

Lorentz and polarization corrections were applied and absorption corrections<sup>30</sup> were performed. Scattering factors for neutral atoms were taken from ref 31 and were corrected for the real and imaginary parts of anomalous dispersion. All calculations and refinements were performed by using SHELX-76.<sup>32</sup>

**Structural Analysis and Refinement.** The systematically absent reflections confirmed the space group as C22<sub>2</sub>, for the a,a and P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> for the e,e form. The positions of the bromine atoms were determined from a Patterson map and the remaining non-hydrogen atoms were located in subsequent Fourier syntheses. The structures were refined initially by a full-matrix least-squares procedure with independent isotropic temperature factors on the atoms. Absorption corrections were then applied. Hydrogen atoms were placed in their geometrically calculated positions [ $d(\text{C-H}) = 1.08 \text{ \AA}$ ]. The final refinements were with anisotropic temperature factors on the non-hydrogen atoms and individual isotropic temperature factors on the H atoms. A two-block matrix least-squares method was employed. The function minimized was  $w(|F_o| - |F_c|)^2$  where  $w$  is the weight. Final  $R = 0.032$  for the a,a and 0.036 for the e,e form.

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No correction was applied for extinction. The final difference maps had no recognizable residual features. The figures were produced with CHEMGRAF.<sup>33</sup> The fractional positional parameters of the non-hydrogen atoms are presented in Table IV.

**Acknowledgment.** T.S.C. is grateful for financial support from the Natural Sciences and Engineering Research Council of Canada. Financial support from the Swedish Natural Science Research Council and the Knut and Alice Wallenberg Foundation to J.S. is gratefully acknowledged. We are also grateful to Docent Roland Isaksson for valuable advice concerning the use of the chromatographic equipment.

**Supplementary Material Available:** Tables of hydrogen atom positional parameters (Table V), anisotropic thermal parameters (Table VI), interatomic distances (Table VII), and interbond (Table VIII), torsional (Table IX), and interplane (Table X) angles (11 pages); observed and calculated structure factors (29 pages). Ordering information is given on any current masthead page.

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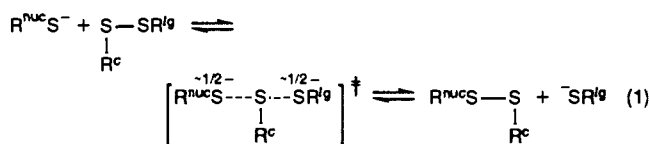
## Comparisons of Rate Constants for Thiolate–Disulfide Interchange in Water and in Polar Aprotic Solvents Using Dynamic <sup>1</sup>H NMR Line Shape Analysis

Rajeeva Singh and George M. Whitesides\*

Contribution from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. Received July 18, 1989

**Abstract:** The rate constants for representative thiolate–disulfide interchange reactions are larger in DMSO and DMF than in water by a factor of approximately 2300 at 24 °C. The log of the rate constant is directly proportional to the mole fraction of D<sub>2</sub>O in mixtures of DMSO and D<sub>2</sub>O, even at small mole fractions of D<sub>2</sub>O. This linear proportionality suggests that thiolate anion is not specifically solvated by water and that hydrogen bonding is relatively unimportant in stabilizing this species. The values of  $\Delta S^\ddagger$  for thiolate–disulfide interchange are approximately  $-10 \text{ cal}/(\text{deg mol})$ , presumptively because of loss in the entropy of the reactants in going from ground to transition state, partially compensated by a gain in entropy from solvent release. Introduction of a hydroxyl group  $\beta$  to the C–S bond slows the reaction by a factor of 2–15; the introduction of methyl groups  $\beta$  to the C–S bond slows the rate by factors of 3–20. A number of substances have been screened as potential catalysts for thiolate–disulfide interchange in water: none showed useful levels of catalytic activity, although phenylselenol did accelerate the interchange significantly.

This paper examines the influence of solvent on rate constants for thiolate–disulfide interchange (eq 1: nuc = nucleophile, c = central, lg = leaving group). This reaction is one of broad



importance in biochemistry.<sup>1–7</sup> Although it is often not catalyzed enzymatically in vivo,<sup>8</sup> the formation of certain cystine linkages

in proteins may be enzyme catalyzed.<sup>9–14</sup> Many aspects of the mechanism of thiolate–disulfide interchange are understood.<sup>15–22</sup>

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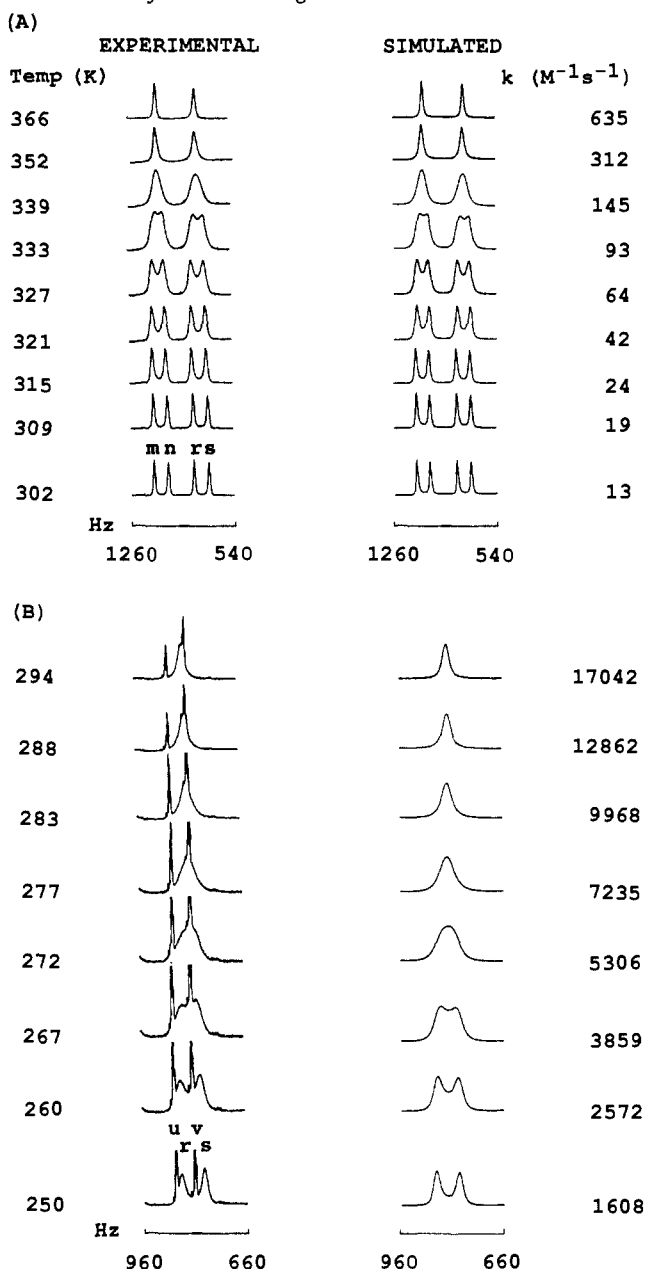


Figure 1. Experimental and calculated line shapes for 300 MHz <sup>1</sup>H NMR spectra at several temperatures of (A) sodium 2-hydroxyethanethiolate (3.8 M) and bis(2-hydroxyethyl) disulfide (1.9 M) in D<sub>2</sub>O; (B) potassium 2-hydroxyethanethiolate (66 mM) and bis(2-hydroxyethyl) disulfide (31 mM) in DMF-*d*<sub>7</sub>. The peak assignments are m = (HOCH<sub>2</sub>CH<sub>2</sub>S)<sub>2</sub>; n = HOCH<sub>2</sub>CH<sub>2</sub>S<sup>-</sup>; r = (HOCH<sub>2</sub>CH<sub>2</sub>S)<sub>2</sub>; s = HOCH<sub>2</sub>CH<sub>2</sub>S<sup>-</sup>; u, v = HCON(CH<sub>3</sub>)<sub>2</sub>.

it is an S<sub>N</sub>2 reaction in which thiolate anion attacks the disulfide bond along the S-S axis. The symmetry and structure of the transition state are not known in detail, although the charge is concentrated on the terminal sulfur atoms.

The influence of solvent on the rate of reactions has not been explored and would provide mechanistically useful information concerning charge distributions and about differences in solvation of ground and transition states. This work reports comparisons of rate constants for representative thiolate-disulfide interchange reactions in water and in the polar aprotic solvents DMSO and DMF. It had three objectives: to clarify the role of solvation; to indicate whether changes in the polarity of the reaction medium would provide a method of controlling the rate of the reaction; and to evaluate the possibility that a weakly solvated thiolate nucleophile might be exceptionally reactive. The observation of a correlation between reactivity and solvation might provide the basis for strategies for the design of catalysts for thiolate-disulfide interchange.

