



## Polar Substituent and Solvent Effects on the Kinetics of Radical Reactions with Thiols

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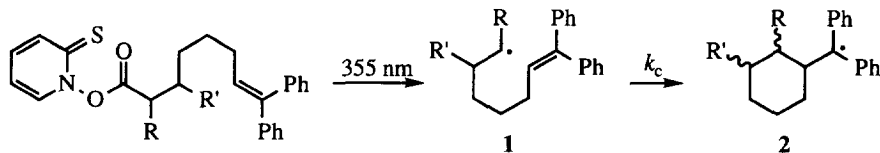
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**Abstract:** The rates of thiol trapping of radicals depend upon polar substituents (Table 1) and solvent effects (Table 2). The rate accelerating effect of water on the reactions of biologically relevant radicals is of special importance. Copyright © 1996 Elsevier Science Ltd

Radical reactions in biological processes have been an area of growing interest in recent years, and the chemistry of DNA radicals is an especially highly targeted area of study because a number of anti-cancer agents are known to act by destroying DNA via formation of radical intermediates.<sup>1</sup> 4'-DNA radicals are of particular interest because they undergo spontaneous strand cleavage in competition with trapping reactions by H-donors or by O<sub>2</sub>.<sup>2</sup> Although qualitative observations concerning product formation and quantitative relative rate constants from competition studies can be determined, absolute rate constants for these radical reactions are not known because an absolute kinetic scale for 4'-DNA radicals does not yet exist. The significant questions to address in the development of such a rate scale are (1) what are the general consequences of multiple oxygenated substituents on radical kinetics, and (2) how are radical kinetics affected by the highly polar aqueous media of biological systems? In this Letter, we report preliminary results of the kinetics of alkoxy substituted radical clocks and thiol trapping that address both points.

The series of isostructural radicals **1a-d** was used to evaluate the general effects of single and double alkoxy substitution on radical kinetics. These radicals, produced by laser flash photolysis (LFP, Nd-YAG, 355 nm) of the corresponding PTOC esters precursors,<sup>3</sup> cyclize in a 6-*exo* fashion to give diphenylalkyl radical products **2a-d** that are readily monitored by UV spectroscopy. The method and cyclization rate constants for radicals **1a** and **1b** in THF were reported previously.<sup>4</sup> Arrhenius functions for the cyclizations of radicals **1** were determined in various solvents over the temperature range of 0–40 °C, and the results are given in Table 1. The data demonstrate that the rate of cyclization is enhanced only slightly by increasing the solvent polarity.<sup>5</sup>



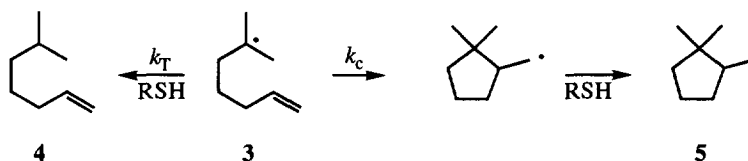
**a:** R = R' = H; **b:** R = OCH<sub>3</sub>, R' = H; **c:** R = H, R' = OCH<sub>3</sub>; **d:** R = R' = OCH<sub>3</sub>

**Table 1.** Rate constants for cyclization reactions ( $k_c$ ) and trapping reactions by octadecanethiol ( $k_T$ ) of radicals **1**.

Radical	Solvent	Arrhenius function <sup>a</sup>	$k_c$ <sup>b</sup> (25 °C) s <sup>-1</sup>	$k_T$ <sup>c</sup> (30 °C) M <sup>-1</sup> s <sup>-1</sup>
<b>1a</b>	THF	9.52 – 5.27/ $\theta$	$4.5 \times 10^5$	$(1.9 \pm 0.3) \times 10^7$
	CH <sub>3</sub> CN	9.61 – 5.18/ $\theta$	$6.4 \times 10^5$	
	CH <sub>3</sub> CN/H <sub>2</sub> O (50:50)	10.12 – 5.67/ $\theta$	$9.1 \times 10^5$	
<b>1b</b>	THF	8.76 – 4.87/ $\theta$	$1.5 \times 10^5$	$(2.0 \pm 0.3) \times 10^7$
	CH <sub>3</sub> CN	9.56 – 5.68/ $\theta$	$2.4 \times 10^5$	
<b>1c</b>	THF	9.47 – 4.38/ $\theta$	$1.8 \times 10^6$	$(1.0 \pm 0.1) \times 10^7$
	CH <sub>3</sub> CN	9.79 – 4.74/ $\theta$	$2.0 \times 10^6$	
<b>1d</b>	THF	9.75 – 6.07/ $\theta$	$2.0 \times 10^5$	$(2.1 \pm 0.4) \times 10^6$
	CH <sub>3</sub> CN	9.18 – 5.12/ $\theta$	$2.6 \times 10^5$	
	CH <sub>3</sub> CN/H <sub>2</sub> O (50:50)	10.19 – 6.38/ $\theta$	$3.2 \times 10^5$	

