# Role of Aqueous Media in Specific Recognition of Nonpolar Groups in Molecular Aggregates

**Tadashi Endo,* Hiromichi Takei, Takashi Isago, Jun-ichi Kato,† Masato M. Ito,‡ Kazuo Guro, Ken-ya Akiyama, Yutaka Nakajima, and Hidekazu Kataoka**

*Contribution from the Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, Chitosedai, Setagaya-ku, Tokyo 157, Japan.* *Received January 4, 1989*

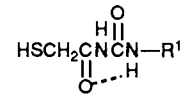
**Abstract:** Oxidation of a pair of associating thiols (**1** and **2**), each having a binding site [—C(=O)NHC(=O)NH—] and a recognition site ($R^1$ or $R^2$), is examined in mainly one set of binary solvent mixtures of ethanol with water and organic cosolvents at various temperatures. The selectivity ($r$)—a measure of the degree of recognition of **1** by **2** (or of **2** by **1**)—in the oxidation is represented by the logarithmic ratio of the yield of an unsymmetrical disulfide to twice that of a symmetrical one. It is found that (i) changes in the selectivity with solvent composition and temperature become much larger in aqueous mixed solvents than in the corresponding nonaqueous ones and (ii) there exist fairly wide temperature and solvent composition ranges where $\Delta r$ ($=r_i - r_n$) [$r_i$ = the selectivity for a branched alkyl group ($R^1$ or $R^2$) and $r_n$ = that for a straight-chain alkyl group of the same carbon number] becomes far larger in aqueous mixed solvents than in the corresponding nonaqueous ones. The "aqueous media effect" on the selectivity is achieved more markedly for flexible groups than for rigid ones. Correlation of the observed selectivity with heterogeneity of reactions, physicochemical properties of aqueous solutions, hydrophobic interaction, and so on is discussed together with a possible role ("amplification") of aqueous media in the degree of recognition of nonpolar groups.

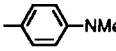
Molecular recognition is essential to living systems. The discrimination between competing molecules of similar structure by a given molecule can practically be ascribed to the discrimination between groups involved in them. For example, methylation, quite a simple chemical modification, of nucleotide bases—which results in the formation of 5-methylcytosine and $N^6$-methyladenine—lying within the recognition sequence serves to protect the cell's own DNA from being degraded by its restriction endonucleases;[1] methylation of cytosine is involved in gene regulation and differentiation.[2] A single amino acid substitution leads to sickle-cell anemia (the change from glutamic acid to valine),[3] alters antigen-binding specificity of an antibody (from glutamic acid to alanine),[4] or appears to be sufficient to confer transforming properties on the gene product of the human bladder carcinoma oncogene (from glycine to valine).[5]

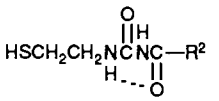
Much attention has been devoted to unique properties of water[6]—circular hydrogen-bond formation,[7] flip-flop hydrogen-bond formation,[8] and bulk physicochemical properties such as high dielectric constant and much higher critical temperature[6c] than expected from its molecular weight. Aqueous solutions[9] also have unusual physicochemical properties: (i) unique heat capacity behavior of aqueous solutions,[10] (ii) viscosity–composition maxima[9a,11] for aqueous alcohols, (iii) striking dependence of excess enthalpies of mixing[9a] on solvent composition for aqueous alcohols, and (iv) a large negative entropy of solution[12] into water of nonpolar substances such as hydrocarbons.

Water is the medium where highly specific enzymatic reactions occur. In contrast, most modern synthetic reactions of current interest[13] are performed in a "nonaqueous" environment probably due to the lability of reagents (or reactive species) to water. *Nonenzymatic* reactions, however, might be expected to proceed specifically in *aqueous* media,[14,15] if conducted in ordered molecular aggregates. We report here remarkable effects of aqueous environment on the selectivity in oxidation of a pair of associating thiols by using mainly one set of binary solvent mixtures of ethanol (EtOH) with water and organic cosolvents at various temperatures.[16]