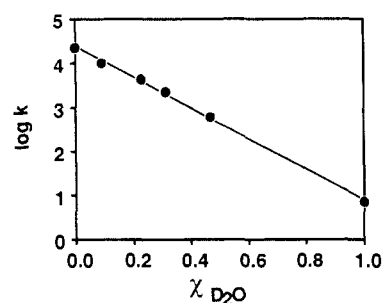


Figure 2. Effect of changing the mole fraction of D<sub>2</sub>O on the second-order rate constant (*k*) of thiolate-disulfide interchange of potassium 2-hydroxyethanethiolate and its disulfide in mixtures of DMSO-*d*<sub>6</sub> and D<sub>2</sub>O at 297 K. The rate constant has units of M<sup>-1</sup> s<sup>-1</sup>.

## Methods

We have investigated self-exchange between RS<sup>-</sup> and RSSR with use of dynamic <sup>1</sup>H NMR spectroscopy<sup>23-27</sup> for four reasons: (i) The rates of representative thiolate-disulfide interchange reactions in polar, aprotic solvents are too fast to be studied conveniently by conventional kinetic techniques. (ii) Thiolate anions are easily oxidized, and exclusion of atmospheric oxygen is comparatively easy in sealed NMR tubes but more difficult in the types of apparatus used in classical kinetic techniques. (iii) The interchange reaction is degenerate and can be examined at equilibrium. Determination of rates of reactions by NMR spectroscopy involved preparation of only a single sample of thiolate and disulfide; it was not necessary to withdraw aliquots or to prepare multiple samples. (iv) The methylene peaks adjacent to the thiolate and disulfide moieties are separated by approximately 0.33 δ (100 Hz at 300 MHz); this separation made the analysis of the line shapes relatively straightforward.

Representative experimental spectra are shown in Figure 1. Estimation of rate constants from these experimental spectra was accomplished by visual comparison of experimental spectra and spectra simulated with the program DNMR4.<sup>28</sup> The temperature dependence of the chemical shifts of the thiolate and disulfide species could be measured independently; the Experimental Section contains details. Solutions of thiolates in polar aprotic solvents were prepared by reaction of thiols with 1 equiv of potassium *tert*-butoxide. The nature of the counterion had no influence on the reaction. Further, addition of 18-crown-6 (1 equiv/equiv thiolate) to the reaction system involving potassium 2-hydroxyethanethiolate and bis(2-hydroxyethyl) disulfide did not influence the rate of thiolate-disulfide interchange.

## Results

**Rate Constants and Activation Parameters.** Table I summarizes rate constants obtained in this work and values of relevant thermodynamic parameters derived from variable-temperature work.<sup>29</sup> The most important observation in this table is that the rate constants for thiolate-disulfide interchange are faster in

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**Table I.** Rate Constants for Degenerate Thiolate–Disulfide Interchange:  $RS^- + RSSR \rightleftharpoons RSSR + ^-SR$ 

RS <sup>-</sup>	M <sup>+</sup>	solvent	$10^{-3}k^{a,b}$ (M <sup>-1</sup> s <sup>-1</sup> ) (297 K)	$\Delta G^\ddagger$ (kcal/mol) (297 K)	$\Delta H^\ddagger$ (kcal/mol)	$\Delta S^\ddagger$ (cal/(deg mol))
HOCH <sub>2</sub> CH <sub>2</sub> S <sup>-</sup>	Na <sup>+</sup>	D <sub>2</sub> O	0.0077	16.2	13	-10
	K <sup>+</sup>	D <sub>2</sub> O	0.0095	16.1	13	-11
	K <sup>+</sup>	DMF- <i>d</i> <sub>7</sub>	20	11.5	8	-13
CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> S <sup>-</sup>	K <sup>+</sup>	DMSO- <i>d</i> <sub>6</sub>	21	11.5		
	Na <sup>+</sup>	DMF- <i>d</i> <sub>7</sub>	43	11.1		
CH <sub>3</sub> C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> S <sup>-</sup>	K <sup>+</sup>	DMSO- <i>d</i> <sub>6</sub>	54	11.0		
	K <sup>+</sup>	DMF- <i>d</i> <sub>7</sub>	15	11.7		
HOC(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> S <sup>-</sup>	K <sup>+</sup>	DMSO- <i>d</i> <sub>6</sub>	16	11.7		
	K <sup>+</sup>	DMSO- <i>d</i> <sub>6</sub>	1.1	13.2	10	-10
HOCH <sub>2</sub> C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> S <sup>-</sup>	K <sup>+</sup>	DMSO- <i>d</i> <sub>6</sub>	0.67	13.5	9	-16

<sup>a</sup>Uncertainties are  $k$ ,  $\pm 10\%$ ;  $\Delta G^\ddagger$ ,  $\pm 0.1$  kcal/mol;  $\Delta H^\ddagger$ ,  $\pm 1$  kcal/mol;  $\Delta S^\ddagger$ ,  $\pm 2$  cal/(deg mol). <sup>b</sup>Rate constants were inferred from visual comparison of the simulated <sup>1</sup>H NMR line shapes with the experimental line shapes; details are given in the Experimental Section.

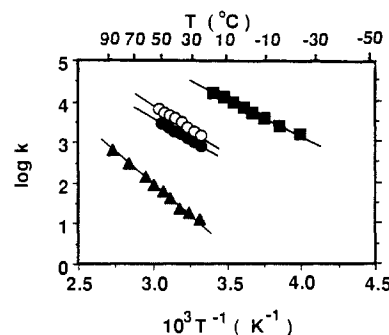
DMSO and in DMF than in water by a factor of approximately  $3 \times 10^3$ . The log of the rate constant depends linearly on the solvent composition in mixtures of D<sub>2</sub>O and DMSO-*d*<sub>6</sub> (eq 2, Figure 2). There is no evidence for specific solvation of thiolate

$$\Delta \log k = \Delta \Delta G^\ddagger \propto \chi_{D_2O} \quad (2)$$

anion by water (specific solvation would be reflected in nonlinearity in this plot, especially at small values of  $\chi_{D_2O}$ ). This observation suggests that hydrogen bonding between water and the thiolate anion is relatively unimportant.

The rate of thiolate–disulfide interchange reaction shows only modest sensitivity to steric effects and to groups capable of intramolecular hydrogen bonding. Introduction of methyl groups  $\beta$  to the C–S bond slows the reaction only by factors of 3–20. This insensitivity is expected for an S<sub>N</sub>2 reaction involving front-side attack of RS<sup>-</sup> along the S–S axis of the disulfide. Intramolecular hydrogen bonding between hydroxyl and thiolate does not significantly modify the rate of interchange of 2-hydroxyethanethiolate: the rate constant for thiolate–disulfide interchange reaction of potassium 1-butanethiolate is only twice that of potassium 2-hydroxyethanethiolate in DMSO. Introduction of a hydroxyl group either  $\beta$  or  $\gamma$  to the C–S bond in sterically hindered alkyl thiolates slows the interchange reaction by approximately a factor of 15 in polar aprotic solvents. A *gem*-dimethyl effect<sup>30</sup> and weaker solvation of the hydroxyl group in the sterically hindered case may result in greater intramolecular hydrogen bonding than in the sterically unhindered 2-hydroxyethanethiolate. On the basis of these observations, we suggest that differences in rates with changes in solvent for 2-hydroxyethanethiolate are representative of differences expected for other alkane thiolates.

Arrhenius plots yielded the thermodynamic parameters summarized in Table I (Figure 3). For HOCH<sub>2</sub>CH<sub>2</sub>S<sup>-</sup>,  $\Delta H^\ddagger$  decreased in going from D<sub>2</sub>O to DMF;  $-\Delta S^\ddagger$  is approximately constant. We rationalize the value of the entropy of activation in these reactions as a compromise between two factors. The reaction involves two particles going to one in the transition state and is thus intrinsically entropically disfavored. The observation that  $\Delta S^\ddagger$  is  $\sim -10$  cal/(deg mol) suggests a partial compensation of the unfavorable loss in translational and rotational entropy by some other factor, presumptively solvent release. Thermodynamic data for other thiolate–disulfide interchange reactions have been



**Figure 3.** Plot of  $\log k$  vs  $1/T$  for the thiolate–disulfide interchange of sodium 2-hydroxyethanethiolate (3.8 M) and its disulfide (1.9 M) in D<sub>2</sub>O ( $\blacktriangle$ ); potassium 2-hydroxyethanethiolate (66 mM) and its disulfide (31 mM) in DMF-*d*<sub>7</sub> ( $\blacksquare$ ); potassium 3-hydroxy-2,2-dimethylpropanethiolate (0.20 M) and its disulfide (0.10 M) in DMSO-*d*<sub>6</sub> ( $\bullet$ ); potassium 2-hydroxy-2-methylpropanethiolate (0.18 M) and its disulfide (0.09 M) in DMSO-*d*<sub>6</sub> ( $\circ$ ). The rate constants have units of M<sup>-1</sup> s<sup>-1</sup>.

interpreted similarly.<sup>18</sup> The determination of  $\Delta S^\ddagger$  and  $\Delta H^\ddagger$  by the dynamic NMR method may involve errors, and their values should be treated with caution.<sup>23,31</sup>

**The Thiolate–Disulfide Interchange Reaction Is Not Easily Catalyzed.** Rates of many thiolate–disulfide reactions in water follow a Brønsted relation in the values of  $pK_a$  of the participating species (eq 3 is one expression describing this relation).<sup>32</sup> We have surveyed a number of aromatic thiols having values of  $pK_a$  centered around pH 7, in the hope that these species might, by virtue of charge delocalization and possible weak solvation in water, prove to be more reactive in thiolate–disulfide interchange than would be predicted on the basis of eq 3.<sup>33</sup> These and a range

$$\log k = 6.3 + 0.59 pK_a^{nuc} - 0.40 pK_a^c - 0.59 pK_a^{ls} \quad (3)$$

of other types of potential catalysts did not show useful catalytic activity. The detailed results are given in the Experimental Section.