<sup>a</sup>  $\theta = 2.3RT$  in kcal/mol; <sup>b</sup> rate constant at 25 °C from Arrhenius function; <sup>c</sup> bimolecular rate constant for reaction of radicals **1** with octadecanethiol in THF at 30 °C; errors are  $2\sigma$ .

Second order rate constants for reactions of radicals **1** with octadecanethiol in THF at 30 °C were measured directly, and the results are also listed in Table 1. Unfortunately, the PTOC precursors for **1** were unstable in aqueous solutions containing thiols precluding solvent effect studies. Therefore alkyl radical **3** was used to study the effect of H<sub>2</sub>O on the rate of H-abstraction from thiols.



Radical **3** was produced from the corresponding PTOC ester at 30 °C in the presence of various concentrations of *t*-BuSH or glutathione (GSH) diethyl ester.<sup>6</sup> Rate constants for cyclization ( $k_c$ ) of **3** determined by competitive trapping by Bu<sub>3</sub>SnH have been reported,<sup>7a</sup> but we recalibrated the cyclization of **3** at 30 °C by competition against PhSH<sup>7b</sup> trapping; at 30 °C in cyclohexane the rate constant was  $1.6 \times 10^5$  s<sup>-1</sup>. With the assumption that the cyclization is nearly insensitive to solvent effects (see Table 1), rate values for the H-transfer ( $k_T$ ) were measured and are collected in Table 2.

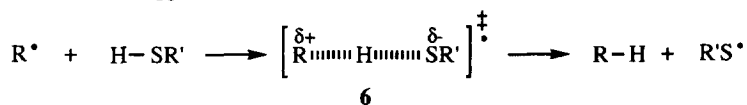
**Table 2.** Rate constants for thiol trapping of radical **3** at 30 °C.

Thiol	Solvent	$k_T/k_c$	$k_T^a$ (30 °C) $M^{-1} s^{-1}$
<i>t</i> -BuSH	CH <sub>3</sub> CN	21.7	$(3.5 \pm 0.3) \times 10^6$
	CH <sub>3</sub> CN/H <sub>2</sub> O (50:50)	83.4	$(1.3 \pm 0.1) \times 10^7$
glutathione diethyl ester	CH <sub>2</sub> Cl <sub>2</sub>	22.9	$(3.7 \pm 0.2) \times 10^6$
	CH <sub>3</sub> OH	53.6	$(8.6 \pm 0.4) \times 10^6$
	CH <sub>3</sub> CN	41.6	$(6.7 \pm 0.4) \times 10^6$
	CH <sub>3</sub> CN/H <sub>2</sub> O (75:25)	71.4	$(1.1 \pm 0.1) \times 10^7$
	CH <sub>3</sub> CN/H <sub>2</sub> O (50:50)	147	$(2.4 \pm 0.1) \times 10^7$
	CH <sub>3</sub> CN/H <sub>2</sub> O (20:80)	372	$(6.0 \pm 0.5) \times 10^7$

<sup>a</sup> Bimolecular rate constant using a cyclization rate  $k_T$  (**3**→**4**) of  $1.6 \times 10^5 s^{-1}$  (cyclohexane, 30 °C).