Our model compound comprises a pair of acylurea derivatives (**1** and **2**), open-chain analogues of pyrimidine bases (e.g., uracil and thymine). Thiols **1** and **2** each have three sites: (i) the reaction site (SH group) where a model reaction takes place, (ii) the binding site [—C(=O)NHC(=O)NH—, acylurea bond]



**1a**: $R^1$ =  **b**: $R^1$ = —Ph **c$_n$**: $R^1$ = $n$-$C_5H_{11}$ **c$_i$**: $R^1$ = $i$-$C_5H_{11}$ **d**: $R^1$ = $n$-$C_4H_9$



**2a$_n$**: $R^2$ = $n$-$C_5H_{11}$ **a$_i$**: $R^2$ = $i$-$C_5H_{11}$ **b**: $R^2$ = $n$-$C_3H_7$ **c**: $R^2$ =  **d**: $R^2$ = $n$-$C_4H_9$

whose inner —NHC(=O)— unit participates in two NH···O intermolecular hydrogen bonds[17] and which extends in the opposite

† Present address: Research Department, The Noguchi Institute, Kaga, Itabashi-ku, Tokyo 173, Japan.

‡ Present address: Department of Chemical Engineering, Faculty of Engineering, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

(1) Smith, H. O. *Science (Washington, D.C.)* **1979**, *205*, 455. Arber, W. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 73.
(2) Razin, A.; Riggs, A. D. *Science (Washington, D.C.)* **1980**, *210*, 604. Bird, A. P. *Nature (London)* **1984**, *307*, 503.
(3) Lehninger, A. L. *Principles of Biochemistry*; Worth: New York, 1982; pp 194–200.
(4) Rudikoff, S.; Giusti, A. M.; Cook, W. D.; Scharff, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 1979.
(5) Tabin, C. J.; Bradley, S. M.; Bargmann, C. I.; Weinberg, R. A.; Papageorge, A. G.; Scolnick, E. M.; Dhar, R.; Lowy, D. R.; Chang, E. H. *Nature (London)* **1982**, *300*, 143. Reddy, E. P.; Reynolds, R. K.; Santos, E.; Barbacid, M. *Nature (London)* **1982**, *300*, 149.
(6) For reviews on water, see: (a) Franks, F., Ed. *Water—a Comprehensive Treatise*; Plenum: New York, 1973–1975; Vol. 2–4. (b) Stillinger, F. H. *Science (Washington, D.C.)* **1980**, *209*, 451. (c) Luck, W. A. P. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 28. (d) Lang, E. W.; Lüdemann, H.-D. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 315.
(7) Saenger, W. *Nature (London)* **1979**, *279*, 343. Saenger, W.; Lindner, K. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 398. Neidle, S.; Berman, H. M.; Shieh, H. S. *Nature (London)* **1980**, *288*, 129.
(8) Saenger, W.; Betzel, Ch.; Hingerty, B.; Brown, G. M. *Nature (London)* **1982**, *296*, 581. Betzel, Ch.; Saenger, W.; Hingerty, B. E.; Brown, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7545.
(9) For reviews on aqueous solutions, see: (a) Franks, F.; Ives, D. J. G *Q. Rev.* **1966**, *20*, 1. (b) Amis, E. S.; Hinton, J. F. *Solvent Effects on Chemical Phenomena*; Academic: New York, 1973; Vol. I, Chapter 4. (c) Symons, M. C. R. *Acc. Chem. Res.* **1981**, *14*, 179.
(10) Mirefovsky, D.; Arnett, E. M. *J. Am. Chem. Soc.* **1983**, *105*, 1112.
(11) (a) Washburn, E. W., Ed. *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*; McGraw-Hill: New York, 1929; Vol. V. Roth, W. A.; Scheel, K., Eds. *Landolt-Börnstein Physikalisch-Chemische Tabellen*; Springer: Berlin, 1923. (b) Beddard, G. S.; Doust, T.; Hudales, J. *Nature (London)* **1981**, *294*, 145.
(12) Némethy, G.; Scheraga, H. A. *J. Chem. Phys.* **1962**, *36*, 3401.
(13) House, H. O. *Modern Synthetic Reactions*, 2nd ed.; Benjamin: Menlo Park, CA, 1972. Nógrádi, M. *Stereoselective Synthesis*; VCH: Weinheim, 1987.
(14) For reviews on organic reactions in aqueous media, see: (a) Blandamer, M. J.; Burgess, J. *Chem. Soc. Rev.* **1975**, *4*, 55. (b) Engberts, J. B. F. N. *Pure Appl. Chem.* **1982**, *54*, 1797 and references cited therein.