We screened for catalysis in water by incubating the potentially catalytic additive with an equimolar mixture of 2-carboxy-1,3-propanedithiol and bis(2-hydroxyethyl) disulfide. We followed

(29) From the data of Creighton (Creighton, T. E. *J. Mol. Biol.* **1975**, *96*, 767–776) we have calculated the rate constant [ $k = 19 \text{ M}^{-1} \text{ s}^{-1}$  (25 °C)] for the reaction of dithiothreitol with bis(2-hydroxyethyl) disulfide in water [ $k = k^{obs}(1 + 10^{pK_a - pH})$ ;  $k^{obs} = 4.5 \text{ M}^{-1} \text{ s}^{-1}$ ; pH = 8.7;  $pK_a$  (dithiothreitol) = 9.2]. We have to divide Creighton's rate constant by two (to account for the presence of two symmetrical thiol groups in dithiothreitol) in order to compare with our rate constant [ $9.5 \text{ M}^{-1} \text{ s}^{-1}$  (24 °C)] for HOCH<sub>2</sub>CH<sub>2</sub>S<sup>-</sup>K<sup>+</sup>/(HOCH<sub>2</sub>CH<sub>2</sub>S)<sub>2</sub> interchange in D<sub>2</sub>O. The identity of these two rate constants is accidental but does indicate that the two independent measurements are in close agreement. Some representative values of rate constants of thiolate–disulfide interchange from the literature are  $k = 57 \text{ M}^{-1} \text{ s}^{-1}$  for the reaction of mercaptoethanol and oxidized glutathione in water, pH 7, 30 °C<sup>15</sup> and  $k = 2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  for the reaction of mercaptoethanol with Ellman's reagent in water, pH 7, 30 °C.<sup>16</sup>

(30) Schleyer, P. v. R. *J. Am. Chem. Soc.* **1961**, *83*, 1368–1373. Ingold, C. K. *J. Chem. Soc.* **1921**, *119*, 305–329.

(31) In the case of potassium 2-hydroxyethanethiolate and its disulfide in DMF-*d*<sub>7</sub>, the values of  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  were found to be 7.7 kcal/mol and -13 cal/(deg mol) with careful calibration of temperature. The values of  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  calculated without calibration of temperature were 10.6 kcal/mol and -2 cal/(deg mol). The value of  $\Delta G^\ddagger$  was almost unaffected by the error in temperature.

(32) Two other equations give similar Brønsted correlations. The  $\beta$  coefficients are therefore not sharply defined by the available data. These Brønsted equations should be considered primarily as kinetic models for thiol–disulfide interchange in water.<sup>15</sup> A Brønsted slope ( $\beta = -0.98$ ) has been observed for the reaction of symmetrical alkyl disulfides with triphenylphosphine in 50% dioxane–water: Overman, L. E.; O'Connor, E. M. *J. Am. Chem. Soc.* **1976**, *98*, 771–775.

(33) Catalysis of acylation of hydroxyl groups using *N,N*-(dimethylamino)pyridine (DMAP) is an example of the strategy of enhancing nucleophilicity and charge delocalization: Hofle, G.; Steglich, W.; Vorbruggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569–583.

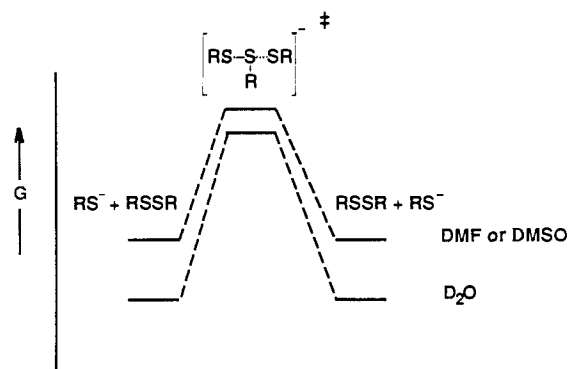
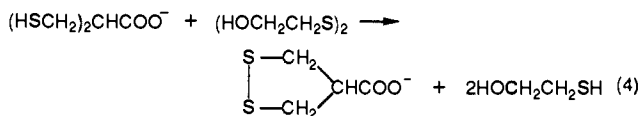


Figure 4. Hypothetical plot of free energy vs reaction coordinate for thiolate-disulfide interchange reaction in water and in polar aprotic solvents (DMSO, DMF).

the rate of reaction by monitoring the increase in absorption at 330 nm due to the product 4-carboxy-1,2-dithiolane (eq 4).



Nucleophiles other than thiolate anion do show high reactivity toward the disulfide bond: for example, the rate constant for reaction of  $\text{P}(\text{CH}_2\text{CH}_2\text{CO}_2^-)_3$  with bis(2-hydroxyethyl) disulfide (eq 5) is  $8 \text{ M}^{-1} \text{ s}^{-1}$  in water (that is, indistinguishable from that  $\text{L}_3\text{P} + (\text{HOCH}_2\text{CH}_2)_2\text{S} \xrightarrow{\text{H}_2\text{O}} \text{L}_3\text{PO} + 2\text{HOCH}_2\text{CH}_2\text{SH}$  (5)

of  $-\text{SCH}_2\text{CH}_2\text{OH}$ ).<sup>34,35</sup> There is, however, no obvious strategy for making this reaction catalytic. Of the substances examined as catalysts, only phenylselenol caused significant enhancement in the rate of interchange. When present at 10% concentration of the disulfide, phenylselenol accelerated the interchange by a factor of approximately 5. This level is not large enough to be useful, but it does suggest that selenols and related compounds may have useful catalytic activity.<sup>27</sup> We will describe studies in this area in a separate paper.

An effort to accelerate the rate of thiolate-disulfide interchange using a two-phase benzonitrile-water system<sup>36</sup> was not successful.

## Discussion

The rate constant for representative thiolate-disulfide interchange reactions increases by  $\sim 10^3$  on going from water as solvent to polar aprotic solvents (DMSO, DMF). The change in  $\Delta G^\ddagger$  is directly proportional to the composition of the solvent (eq 2). This proportionality—particularly the absence of a sharp drop in rate on adding small quantities of water to DMSO—suggests that hydrogen bonding to thiolate anion is a relatively unimportant contribution to the free energy of solvation of this species.<sup>37</sup> We infer, then, that the solvent influences the rate of thiolate-disulfide interchange primarily through its dielectric constant: In the lower dielectric constant solvents (DMSO, DMF) both thiolate anion and the transition state are energetically destabilized relative to water, but the thiolate anion, with its more localized charge, is

Table II. Comparisons of Rates of Reactions in Water and in Polar Nonprotic Solvents

reaction (X = S or O)	solvent	$10^{-3}(k_{\text{sol}}/k_{\text{H}_2\text{O}})$ or $10^{-3}(K_{\text{sol}}/K_{\text{H}_2\text{O}})$		ref
		X = S	X = O	
$\text{RX}^- + \text{RXXR} \rightarrow \text{RXXR} + \text{RX}^-$	DMSO- <i>d</i> <sub>6</sub>	2.3		
	DMF- <i>d</i> <sub>7</sub>	2.2		
$\text{C}_2\text{H}_5\text{X}^- + \text{H}^+ \rightleftharpoons \text{C}_2\text{H}_5\text{XH}$	DMSO	700	$1.6 \times 10^9$	<i>a</i>
$\text{C}_6\text{H}_5\text{X}^- + \text{MeI} \rightarrow$	DMF	32	1900	<i>b</i>
$p\text{-O}_2\text{NC}_6\text{H}_4\text{X}^- + \text{MeI} \rightarrow$	DMF	0.24	15	<i>b</i>
$\text{C}_6\text{H}_5\text{X}^- + p\text{-O}_2\text{NC}_6\text{H}_4\text{I} \rightarrow$	DMF	4.0	$1 \times 10^4$	<i>b</i>

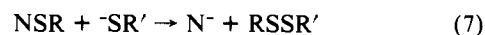
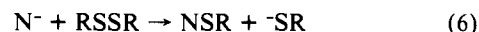
<sup>a</sup>Arnett, E. M.; Small, L. E. *J. Am. Chem. Soc.* **1977**, *99*, 808–816. Hupe, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 451–464. <sup>b</sup>Clare, B. W.; Cook, D.; Ko, E. C. F.; Mac, Y. C.; Parker, A. J. *J. Am. Chem. Soc.* **1966**, *88*, 1911–1916. Parker, A. J. *Chem. Rev.* **1969**, *69*, 1–32. The rate comparisons are reported for DMF and methanol at 0 °C.

the more strongly destabilized (Figure 4). This inference does not depend strongly on the structure of the transition state, provided that the charge is delocalized over the three sulfur centers, as is indeed inferred from the Brønsted relation that describes the reaction in water.