The data of Tables 1 and 2 demonstrate that the rates of H-transfer from alkyl thiols to alkyl radicals depend upon the radical substituents and the polarity of the solvent. Thus, the  $\alpha,\beta$ -dimethoxy substituted radical **1d** reacts about 10 times slower than the unsubstituted radical **1a** (Table 1). This rate decrease is caused by polar and stability effects of the methoxy substituents at the radical. A rate decreasing polar effect of the  $\beta$ -methoxy substituent is demonstrated by radical **1c** which reacts two times slower than the unsubstituted alkyl radical **1a**. A more extreme example for this polar effect is the reaction of an electron deficient perfluoroalkyl radical with PhSH which is a factor of 500 less rapid than reaction of an alkyl radical.<sup>8</sup> Because of polar effects, one could expect a rate increase with  $\alpha$ -methoxy substituted radical **1b** compared to the unsubstituted radical **1a**. However, both radicals abstract a H-atom from octadecanethiol with the same rate. Presumably the influence of the polar effect is compensated by the radical stabilizing effect of the  $\alpha$ -methoxy group. In radical **1d** this  $\alpha$ -methoxy stabilizing effect is even more pronounced because of the synergistic effect of the two methoxy substituents.<sup>9</sup> The same trend has been observed in pulse radiolysis studies in water where the  $\alpha$ -hydroxy radical formed from ethanol reacts with dithiothreitol about four times faster than does the  $\alpha,\beta$ -dihydroxy radical produced from ethylene glycol.<sup>10</sup>

The kinetic measurements listed in Table 2 show that the thiol trapping rate constants increase as the water content of the solution is increased. For the glutathione diethyl ester reaction with **3**, the rate enhancement upon proceeding from CH<sub>3</sub>CN to a 4:1 H<sub>2</sub>O/CH<sub>3</sub>CN mixture is about an order of magnitude. Replacing the solvent CH<sub>2</sub>Cl<sub>2</sub> by the more polar CH<sub>3</sub>CN or CH<sub>3</sub>OH increases rates of the hydrogen abstraction by a factor of 1.8 and 2.3, respectively.<sup>11</sup> From these kinetic measurements in different solvents, a highly polarized transition state **6** for hydrogen transfer from thiols is apparent.<sup>12</sup>



In conclusion, polar substituent effects and the rate accelerating effect of water on the kinetics of thiol reactions with radicals must be taken into consideration for reactions occurring under biochemical conditions.<sup>13</sup>

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## References and Notes

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3. The PTOC precursors to **1a** and **1b** have been reported,<sup>4</sup> and the PTOC precursors to radicals **1c** and **1d** were prepared from the corresponding carboxylic acids by conversion to the acid chlorides (CO<sub>2</sub>Cl<sub>2</sub>) and reaction with the sodium salt of *N*-hydroxypyridine-2-thione by the reported method.<sup>4</sup> All PTOC precursors had appropriate <sup>1</sup>H and <sup>13</sup>C NMR spectra. Reaction of two equivalents of PhMgBr with 5-bromopentanoyl chloride, acid-catalyzed dehydration of the resulting alcohol, reaction of the resulting bromoalkene with NaCN, and hydrolysis of the nitrile gave 6,6-diphenyl-5-hexenoic acid. The corresponding acid chloride was allowed to react with the anions from methyl acetate (for **1c**) and methyl methoxyacetate (for **1d**). The resulting β-keto esters were reduced with NaBH<sub>4</sub>, the alcohols were methylated, and the esters were saponified to give 3-methoxy-8,8-diphenyl-7-octenoic acid and 2,3-dimethoxy-8,8-diphenyl-7-octenoic acid, respectively. All intermediates were characterized by NMR spectroscopy, and the carboxylic acids were characterized by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy and high resolution mass spectrometry.
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11. Since the rates of cyclization reactions are slightly increased by increasing the polarity of the solvent (Table 1), the thiol trapping rates of Table 2 are the minimum values.
12. An inverse solvent effect in H-transfer from the OH-group of phenols and hydroperoxides to alkoxy radicals was observed by Luszyk and coworkers: Avila, D. V.; Ingold, K. U.; Luszyk, J.; Green, W. H.; Procopio, D. R. *J. Am. Chem. Soc.* **1995**, *117*, 2929–2930. The inverse solvent effect was explained by hydrogen bond donor effects of the solvent towards the OH group of the educts.
13. According to the data of Tables 1 and 2, the 4'-DNA radicals should be trapped by thiols such as glutathione diethyl ester with a rate of ca. 10<sup>7</sup> M<sup>-1</sup> s<sup>-1</sup> at 30 °C in water as solvent (see also ref 10).