0002-7863/89/1511-8166$01.50/0 

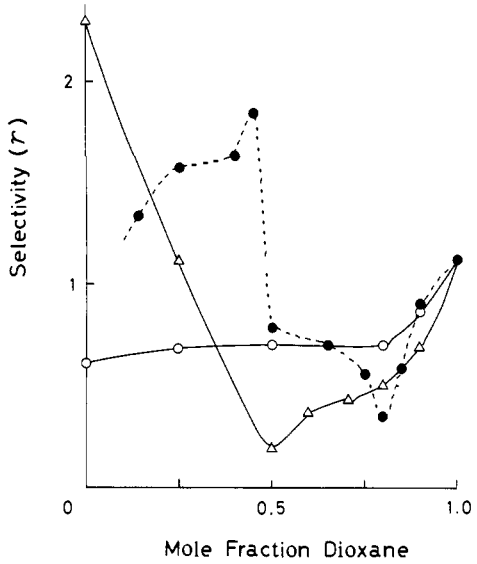



**Figure 1.** Dependence of selectivity ($r$) for system $A_i$ at 35.0 °C on mole fraction ($x$) of 1,4-dioxane (DI) in binary solvent systems containing dioxane. Mixed solvents used were water–dioxane (●), MeOH–dioxane (○), and *i*-PrOH–dioxane (△). Errors (3 times the standard deviations) for $r$ values range from ±0.01 to ±0.04 (water–dioxane) and from ±0.01 to ±0.03 (the other solvents) except in pure *i*-PrOH (±0.06). The $r$ values in water–dioxane at $x_{DI}$ = 0.40, 0.45, and 0.50 were 1.63 ± 0.04, 1.84 ± 0.02, and 0.79 ± 0.01, respectively.

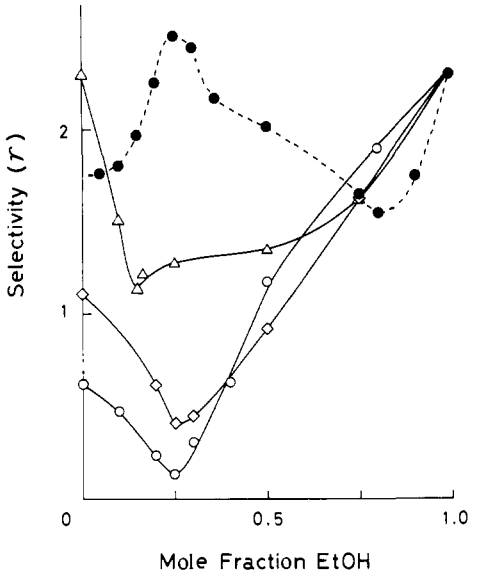
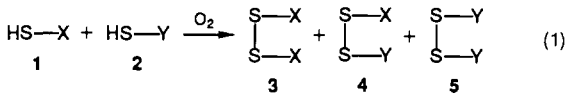



**Figure 2.** Dependence of selectivity ($r$) for system $A_i$ at 35.0 °C on mole fraction ($x$) of EtOH (EA) in binary solvent systems containing EtOH. Mixed solvents used were water–EtOH (●), MeOH–EtOH (○), *i*-PrOH–EtOH (△), and dioxane–EtOH (◇). Errors (3 times the standard deviations) for $r$ values range from ±0.02 to ±0.07 (water–EtOH) and from ±0.01 to ±0.04 (the other solvents) except in pure *i*-PrOH (±0.06) and pure EtOH (±0.15). The $r$ values in water–EtOH at $x_{EA}$ = 0.20, 0.25, and 0.30 were 2.25 ± 0.06, 2.50 ± 0.02, and 2.44 ± 0.02, respectively.