The proposal that the increase in rate in going from water to DMSO or DMF correlates primarily with the dielectric constant of the medium is compatible with much higher rates ( $\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) observed in the gas phase.<sup>38–40</sup> The progression of rates and dielectric constants— $\text{H}_2\text{O}$  ( $k \sim 10 \text{ M}^{-1} \text{ s}^{-1}$ ,  $\epsilon = 78$ ), DMSO ( $k \sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ,  $\epsilon = 47$ ), DMF ( $k \sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ,  $\epsilon = 37$ ), vapor ( $k \sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ,  $\epsilon = 1$ )—suggests a proportionality between  $\log k$  and  $\epsilon$  and both supports the hypothesis of a general medium effect and indicates that the fastest rates for thiolate-disulfide interchange in solution should be obtained in the media of lowest dielectric constant. The practical limits to such approaches are usually set by the onset of ion pairing and aggregation in low dielectric media.

Table II compares the effects of solvents on thiolate-disulfide interchange with those for other nucleophilic reactions involving sulfur (and, for comparison, oxygen) nucleophiles. In going from polar protic solvents (water, methanol) to polar nonprotic solvents (DMSO, DMF), the rates of reactions involving thiolate and alkoxy anions increase; the increase is, however, much more for the reactions involving alkoxy anions than thiols.<sup>40</sup> The alkoxy anions are solvated in water to a higher degree than thiols.

What are the implications of these results for catalysis of thiolate-disulfide interchange? Most strategies for catalyzing this reaction would require developing a species N: that was both an exceptional nucleophile toward sulfur (eq 6) and an exceptional leaving group from sulfur (eq 7). Our studies indicate that it



is more difficult to develop hypernucleophilic species based on sulfur than on oxygen. It appears that it will be difficult to increase the nucleophilicity of thiolate ions relative to their basicity by interfering with hydrogen bonding to them, since hydrogen bonding does not seem to be very important in any event. The parallel to the  $\alpha$  effect that is important for oxygen nucleophiles such as  $\text{HOO}^-$ ,  $\text{ClO}^-$ , and  $\text{Me}_2\text{NO}^-$ <sup>41</sup> is not known to exist with sulfur

(34) Butler, J. C.; Whitesides, G. M. Unpublished results.

(35) For a review of the reaction of disulfides with trivalent phosphorus compounds, see: Mukaiyama, T.; Takei, H. In *Topics in Phosphorus Chemistry*; Griffith, E. J., Grayson, M., Eds.; Wiley-Interscience: New York, 1976; Vol. 8, pp 587–645.

(36) The rate constant for thiol-disulfide interchange of 2-carboxy-1,3-propanedithiol and bis(2-hydroxyethyl) disulfide in benzonitrile/water mixture was the same as that in water. Our choice of benzonitrile/water system was based on the rapid decarboxylation of 3-carboxybenzoxazoles observed in this system by Kemp et al. (Kemp, D. S.; Paul, K. G. *J. Am. Chem. Soc.* **1975**, *97*, 7305–7312. Kemp, D. S.; Cox, D. D.; Paul, K. G. *J. Am. Chem. Soc.* **1975**, *97*, 7312–7318).

(37) The weak contribution of ionic hydrogen bond to solvation in  $\text{RS}^-(\text{H}_2\text{O})_n$  complexes is dissipated effectively within the first 2–3 solvent molecules ( $n = 2-3$ ) as the dissociation energy decreases rapidly upon successive hydration: Sieck, L. W.; Meot-Ner (Mautner), M. *J. Phys. Chem.* **1989**, *93*, 1586–1588.

(38) A value of  $k \sim 3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  has been estimated for reaction of  $\text{CH}_3\text{CH}_2\text{S}^-$  with  $\text{CH}_3\text{SSCH}_3$  in vapor phase by using flowing afterglow techniques. Comparison of this rate constant with the collisional rate constant suggests that the reaction occurs with a probability of  $\sim 3 \times 10^{-3}$  per collision: Grabowski, J. J.; Zhang, L. *J. Am. Chem. Soc.* **1989**, *111*, 1193–1203.

(39) For comparison, the rate constant for a representative  $\text{S}_\text{N}2$  reaction at carbon— $\text{Cl}^- + \text{CH}_3\text{Cl} \rightarrow \text{ClCH}_3 + \text{Cl}^-$ —increases by a factor of approximately  $10^{15}$  on going from water to the vapor phase. Jorgensen and Buckner summarize leading references in this area: Jorgensen, W. L.; Buckner, J. K. *J. Phys. Chem.* **1986**, *90*, 4651–4654.

(40) For related calculations concerning the relative nucleophilicities of  $\text{OH}^-$  and  $\text{SH}^-$ , see: Howard, A. E.; Kollman, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 7195–7200.

(41) Edwards, J. O.; Pearson, R. G. *J. Am. Chem. Soc.* **1962**, *84*, 16–24. Hoz, S.; Buncel, E. *Isr. J. Chem.* **1985**, *26*, 313–319. Buncel, E.; Um, I. *J. Chem. Soc., Chem. Commun.* **1986**, 595. Hudson, R. F.; Hansell, D. P.; Wolfe, S.; Mitchell, D. J. *J. Chem. Soc., Chem. Commun.* **1985**, 1406–1407.

nucleophiles. Because  $\pi$ -bonding to sulfur is less important than to oxygen, it will be more difficult to manipulate the polarizability and nucleophilicity of sulfur than of oxygen through remote substituent effects. It may be possible to develop species highly nucleophilic toward the disulfide group using phosphorus, selenium, or other elements, but we do not have firm experimental support for this approach.

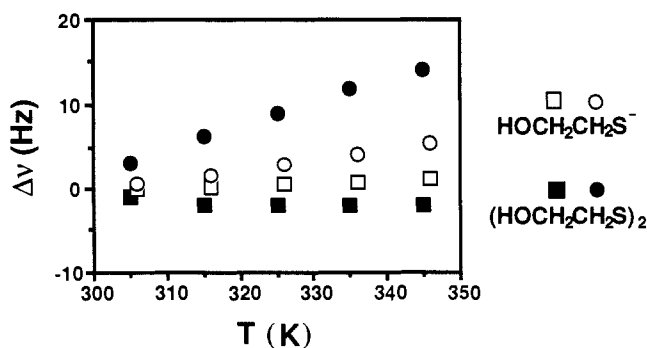
Thus, we conclude that the best strategies for "catalyzing" thiolate–disulfide interchange, especially under conditions compatible with biological reactions, probably involve transferring the reactants from water to an environment of lower dielectric constant and preorganizing them into a configuration resembling the transition state. A strategy involving reaction in a low dielectric medium is based on *destabilization* of the reactant thiolate ( $\text{RS}^-$ ) relative to the more delocalized transition state  $[\text{R}_3\text{S}_3]^\ddagger$ . *Stabilizing* the transition state electrostatically relative to the reactant thiolate anion will require both preorganization and precise arrangement of the electrostatic potential around the  $[\text{R}_3\text{S}_3]^\ddagger$  unit.

### Experimental Section

**General Methods.**  $^1\text{H}$  NMR spectra were recorded with Bruker AM300 and AM500 instruments. The difference between the  $^1\text{H}$  NMR chemical shifts of the methylene and hydroxyl peaks of ethylene glycol was used to calibrate the temperature recorded by the NMR spectrometer in the range of temperatures from 295–370 K; methanol was used for calibration from 230–290 K.<sup>42–45</sup> Argon was deoxygenated by passing over Radox (Fisher Scientific) and molecular sieves before use. Distilled water from a Corning AG-1b still was used to wash all glassware.<sup>46</sup>

**Materials.** Cambridge Isotope Laboratories and Aldrich supplied  $\text{D}_2\text{O}$  (99.9%),  $\text{DMSO-}d_6$  (99.96%), and  $\text{DMF-}d_7$  (99%). Mercaptoethanol, bis(2-hydroxyethyl) disulfide, methyl thioglycolate, 2,2-dimethylpropanol, *p*-toluenesulfonyl chloride, sodium deuterioxide (40% solution in  $\text{D}_2\text{O}$ ), potassium deuterioxide (40% solution in  $\text{D}_2\text{O}$ ), sodium hydrogen sulfide, potassium *tert*-butoxide, thiolacetic acid, diethyl malonate, and methylmagnesium bromide (a 3 M solution in diethyl ether) were all purchased from Aldrich. Sodium 1-butanethiolate was purchased from Fluka. Protein disulfide isomerase (E.C. 5.3.4.1) was a gift from Genzyme.

