α-tocopherol by $PenS^{\cdot}$ (reaction (11)). Equation (VII) neglects reaction (7) and thus represents only a lower limit for the calculation of k_1.

At the low CCl_4 concentrations used $k_{-1}\,[PenSH] \gg k_9[CCl_4]$. Equation (VII) can then be approximated to

$$\frac{A_0}{A} = 1 + \frac{k_9}{k_{11}} \times \frac{k_1}{k_{-1}} \times \frac{[(CH_3)_2CHOH]}{[PenSH]} \times \frac{[CCl_4]}{[VitE\text{-}OH]} \qquad (VIIa)$$

Figure 6 shows a plot of A_0/A vs. $[CCl_4]/[VitE\text{-}OH]$. The obtained straight line intercepts the ordinate at unity, as required by eq. (VIIa). From the slope $k_1 = 0.7 \times 10^4\,M^{-1}s^{-1}$ is obtained taking the known values of k_9 and k_{11}, and $k_{-1} = 1.2 \times 10^8\,M^{-1}s^{-1}$ as an average value from the previous three independent experiments.

Inclusion of reaction (7) in equation (VII) as a second order reaction is mathema-

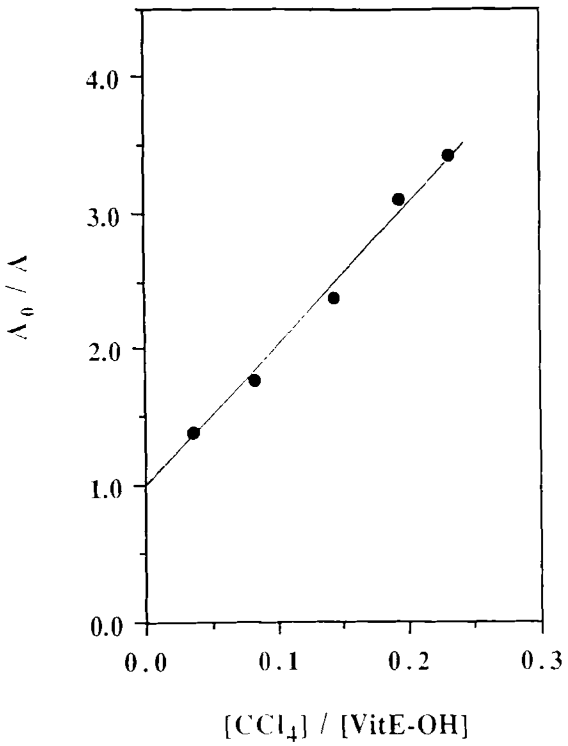


FIGURE 6 Competition for PenS˙ radicals between α-tocopherol (4.0×10^{-3}M) and CCl_4 (via $(CH_3)_2\dot{C}OH$, see text). Relative absorption at 425 nm versus relative solute concentration obtained in pulse-irradiated N_2O-saturated 50 vol% 2-propanol/water mixture, pH 5, and 1×10^{-2}M PenSH.

tically difficult because the exact concentration of RS˙ at various CCl_4 concentrations is not known over the entire time range. An upper limit of k_1 could be obtained (on the basis of a purely mathematical consideration, though) if reaction (7) was assumed to be first-order with respect to [RS˙], i.e. any RS˙ would always find a constant rather

TABLE 1
Rate constants for the equilibrium reactions (1) and for the processes involved in their determination.

Reaction	Rate constant/$M^{-1}s^{-1}$
PenS˙ + $(CH_3)_2CHOH$	$k_1^* = (1.4 \pm 0.3) \times 10^4$
$(CH_3)_2\dot{C}OH$ + PenSH	$K_{-1}^* = (1.2 \pm 0.3) \times 10^8$
$(CH_3)_2\dot{C}OH$ + CCl_4	$k_9^{**} = (1.2 \pm 0.2) \times 10^8$
$(CH_3)_2\dot{C}OH$ + PNAP	$k_{10}^{**} = (1.5 \pm 0.2) \times 10^9$
PenS˙ + VitE-OH	$k_{11}^{**} = (4.4 \pm 1.0) \times 10^5$

*average value for all methods used
**for 50 vol% 2-propanol water mixtures

than a gradually decreasing RS˙ concentration. Equation (VIIa) would then change into (VIII). A plot of A_0/A vs. $[CCl_4]$ gave the expected straight line.

$$\frac{A_0}{A} = 1 + \frac{k_9[CCl_4]}{k_{11}[\text{VitE-OH}] + k_7[\text{PenS}\cdot]} \times \frac{k_1}{k_{-1}} \times \frac{[(CH_3)_2CHOH]}{[\text{PenSH}]} \qquad \text{(VIII)}$$

From the slope the upper limit of k_1 is calculated to be $k_1 = 1.4 \times 10^4 M^{-1}s^{-1}$. This is in better agreement with the other data than the lower limit of $0.7 \times 10^4 M^{-1} s^{-1}$.

CONCLUSION

The existence of equilibrium (1) has unambiguously been proven, and the rate constant for the most interesting reaction, namely the H-atom abstraction from 2-propanol by PenS˙ was determined by several independent methods to be $k_1 = (1.4 \pm 0.3) \times 10^4 M^{-1} s^{-1}$ as mean value.

For each method evaluation of k_1 required determination of rate constants for several other reactions. Most of them were already known for dilute aqueous solutions but had to be redetermined for the less polar 2-propanol/water mixtures used. The rate constants for the reactions of the $(CH_3)_2\dot{C}OH$ radical with PenSH and α-tocopherol have been measured for the first time in this work.

H-Atom abstraction reactions by thiyl radicals from activated C–H bonds are generally possible. This was shown for some cases previously in the literature, where rate constants were found to vary between 10^3 and $10^7 M^{-1}s^{-1}$.[9–15] An interesting although probably extreme example was given by Alder *et al.*[19] who measured a nearly diffusion-controlled rate constant of $3 \times 10^9 M^{-1}s^{-1}$ for a hydrogen abstraction from the 1,6-diazabicyclo [4.4.4] dodecane radical cation by t-butS˙ radicals.

From the thermodynamic point of view H-atom abstraction from activated C–H bonds is not unexpected, because the bond energies of S–H in thiols and activated C–H bonds are not very different. For alcohols this difference amounts to only a few kcal/mol, if any, e.g. bond energies are listed as 91 ± 1 kcal/mol for $(CH_3)_2(OH)C \ldots H$, and 91 ± 1.5 kcal/mol for aliphatic RS . . . H.[34]

The mechanism presented and all considerations discussed in this paper apply, in principle, to other thiols, alcohols and other compounds with activated C–H bonds. Also, the use of electron acceptors and donors is not limited to CCl_4, PNAP and α-tocopherol used in the present study as will be reported separately.

Our present findings may be of particular relevance for the *in vivo* action of CCl_4. The well-known toxicity of this compound is mainly related to the formation of $\dot{}CCl_3$ radicals in the liver.[3–5] In the presence of alcohol, and possibly many other compounds with activated C–H bonds, CCl_4 could thus be degraded via equilibrium (1) even in the presence of relatively high α-tocopherol concentrations as shown in this study. An interesting synergism in CCl_4 toxicity was found in vivo if CCl_4 and ethanol were taken up simultaneously (T.F. Slater, personal communication).

In conclusion, it seems that thiol toxicity, mediated via thiyl radicals, has to be taken into consideration under certain conditions. The fact, that glutathione and cysteine are mutagenic in the Ames test[35] and, more generally, that radical repair and protection by thiols is practically never quantitative may be directly related to this.

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