direction to each other, and (iii) the recognition site ($R^1$ or $R^2$) that participates in the discrimination. Thiol **1** has the same group as a cysteine side chain ($HSCH_2$), and **2** is a derivative of cysteamine (the decarboxylated compound of cysteine). In this study, we used eight reaction systems: systems $A_n$, $A_i$, $B_i$, $C_n$, $C_i$, $D_3$, $D_4$, and $D_5$. System $A_n$ consists of a 1:1 mixture of **1a** and $\mathbf{2a}_n$ [$R^2 = (CH_2)_4CH_3$], $A_i$ of **1a** and $\mathbf{2a}_i$ [$R^2 = (CH_2)_2CH(CH_3)_2$], and so on.

| | |
|---|---|
| system $A_n$: **1a** + $\mathbf{2a}_n$ | system $A_i$: **1a** + $\mathbf{2a}_i$ |
| system $B_i$: **1b** + $\mathbf{2a}_i$ | system $C_n$: $\mathbf{1c}_n$ + **2c** |
| system $C_i$: $\mathbf{1c}_i$ + **2c** | system $D_3$: **1d** + **2b** |
| system $D_4$: **1d** + **2d** | system $D_5$: **1d** + $\mathbf{2a}_n$ |

As a model reaction, oxidation with oxygen was chosen in connection with the correct pairing of half-cystine residues in proteins,[18] namely, specific S–S bond formation. Oxidation of a 1:1 mixture of **1** and **2** with oxygen in the presence of a catalytic amount of triethylamine gives one unsymmetrical (**4**) and two symmetrical disulfides (**3** and **5**) (eq 1). The selectivity ($r$)—a




(1)

measure of the degree of the recognition of **1** by **2** (or of **2** by **1**)—is represented by the logarithmic ratio of the yield of unsymmetrical disulfide **4** to twice that of symmetrical disulfide **3** (eq 2).

$$r = \ln ([\mathbf{4}]/2[\mathbf{3}]) \quad (2)$$

## Results

**Selectivity in Binary Solvent Mixtures.** The selectivity was investigated in various binary solvent mixtures, with solvent composition and/or temperature being changed.

Figure 1 shows a plot of $r$ for system $A_i$ against mole fraction ($x$) of 1,4-dioxane (i.e., $x_{DI}$) in binary mixtures of 1,4-dioxane with water and organic cosolvents. In mixtures with isopropyl alcohol (*i*-PrOH), $r$ reaches a minimum ($x_{DI}$ = ca. 0.5); $r$ values in methanol (MeOH)–dioxane change progressively with solvent composition. By contrast, in aqueous dioxane the selectivity passes through both a sharp maximum ($x_{DI}$ = 0.45) and a sharp minimum ($x_{DI}$ = 0.8). In Figure 2 is given the dependence of $r$ for system $A_i$ on mole fraction of EtOH (EA) in binary mixtures containing EtOH. In mixtures with MeOH, *i*-PrOH, or dioxane, $r$ exhibits a minimum at $x_{EA}$ = 0.25, 0.15, or 0.25, respectively. By contrast, in aqueous EtOH[19] the selectivity passes through both a pronounced maximum ($x_{EA}$ = 0.25) and a pronounced minimum ($x_{EA}$ = 0.8). A striking feature of the $r$–$x$ profiles for system $A_i$ (Figures 1 and 2) is that it is in the aqueous binary solvents among each of two sets of binary solvent systems that the selectivity alters most drastically with solvent composition.

Figure 3 plots $r$ for system $D_4$ as a function of $x_{EA}$ in binary solvent mixtures containing EtOH. $r$ has turned out to alter with $x_{EA}$ more markedly in aqueous EtOH than in the corresponding nonaqueous mixed solvents. Similarly, for system $B_i$ (Table I), $r$ in aqueous EtOH changes largely with solvent composition compared with that in dioxane–EtOH.