**Calculations of Rate Constants and Activation Parameters of Thiolate–Disulfide Interchange by Dynamic  $^1\text{H}$  NMR Analysis.** The pseudomolecular rate constants ( $k' = 1/\tau$ ) for thiolate–disulfide interchange were determined by complete line shape analysis on a VAX 8600 by using the computer program DNMR4.<sup>28</sup> The  $\text{HOCH}_2\text{CH}_2\text{S}^-/(\text{HOCH}_2\text{CH}_2\text{S})_2$  system was treated as an  $\text{A}_2\text{B}_2 \leftrightarrow \text{C}_2\text{D}_2$  spin system with  $J_{\text{AB}} = 7.1$  Hz,  $J_{\text{CD}} = 6.0$  Hz.<sup>47</sup> The values of the chemical shifts of the A, B, C, and D methylene protons in  $\text{D}_2\text{O}$  (300 MHz  $^1\text{H}$  NMR) were taken as 1007.7, 719.4, 1102.2, and 818.5 Hz, respectively. The relative populations of the two configurations were taken as 0.5 each.  $T_2^*$  was taken as 0.15 s (calculated from the reciprocal of the product of  $\pi$  and the peak width (Hz) at half height of the singlet peak corresponding to the methyl groups of *t*-BuOH). In our experiments, the NMR line-shapes were not very sensitive to  $T_2^*$  for two reasons: (i)  $\Delta\nu$  ( $\sim 100$  Hz at 300 MHz)  $\gg 1/T_2^*$  ( $\sim 6$  Hz), and (ii) we avoided very fast and very slow exchange regimes in our study by manipulating the concentration of thiolate and disulfide and the temperature. We resimulated the plots for  $\text{HOCH}_2\text{CH}_2\text{S}^-/\text{K}^+(\text{HOCH}_2\text{CH}_2\text{S})_2$  interchange in  $\text{D}_2\text{O}$  with correct values of  $T_2^*$  [0.15 s ( $\text{HOCH}_2\text{CH}_2\text{S}^-/\text{K}^+$ ), 0.26 s ( $(\text{HOCH}_2\text{CH}_2\text{S})_2$ )] and found the plots to be similar to those obtained previously with  $T_2^* = 0.15$  s. For various values of rate constants, simulated NMR plots were generated for the frequency range 540–1260 Hz. The rate constants were inferred by comparison of the simulated plots with the experimental plots to the best fit by eye. The coalescence approximation [ $\tau = 1/k' =$



**Figure 5.** Effect of temperature on the 300 MHz  $^1\text{H}$  NMR chemical shifts of the methylene protons of (a) sodium 2-hydroxyethanethiolate (1.2 M) and (b) bis(2-hydroxyethyl) disulfide (2.0 M) in  $\text{D}_2\text{O}$ . The temperature dependence of the chemical shifts for the methylene protons of the thiolate (a) and the disulfide (b) are small in comparison to the difference in chemical shifts between the methylene protons of the thiolate and disulfide ( $\sim 100$  Hz at 300 MHz). The standards for measurements of the chemical shifts were silicone grease (for a) and DSS (for b).

$(\sqrt{2})/(\pi\Delta\nu)$  is quite accurate (within 5% of the value determined by complete line shape analysis). The temperature dependence of the chemical shifts for the methylene protons of the thiolate and disulfide were measured independently and were found to be small compared to the difference in chemical shifts between the methylene protons of the thiolate and disulfide (Figure 5).

In each reactive collision of thiolate and disulfide, only half of each disulfide molecule converts to thiolate, although each thiolate converts to one-half of a disulfide molecule. In the degenerate thiolate–disulfide interchange, if we denote the rate of exchange seen by NMR as  $-d[\text{RSSR}]/dt$  and  $-d[\text{RS}^-]/dt$  for the disulfide and thiolate, respectively, we obtain  $-d[\text{RSSR}]/dt = (1/2)k[\text{RS}^-][\text{RSSR}]$  and  $-d[\text{RS}^-]/dt = k[\text{RS}^-][\text{RSSR}]$ . If  $\tau_{\text{RS}^-}$  and  $\tau_{\text{RSSR}}$  are lifetime of the thiolate and disulfide, then

$$-d[\text{RS}^-]/dt = (1/\tau_{\text{RS}^-})[\text{RS}^-] = k[\text{RS}^-][\text{RSSR}] \quad (8)$$

$$-d[\text{RSSR}]/dt = (1/\tau_{\text{RSSR}})[\text{RSSR}] = (1/2)k[\text{RS}^-][\text{RSSR}] \quad (9)$$

In our experiments,  $[\text{RS}^-] = 2[\text{RSSR}]$ . We therefore obtain from eq 8 and 9:  $(1/\tau_{\text{RS}^-}) = (1/\tau_{\text{RSSR}}) = k[\text{RSSR}]$ .

The activation parameter  $\Delta G^\ddagger$  was determined from the rate constants by using transition state theory (eq 10). The activation parameters  $\Delta H^\ddagger$

$$k = (k_B T/h) \exp(-\Delta G^\ddagger/RT) \quad (10)$$

and  $\Delta S^\ddagger$  were calculated from the plot of  $\ln(k/T)$  vs  $1/T$ . The experimental plots of  $\ln(k/T)$  vs  $1/T$  were linear with  $R^2 \geq 0.995$  for 7–9 points (Figure 3).<sup>48</sup> The determination of  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  by dynamic NMR spectroscopy is known to not be very accurate.<sup>23</sup> We have estimated the errors in  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  by calculating the standard deviations at 90% confidence for the slope and intercept of the plot of  $\ln(k/T)$  vs  $1/T$ .<sup>23</sup>

**Preparation of Samples Containing Thiolates and Disulfides for Dynamic NMR Spectroscopy: General Procedure.** The solvent was deoxygenated by bubbling argon through it for  $>30$  min if the sample was to contain thiolate at  $>0.1$  M concentration. For a sample containing  $<0.1$  M concentration of thiolate, the solvent was degassed with at least four freeze–pump–thaw cycles. The NMR tube was stoppered with a rubber septum, and the top of the NMR tube was sealed with paraffin wax (or Apiezon W wax for high-temperature NMR measurements) to prevent oxidation of the sample.<sup>49</sup> Sealing by paraffin wax was especially useful in rate studies in mixtures of  $\text{DMSO-}d_6$  and  $\text{D}_2\text{O}$ ; the wax seal could be broken easily (to introduce small amounts of deoxygenated  $\text{D}_2\text{O}$ ) and replaced. Potassium *tert*-butoxide was used as base to generate thiolate from thiol in  $\text{DMSO-}d_6$  and  $\text{DMF-}d_7$ . Sodium 1-butanethiolate was used directly for one experiment in  $\text{DMF-}d_7$ . Potassium deuterioxide

(42) van Geet, A. L. *Anal. Chem.* **1968**, *40*, 2227–2229.

(43) van Geet, A. L. *Anal. Chem.* **1970**, *42*, 679–680.

(44) Raiford, D. S.; Fisk, C. L.; Becker, E. D. *Anal. Chem.* **1979**, *51*, 2050–2051.

(45) Ammann, C.; Meier, P.; Merbach, A. E. *J. Magn. Reson.* **1982**, *46*, 319–321.

(46) Thiolates are oxidized rapidly by oxygen; the oxidation is catalyzed by metal ions.<sup>7</sup>

(47) We prepared 2,2-dideuterio-2-hydroxyethanethiol by reduction of methyl thioglycolate with lithium aluminum deuteride. The rate of the thiolate/disulfide interchange for sodium 2,2-dideuterio-2-hydroxyethanethiolate and its disulfide was the same as that for sodium 2-hydroxyethanethiolate and its disulfide. The peak corresponding to the methylene protons in sodium 2,2-dideuterio-2-hydroxyethanethiolate appeared as a singlet in the 300 MHz  $^1\text{H}$  NMR spectrum ( $J_{\text{CH}_2\text{CD}_2} \sim 0.15 J_{\text{CH}_2\text{CH}_2}$ ).

(48) The plots of  $(\ln k/T)$  vs  $(1/T)$  for the thiolate–disulfide interchange reactions of sodium 1-butanethiolate in  $\text{DMF-}d_7$  and potassium 2,2-dimethylpropanethiolate in  $\text{DMF-}d_7$  are also linear. The values of  $\Delta S^\ddagger$  are negative in both cases; the values of  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$ , however, could be inaccurate because the chemical shifts of methylene protons of the thiolate and disulfide vary with temperature in both cases. The simulations of the NMR spectra were done by varying both the rate constant and  $\Delta\nu$ .

(49) The results were the same whether the top of the NMR tube was sealed with wax or flame-sealed.

and sodium deuteroxide were used as bases for generation of thiolate in D<sub>2</sub>O.

**Preparation of a Sample in D<sub>2</sub>O: Representative Procedure.** All flasks and the NMR tube were stoppered with rubber septa and were flushed with argon before use. Mercaptoethanol (0.238 g, 3.04 mmol), bis(2-hydroxyethyl) disulfide (0.230 g, 1.49 mmol) and D<sub>2</sub>O (0.30 mL) were placed in a flask. The contents of the flask were degassed with four freeze-pump-thaw cycles. In another flask, NaOD (40 wt % solution in D<sub>2</sub>O, 0.306 g, 2.98 mmol) was deoxygenated by bubbling argon through it for 30 min. The solution containing mercaptoethanol and the corresponding disulfide was transferred to the flask containing sodium deuteroxide solution by cannula under argon. The resulting solution was transferred to an NMR tube by cannula under argon. The top of the NMR tube was sealed with Apiezon W wax. The total volume of the solution was 0.79 mL. The solution was 3.78 M in thiolate and 1.89 M in disulfide. The <sup>1</sup>H NMR spectra were recorded at various temperatures at 300 MHz (pulse width = 4 μs, pulse delay = 4 s, acquisition time = 2.7 s). The time delay between pulses was greater than the value of T<sub>1</sub>. The values of T<sub>1</sub> were determined by the inversion-recovery method as 1.9 s (CH<sub>2</sub>OH) and 1.3 s (CH<sub>2</sub>S<sup>-</sup>) for sodium 2-hydroxyethanethiolate in D<sub>2</sub>O and 2.2 s (CH<sub>2</sub>OH) and 2.1 s (CH<sub>2</sub>S) for bis(2-hydroxyethyl) disulfide in D<sub>2</sub>O.