Temperature dependence of the selectivity is illustrated for system $D_4$ in Figure 4. $r$ alters with temperature more sharply

**Table I.** Dependence of Selectivity ($r$) for System $B_i$ at 35.0 °C on Mole Fraction ($x$) of EtOH (EA) in Binary Solvent Systems Containing EtOH

| | $r$ | |
|---|---|---|
| $x_{EA}$ | $H_2O$–EtOH | DI[a]–EtOH |
| 0.25 | 4.80 (0.25)[b] | 0.84 (0.06) |
| 0.50 | 3.06 (0.18) | 0.61 (0.09) |
| 0.75 | 2.70 (0.17) | 0.41 (0.06) |
| 1.00 | 1.36 (0.07) | 1.36 (0.07) |

[a] DI, 1,4-dioxane. [b] Errors given in parentheses are 3 times the standard deviations.

(15) For studies on molecular recognition in aqueous media, see: Hiraki, Y.; Tai, A. *Chem. Lett.* **1982**, 341. Hiraki, Y.; Oda, T.; Tai, A. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2330. Hiraki, Y.; Tai, A. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 1570.

(16) A preliminary report: Endo, T.; Takei, H.; Guro, K.; Yamashita, M.; Yonenaga, S.; Ito, M. M.; Matsushita, K. *Chem. Lett.* **1984**, 1717.

(17) (a) Endo, T.; Takeda, Y.; Orii, T.; Kaneko, Y.; Kondo, M. *Chem. Lett.* **1979**, 1455. (b) Uchida, A.; Ohashi, Y.; Sasada, Y.; Kaneko, Y.; Endo, T. *Acta Crystallogr.* **1984**, *C40*, 115. (c) Endo, T. *Top. Curr. Chem.* **1985**, *128*, 91 and references cited therein.

(18) The correct pairing of half-cystine residues has been suggested to depend upon specific noncovalent bonds: Anfinsen, C. B.; Sela, M.; Cooke, J. P. *J. Biol. Chem.* **1962**, *237*, 1825.

(19) Also in aqueous *i*-PrOH, there is a maximum in the selectivity–composition curve.

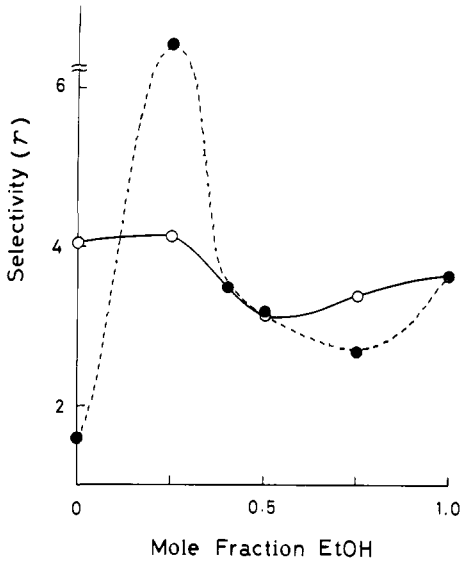



**Figure 3.** Dependence of selectivity ($r$) for system $D_4$ at 35.0 °C on mole fraction of EtOH in binary solvent systems containing EtOH. (●) Water–EtOH; (○) MeCN–EtOH. Errors (3 times the standard deviations) for $r$ values range from ±0.02 to ±0.10 (water–EtOH) and from ±0.06 to ±0.10 (MeCN–EtOH).

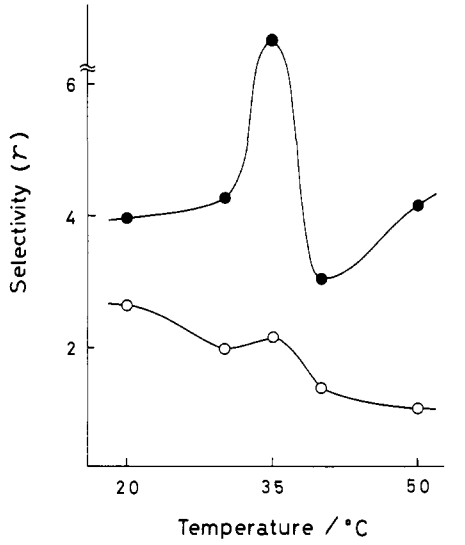



**Figure 4.** Temperature dependence of selectivity ($r$) for system $D_4$ in binary solvent systems containing EtOH. (●) Water–EtOH ($x_{EA}$ = 0.25); (○) dioxane–EtOH ($x_{EA}$ = 0.25). Errors (3 times the standard deviations) for $r$ values range from ±0.09 to ±0.18 (water–EtOH) and from ±0.01 to ±0.11 (dioxane–EtOH).