**Preparation of a Sample in DMSO-*d*<sub>6</sub>: Representative Procedure.** All flasks and the NMR tube were stoppered with rubber septa and were flushed with argon before use. Mercaptoethanol (0.0049 g, 0.063 mmol), bis(2-hydroxyethyl) disulfide (0.0042 g, 0.027 mmol), and DMSO-*d*<sub>6</sub> (0.50 mL) were placed in a flask. The solution was degassed with four freeze-pump-thaw cycles. The degassed solution was transferred to the NMR tube by cannula under argon. In another flask, potassium *tert*-butoxide (0.0089 g, 0.079 mmol) was weighed in the glovebox. From a solution of DMSO-*d*<sub>6</sub> that had been deoxygenated previously by bubbling argon through it for 45 min, 72 μL was transferred by a gas-tight syringe to the flask containing potassium *tert*-butoxide. From the resulting potassium *tert*-butoxide solution (1.10 M), 50 μL was transferred by a gas-tight syringe to the NMR tube. The top of the NMR tube was sealed with paraffin wax. The solution was 49 mM in disulfide and 100 mM in thiolate. The <sup>1</sup>H NMR spectra were recorded at 500 MHz. After the <sup>1</sup>H NMR spectra had been taken, the solution was quenched by addition of DCl (10 μL of a 37 wt % solution in D<sub>2</sub>O).<sup>50,51</sup> The <sup>1</sup>H NMR spectrum of the resulting solution showed that no oxidation (<5%) of thiolate to disulfide had occurred.

**Screening for Catalysis in Water Using UV Spectroscopy.** The thiol-disulfide interchange of 2-carboxy-1,3-propanedithiol<sup>52</sup> and bis(2-hydroxyethyl) disulfide was monitored at 330 nm; the product 4-carboxy-1,2-dithiolane (ε = 144 M<sup>-1</sup> cm<sup>-1</sup>) is the only species that absorbs at 330 nm. Separate stock solutions of 2-carboxy-1,3-propanedithiol and bis(2-hydroxyethyl) disulfide (each 8 mM) were prepared in deoxygenated aqueous phosphate buffer solution (50 mM, pH 7). A quartz cuvette with the material to be tested as catalyst (1.2 μmol) was stoppered with a rubber septum and flushed with argon. The solution of 2-carboxy-1,3-propanedithiol (1.5 mL) was added by a gas-tight syringe and the cuvette equilibrated at 25 °C. The bis(2-hydroxyethyl) disulfide solution (1.5 mL) was added to the cuvette with a gas-tight syringe, and the increase in absorbance at 330 nm was monitored. The initial concentrations in the assay mixture were [2-carboxy-1,3-propanedithiol] = 4 mM, [bis(2-hydroxyethyl) disulfide] = 4 mM, and [catalyst] = 0.4 mM. The uncatalyzed apparent rate constant was 2.3 M<sup>-1</sup> min<sup>-1</sup> (see Table III).

When Cd<sup>2+</sup>, Ag<sup>+</sup>, and Co<sup>2+</sup> were tried as catalysts, precipitation occurred. When aromatic thiols were used as catalysts, an initial increase in absorbance was observed due to the formation of mixed disulfide between the aromatic thiol and mercaptoethanol.<sup>55</sup> This interference was avoided by incubating the aromatic thiol (catalyst) with bis(2-hydroxyethyl) disulfide in the cuvette for 10 min before addition of 2-carboxy-1,3-propanedithiol.

#### <sup>1</sup>H NMR Assay of Catalysis of Thiol-Disulfide Interchange by *p*-

(50) Lowe, O. G. *J. Org. Chem.* **1975**, *40*, 2096-2098.

(51) The addition of excess DCl was avoided during quenching because thiols are oxidized to disulfides in DMSO in the presence of excess HCl;<sup>50</sup> we prepared bis(2,2-dimethylpropyl) disulfide by oxidation of 2,2-dimethylpropanethiol in DMSO in the presence of HCl (see Experimental Section).

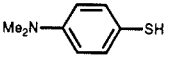
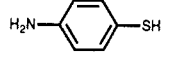
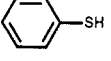
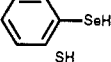
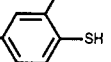
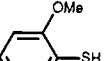
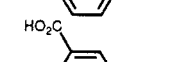
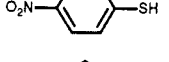
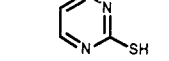
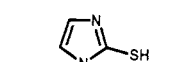
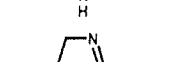
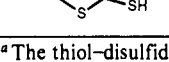
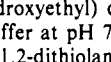
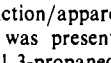
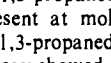
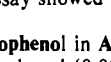
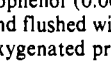
(52) 2-Carboxy-1,3-propanedithiol is a useful substrate for the assay because it is an easily prepared crystalline solid, and it does not smell strongly.

(53) The observed rate enhancement of 1.7 times compares well with the theoretical value of 1.6 calculated from eq 3.

(54) Protein disulfide isomerase does not catalyze reactions of dithiothreitol with oxidized glutathione: Creighton, T. E.; Hillson, D. A.; Freedman, R. B. *J. Mol. Biol.* **1980**, *142*, 43-62.

(55) Campaigne, E.; Tsurugi, J.; Meyer, W. W. *J. Org. Chem.* **1961**, *26*, 2486-2491.

**Table III.** The Influence of Potentially Catalytic Additives on the Rate of Thiol-Disulfide Interchange in Water at pH 7

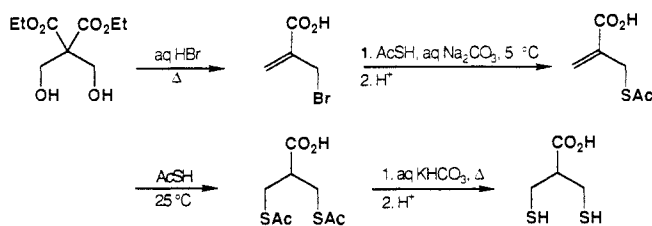
additive	$v_{\text{cat}}/v_{\text{uncat}}^a$	additive	$v_{\text{cat}}/v_{\text{uncat}}$
	2	papain	1
	2 <sup>c</sup>	CF <sub>3</sub> CH <sub>2</sub> SH	1
	2	thiamine hydrochloride	1
	5	I <sup>-</sup> (KI)	1
	2	SO <sub>3</sub> <sup>2-</sup> (Na <sub>2</sub> SO <sub>3</sub> )	1
	2	CNS <sup>-</sup> (NaCNS)	1
	2	PO <sub>3</sub> S <sup>3-</sup> (Na <sub>3</sub> PO <sub>3</sub> S)	1
	2	Zn <sup>2+</sup> [Zn(OAc) <sub>2</sub> ]	1
	2	Cd <sup>2+</sup> [Cd(OAc) <sub>2</sub> ]	<i>b</i>
	2	Fe <sup>2+</sup> (FeSO <sub>4</sub> )	1
	2	Ag <sup>+</sup> (AgNO <sub>3</sub> )	<i>b</i>
	2	Co <sup>2+</sup> [Co(NO <sub>3</sub> ) <sub>2</sub> ]	<i>b</i>
	1	alumina	1
	1	activated carbon	1
	1	P(CH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup> K <sup>+</sup> ) <sub>3</sub>	1
	1	(MeO) <sub>3</sub> P	1
	1	protein disulfide isomerase	1 <sup>d</sup>

<sup>a</sup> The thiol-disulfide interchange of 2-carboxy-1,3-propanedithiol and bis(2-hydroxyethyl) disulfide (4 mM each) in 50 mM aqueous phosphate buffer at pH 7 was followed by monitoring the formation of 4-carboxy-1,2-dithiolane at 330 nm;  $v_{\text{cat}}/v_{\text{uncat}}$  = apparent rate of catalyzed reaction/apparent rate of uncatalyzed reaction. In each case the additive was present at a molar concentration = 10% that of 2-carboxy-1,3-propanedithiol. Papain and protein disulfide isomerase were present at molar concentrations = 1% and 0.06% that of 2-carboxy-1,3-propanedithiol. <sup>b</sup> Precipitate was observed in solution. <sup>c</sup> <sup>1</sup>H NMR assay showed  $v_{\text{cat}}/v_{\text{uncat}}$  = 1.7 (ref 53). <sup>d</sup> Reference 54.

**Aminothiophenol in Aqueous Solution at pH 7.** A flask containing *p*-aminothiophenol (0.0059 g, 0.047 mmol) was stoppered with a rubber septum and flushed with argon. Methylene chloride (0.94 mL) that had been deoxygenated previously by bubbling argon through it for 30 min was added to generate a stock solution of *p*-aminothiophenol. The solution was estimated by Ellman's analysis to be 51 mM in thiol.<sup>56</sup> By using a gas-tight syringe, 20 μL of this solution was added to a flask stoppered with a rubber septum, and the methylene chloride was removed by evaporation under argon to leave *p*-aminothiophenol as a solid residue. Into two flasks were weighed bis(2-hydroxyethyl) disulfide (0.0032 g, 0.02 mmol) and 2-carboxy-1,3-propanedithiol (0.0031 g, 0.02 mmol) separately. The two flasks were stoppered with rubber septa and flushed with argon. To each flask was added by using a gas-tight syringe 1 mL of D<sub>2</sub>O (50 mM phosphate buffer, pH 7) that had been deoxygenated previously by bubbling argon through it for 2 h. The contents of the two flasks were mixed by transfer through cannula under argon; 4 min after mixing, 1 mL of the solution was transferred to the flask containing *p*-aminothiophenol under argon. The initial concentrations were [(HOCH<sub>2</sub>CH<sub>2</sub>S)<sub>2</sub>]<sub>initial</sub> = 10 mM, [HSC<sub>2</sub>H<sub>4</sub>(COOH)CH<sub>2</sub>SH]<sub>initial</sub> = 10 mM, and [HSC<sub>6</sub>H<sub>4</sub>NH<sub>2</sub>(*p*)]<sub>initial</sub> = 1 mM in the catalyzed solution. The catalyzed reaction was quenched after 13 min by adding 15 μL of DCl solution (37 wt % in D<sub>2</sub>O), and the uncatalyzed solution was quenched after 14 min by addition of 15 μL of DCl solution. The acid-quenched solutions were then transferred under argon to NMR tubes stoppered with rubber septa. The top of the NMR tubes were sealed with paraffin wax. The rates of the reactions were estimated from the peak areas for the methylene peaks of mercaptoethanol and its disulfide in the <sup>1</sup>H NMR spectrum.

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## Scheme I



**3-Hydroxy-2,2-dimethylpropanethiol.** A flask containing 3,3-dimethyloxetane<sup>57</sup> (8.6 g, 100 mmol) and thioacetic acid (9.0 mL, 126 mmol) was fitted with a reflux condenser and warmed in an oil bath at 60 °C for 20 h. The <sup>1</sup>H NMR spectrum of the reaction mixture showed 75% conversion to the product 3-(acetylthio)-2,2-dimethylpropanol. To the reaction mixture was added methanol (50 mL) and concentrated HCl (0.5 mL of a 37 wt % solution in water). The resulting solution was stirred in an oil bath at 60–65 °C for 24 h. The reaction mixture was cooled in dry ice–acetone bath, and the upper liquid layer was removed by cannula under argon. The lower solid layer which remelted at room temperature was suspended in a mixture of petroleum ether (40–60 °C fraction, 50 mL), diethyl ether (50 mL), and water (100 mL). The organic layer was dried (MgSO<sub>4</sub>) and concentrated under reduced pressure to yield a colorless liquid (4.47 g). The crude product was distilled (0.1 Torr, 60–70 °C) to yield a colorless liquid (3.53 g, 30%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.46 (d, 2 H, *J* = 5.7 Hz), 2.53 (d, 2 H, *J* = 8.7 Hz), 1.53 (t, 1 H, *J* = 5.7 Hz), 1.26 (t, 1 H, *J* = 8.7 Hz), 0.96 (s, 6 H). Anal. Calcd for C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>S: C, 49.96; H, 10.06. Found: C, 49.99; H, 10.11.

**Bis(3-hydroxy-2,2-dimethylpropyl) Disulfide.** 3-Hydroxy-2,2-dimethylpropanethiol (0.85 g, 7.1 mmol) and FeCl<sub>3</sub> (0.0040 g, 0.015 mmol) were dissolved in KHCO<sub>3</sub> solution (100 mL of a 10 wt % aqueous solution). Oxygen was bubbled through the solution for 7 h with stirring until the solution became colorless. The cloudy reaction mixture was extracted with chloroform (50 mL). The chloroform layer was dried (MgSO<sub>4</sub>) and concentrated at reduced pressure to yield oil. The crude oil was recrystallized from toluene/petroleum ether to yield a white crystalline solid (0.33 g, 40%): mp 92 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.43 (br s, 2 H), 2.84 (s, 2 H), 2.16 (br s, 1 H), 0.96 (s, 6 H). Anal. Calcd for C<sub>10</sub>H<sub>22</sub>O<sub>2</sub>S<sub>2</sub>: C, 50.38; H, 9.30. Found: C, 50.38; H, 9.53.

**2-Hydroxy-2-methylpropanethiol.** To a solution of methylmagnesium bromide in diethyl ether (100 mL of a 3.0 M solution, 300 mmol) in a flask stoppered with a rubber septum was added dropwise a solution of methyl thioglycolate (8.79 g, 83 mmol) in dry diethyl ether (100 mL) by cannula with stirring at room temperature under argon. The reaction mixture was stirred further at room temperature for 10 min and poured into a mixture of crushed ice–water (100 mL) and concentrated HCl (33 mL of a 37 wt % solution in water) with stirring. Diethyl ether was added to make the total volume of the organic layer ~300 mL. The aqueous layer was saturated with NaCl, and the two layers were separated with a separatory funnel. The diethyl ether layer was washed with brine solution (20 mL), dried (MgSO<sub>4</sub>), and concentrated at reduced pressure to yield a foul-smelling liquid (6.84 g, 78%). The crude product was distilled (30 Torr, 110 °C) to yield a colorless liquid (3.65 g, 41%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.63 (d, 2 H, *J* = 9.0 Hz), 2.2 (br, 1 H), 1.37 (t, 1 H, *J* = 9.0 Hz), 1.28 (s, 6 H). Anal. Calcd for C<sub>4</sub>H<sub>10</sub>OS: C, 45.25; H, 9.49. Found: C, 44.69; H, 9.13. (This sample was very hygroscopic, and we believe that this analysis reflects water we were unable to remove.)

**Bis(2-hydroxy-2-methylpropyl) Disulfide.** 2-Hydroxy-2-methylpropanethiol (1.25 g, 11.8 mmol) and FeCl<sub>3</sub> (0.0060 g, 0.022 mmol) were dissolved in KHCO<sub>3</sub> solution (200 mL of a 10 wt % aqueous solution). The brown solution was stirred, and oxygen was bubbled through it for 10 h until the solution became colorless. The solution was extracted with methylene chloride (50 mL). The CH<sub>2</sub>Cl<sub>2</sub> layer was dried (MgSO<sub>4</sub>) and concentrated at reduced pressure to yield a crude oil (0.81 g, 65%). The crude mixture was purified by flash chromatography (25% EtOAc/petroleum ether) to yield a colorless liquid (0.45 g, 36%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.01 (s, 2 H), 2.3–2.2 (br, 1 H), 1.30 (s, 6 H). Anal. Calcd for C<sub>8</sub>H<sub>18</sub>O<sub>2</sub>S<sub>2</sub>: C, 45.68; H, 8.62. Found: C, 45.83; H, 8.68.

**Bis(2,2-dimethylpropyl) Disulfide.** To a solution of 2,2-dimethylpropanethiol<sup>58</sup> (0.36 g, 3.5 mmol) in DMSO (7.5 mL) was added concentrated HCl (0.3 mL of a 37 wt % solution in water). The resulting

solution was stirred at room temperature for 20 h. The reaction mixture tested negative with Ellman's reagent, thereby indicating the complete oxidation of thiol. The reaction mixture was poured into 200 mL of ice–cold water. The resulting suspension was warmed to room temperature and extracted with petroleum ether (30–60 °C, 2 × 75 mL). The petroleum ether layer was dried (MgSO<sub>4</sub>) and concentrated at reduced pressure to yield a colorless liquid (0.29 g, 82%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.76 (s, 2 H), 0.99 (s, 9 H).<sup>59</sup>

**2-Bromomethylpropenoic Acid.**<sup>60</sup> Crude diethyl bis(hydroxymethyl)malonate<sup>61</sup> (114 g, 0.50 mol) was dissolved in HBr (226 mL of a 48 wt % aqueous solution, 2.0 mol), and the solution was heated at 120–125 °C in an oil bath for 45 min (Scheme I). The solution was cooled in dry ice–acetone bath; after appearance of white crystals the solution was cooled in an ice bath. The crystals were removed by filtration, and the solution was heated again at 120–125 °C for 45 min. The solution was cooled, and additional crystals separated. The procedure was repeated four times. The six crops were combined and suspended in a mixture of methylene chloride (200 mL) and 1 N HCl aqueous solution (60 mL) in a separatory funnel. The CH<sub>2</sub>Cl<sub>2</sub> layer was separated, dried (Na<sub>2</sub>SO<sub>4</sub>), and concentrated at reduced pressure to yield a white crystalline solid (26.0 g, 32%): mp 72 °C (lit.<sup>62</sup> mp 74 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.50 (s, 1 H), 6.11 (s, 1 H), 4.18 (s, 2 H).