**Table II.** Selectivity ($r$) for Systems $D_3$–$D_5$ at 35.0 °C in Binary Solvent Systems Containing EtOH ($x_{EA}$ = 0.25)

| | $r$ | |
|---|---|---|
| $R^2$ | $H_2O$–EtOH | DI[a]–EtOH |
| $n$-$C_3H_7$ | 3.42 (0.10)[b] | 1.04 (0.04) |
| $n$-$C_4H_9$ | >6[c] | 2.16 (0.06) |
| $n$-$C_5H_{11}$ | 3.97 (0.04) | 1.53 (0.07) |

[a] DI, 1,4-dioxane. [b] Errors given in parentheses are 3 times the standard deviations. [c] The error for the $r$ value for $R^2$ = $n$-$C_4H_9$ is not shown, since the yield of symmetrical disulfide **3** cannot be determined due to the limitations (0.1%) of sensitivity of the integrator employed.

in water–EtOH than in dioxane–EtOH.

Table II shows the results for another approach using systems $D_3$–$D_5$. $r$ has a larger dependence on the carbon number of $R^2$ in water–EtOH than in dioxane–EtOH.

The above findings clearly indicate that more drastic changes in the selectivity are produced in aqueous mixed solvents than in the corresponding nonaqueous ones. It is interesting that the

**Table III.** Temperature Dependence of Selectivity ($r$) for Systems $A_i$ and $A_n$ in Binary Solvent Systems Containing EtOH ($x_{EA}$ = 0.25)

| | $r_i$ | | $r_n$ | |
|---|---|---|---|---|
| $t$/°C | $H_2O$–EtOH | DI[a]–EtOH | $H_2O$–EtOH | DI–EtOH |
| 20.0 | 2.62 (0.05)[b] | 2.05 (0.04) | −4.07 (0.05) | 1.38 (0.04) |
| 35.0 | 2.50 (0.04) | 0.41 (0.02) | −3.58 (0.05) | 0.36 (0.03) |
| 50.0 | 3.83 (0.07) | 1.01 (0.02) | −2.98 (0.02) | 0.93 (0.02) |
| 70.0 | 2.85 (0.07) | 1.15 (0.03) | 0.34 (0.03) | 1.64 (0.03) |

[a] DI, 1,4-dioxane. [b] Errors given in parentheses are 3 times the standard deviations.

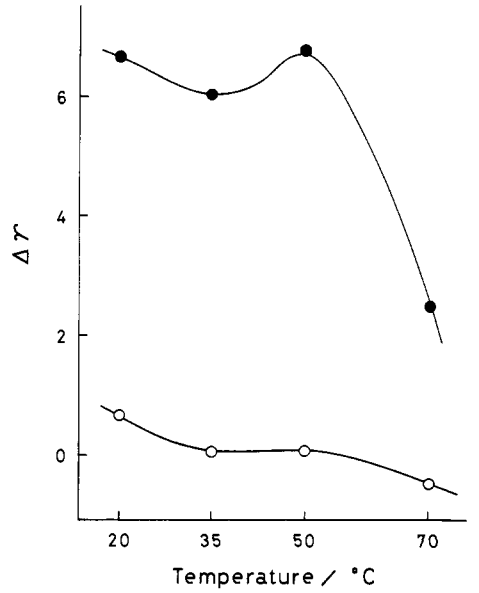



**Figure 5.** Temperature dependence of the differential selectivity ($\Delta r$) for systems $A_i$ and $A_n$ in binary solvent systems containing EtOH. (●) Water–EtOH ($x_{EA}$ = 0.25); (○) dioxane–EtOH ($x_{EA}$ = 0.25). Errors for $r$ values used to calculate $\Delta r$ are listed in Table III.

"aqueous media effect" mentioned here depends upon the structures of $R^1$ and $R^2$ to some extent: the selectivity becomes highest for a system (i.e., system $D_4$) having a *flexible* group[20] such as an alkyl group rather than for a system (e.g., system $A_i$) having a *rigid* group such as a phenyl group, an $r$[21] of larger than 6 for system $D_4$ (Table II) being the largest of all the values so far obtained for systems A–D.