**2-Carboxy-1,3-propanedithiol.**<sup>63,64</sup> To a stirred suspension of 2-bromomethylpropenoic acid (4.95 g, 30.0 mmol) in 100 mL of water in an ice bath was added a solution of sodium carbonate (5.50 g, 52.0 mmol) in 20 mL of water in small portions. To the resulting solution was added thioacetic acid (2.20 mL, 30.5 mmol), and the solution was stirred in an ice bath for 15 min. The solution was acidified with HCl to pH 1 and extracted with ethyl acetate (2 × 50 mL). The EtOAc layer was dried (MgSO<sub>4</sub>) and concentrated at reduced pressure to yield a white solid (6.4 g): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 6.40 (s, 1 H), 6.04 (s, 1 H), 3.77 (s, 2 H), 2.34 (s, 3 H). To the crude 3-(acetylthio)-2-methyl-1-propanoic acid was added thioacetic acid (3.25 mL, 45.0 mmol). After 15 h at room temperature, <sup>1</sup>H NMR spectroscopy showed no olefinic peaks. The reaction mixture was concentrated at reduced pressure to remove the excess thioacetic acid and heated at reflux with 200 mL of deoxygenated 10 wt % aqueous KHCO<sub>3</sub> solution for 5 h under argon. The reaction mixture was cooled in an ice bath, acidified to pH 1 with HCl, and extracted with ethyl acetate (200 mL). The EtOAc layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated at reduced pressure to yield an oily residue which was recrystallized in hexane (by cooling to –80 °C) to yield a white crystalline solid (3.43 g, 75%): mp 60 °C (lit.<sup>63</sup> mp 59.5–60.5 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.90 (m, 5 H), 1.53 (t, 2 H, *J* = 8.6 Hz).

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**Registry No.** 3-Hydroxy-2,2-dimethylpropanethiol, 15718-66-8; 3,3-dimethyloxetane, 6921-35-3; thioacetic acid, 507-09-5; bis(3-hydroxy-2,2-dimethylpropyl) disulfide, 124357-88-6; 2-hydroxy-2-methylpropanethiol, 73303-88-5; methyl thioglycolate, 2365-48-2; bis(2-hydroxy-2-methylpropyl) disulfide, 124357-89-7; bis(2,2-dimethylpropyl) disulfide, 37552-63-9; 2,2-dimethylpropanethiol, 1679-08-9; 2-bromomethylpropenoic acid, 72707-66-5; diethyl bis(hydroxymethyl)malonate,

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(62) Duttmann, H.; Weyerstahl, P. *Chem. Ber.* **1979**, *112*, 3480–3485.

(63) This scheme is a modification of the reported syntheses of 2-carboxy-1,3-propanedithiol by Yanagawa et al. (Yanagawa, H.; Kato, T.; Sagami, H.; Kitahara, Y. *Synthesis* **1973**, 607–608) and by Schotte and Strom (Schotte, L.; Strom, H. *Acta Chem. Scand.* **1956**, *10*, 687–688).

(64) We used the procedure of Danehy and Elia (Danehy, J. P.; Elia, V. *J. Org. Chem.* **1972**, *37*, 369–373) for the oxidation of 2-carboxy-1,3-propanedithiol to 4-carboxy-1,2-dithiolane.

(57) 3,3-Dimethyloxetane was prepared by the method of Schmoyer and Case (Schmoyer, L. F.; Case, L. C. *Nature* **1959**, *183*, 389) in the same yield as reported (30%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.39 (s, 4 H), 1.29 (s, 6 H).

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20605-01-0; 2-carboxy-1,3-propanedithiol, 20605-01-0; 3-(acetylthio)-2-methenyl-1-propanoic acid, 56140-22-8; sodium 2-hydroxyethanethiolate, 37482-11-4; potassium 2-hydroxyethanethiolate, 7450-31-9; sodium butylthiolate, 4779-86-6; potassium butylthiolate, 26385-25-1; potassium 2,2-dimethylpropylthiolate, 124357-90-0; potassium 2-hydroxy-2-

methylpropylthiolate, 124357-91-1; potassium 3-hydroxy-2,2-dimethylpropylthiolate, 124357-92-2; bis(2-hydroxyethyl) disulfide, 1892-29-1; 4-carboxy-1,2-dithiolane, 2224-02-4; sodium 2,2-dideuterio-2-hydroxyethanethiolate, 124357-93-3; bis(2,2-dideuterio-2-hydroxyethyl) disulfide, 124357-94-4; butyl disulfide, 629-45-8; selenylbenzene, 645-96-5.

## Quantitative Analysis of Solvent Effects in Highly Aqueous Media. Application of the SWAG Procedures and a Critical Appraisal of the Additivity Principle

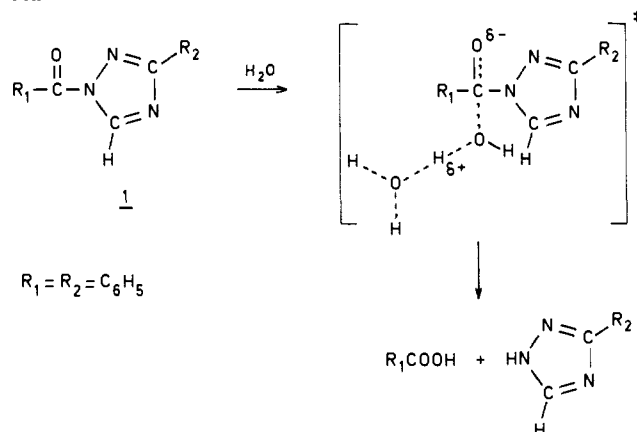
Wilfried Blokzijl,<sup>†</sup> Jan B. F. N. Engberts,<sup>\*,†</sup> and Michael J. Blandamer<sup>†</sup>

Contribution from the Department of Organic Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands, and Department of Chemistry, University of Leicester, Leicester LE1 7RH, England. Received January 27, 1989

**Abstract:** This paper describes a study on medium effects of 25 monohydric and polyhydric alcohols on the neutral hydrolysis of 1-benzoyl-3-phenyl-1,2,4-triazole in highly aqueous media. Dependences of pseudo-first-order rate constants on molality of added alcohol are quantitatively analyzed in terms of pairwise Gibbs energy interaction parameters for initial state and transition state. Additivity of pairwise group interaction parameters (SWAG procedure) is applied, and the validity of additivity is critically examined. Excellent additivity is observed in series of monohydric alcohols and vicinal diols. In series of polyhydric alcohols, group contributions to the observed rate effects, as expressed in pairwise group interaction parameters, are, however, strongly dependent on the position of hydroxyl groups in the alcohol. This (apparent) nonadditivity behavior is discussed in terms of the hydration characteristics of the cosolvents. The importance of hydration shell overlap in determining medium effects is supported by kinetic data in ternary 1-propanol-urea-water mixtures.

Specific noncovalent interactions in aqueous media are responsible for stabilization of proteins,<sup>1</sup> biological membranes,<sup>2</sup> micelles, and other aggregates<sup>3</sup> in aqueous solutions. Chemical reactivity in aqueous media is also largely governed by these types of interactions, and the presence of inert cosolvents in the reaction medium can seriously affect the rate of the reaction.<sup>4</sup> A valuable approach to analyze intermolecular interactions in aqueous solutions has been introduced by Wood and co-workers,<sup>5,6</sup> who proposed that thermodynamic pairwise intermolecular interaction parameters can be calculated as the sum of independent pairwise group interaction parameters (SWAG procedure). Recently we used this "additivity principle" in a quantitative analysis of rate effects of inert cosolvents on a hydrolysis reaction in highly aqueous media.<sup>7,8</sup> Here, we present a study of the medium effects of 25 monohydric and polyhydric alcohols on the neutral hydrolysis of the activated amide bond in 1-benzoyl-3-phenyl-1,2,4-triazole (**1**) in water-rich mixtures. These cosolvents retard the hydrolysis reaction. The observed rate effects, expressed in pseudo-first-order rate constants, are quantitatively analyzed in terms of the Gibbs energies of pairwise intermolecular interactions of the cosolvents with both the initial state (IS) and the transition state (TS) of the reaction. The consequence of gradual changes of the structure of the cosolvent for the rate effect is monitored. Additivity schemes have been frequently invoked to analyze intermolecular solute-solute interactions in aqueous mixtures.<sup>6,9-24</sup> Anomalies in these analyses have been noted because additivity schemes do not accommodate conformational and configurational aspects of intermolecular interactions in solutions.<sup>10,14,20,21,25-30</sup> The rate effect of monohydric and polyhydric alcohols on the hydrolysis of **1** can be expressed in terms of group contributions of merely two groups, i.e., CH and OH. In this analysis, these are the groups in the cosolvent that directly interact with solutes and solvent molecules in the reaction medium. Within series of structurally related alcohols, the rate effects can be understood in terms of two unique

Scheme I



values of these group contributions and excellent additivity is observed. However, attempts to express the rate effects of all

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<sup>†</sup>University of Groningen.

<sup>†</sup>University of Leicester.