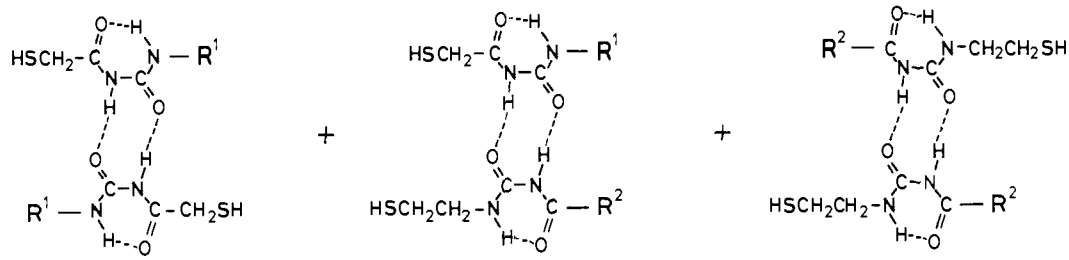
**Differences in Selectivity between a Straight-Chain Alkyl Group and a Branched One.** There are many factors that govern the selectivity in an organic reaction. In order to elucidate the aqueous media effect on chemical selectivity (that is, on molecular recognition), it is not sufficient to compare the selectivity itself in aqueous binary solvents with that in the corresponding nonaqueous ones. Another way is to examine differential selectivity for straight-chain and branched alkyl groups, each having the same number of carbon atoms, for example. A parameter for this analysis ($\Delta r$) is given by eq 3, where $r_i$ refers to the selectivity for an isopentyl group ($i$-$C_5H_{11}$) as $R^2$ (or $R^1$) and $r_n$ to the selectivity for a $n$-pentyl group as $R^2$ (or $R^1$), the structure of $R^1$ (or $R^2$) being fixed.

$$\Delta r = r_i - r_n \tag{3}$$

Table III lists the selectivity ($r_i$) for system $A_i$ and that ($r_n$) for system $A_n$ in dioxane–EtOH and water–EtOH ($x_{EA}$ = 0.25) as a function of temperature. In going from the nonaqueous mixed

(20) In this connection, it is interesting to note that antibodies preferentially bind to the more mobile (or flexible) segments of protein molecules (antigens): (a) Marx, J. L. *Science (Washington, D.C.)* **1984**, *226*, 819 and references cited therein. (b) Geysen, H. M.; Tainer, J. A.; Rodda, S. J.; Mason, T. J.; Alexander, H.; Getzoff, E. D.; Lerner, R. A. *Science (Washington, D.C.)* **1987**, *235*, 1184. See also Huber, R. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 80 and references cited therein.

(21) Endo, T.; Ueda, T.; Isago, T.; Kato, J.; Suzuki, T.; Misaka, M.; Ito, M. M. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2298.



6 7 8

**Figure 6.** Association patterns of dimers **6–8**. (---) Hydrogen bonding.

**Table IV.** Dependence of Selectivity ($r$) and $\Delta r$ for Systems $A_i$ and $A_n$ at 35.0 °C on Mole Fraction ($x$) of *i*-PrOH (IA) in Binary Solvent Systems Containing *i*-PrOH

| | $r_i$ | | $r_n$ | | $\Delta r$ | |
|---|---|---|---|---|---|---|
| $x_{IA}$ | $H_2O$–IA | DI[a]–IA | $H_2O$–IA | DI–IA | $H_2O$–IA | DI–IA |
| 0.20 | 2.17 (0.05)[b] | 0.49 (0.01) | −3.17 (0.03) | 0.05 (0.11) | 5.3 | 0.44 |
| 0.50 | 1.64 (0.03) | 0.19 (0.06) | −2.45 (0.01) | 0.74 (0.02) | 4.1 | −0.55 |
| 0.75 | 2.56 (0.03) | 1.11 (0.02) | −2.33 (0.03) | 1.42 (0.07) | 4.9 | −0.31 |

[a] DI, 1,4-dioxane. [b] Errors given in parentheses are 3 times the standard deviations.

**Table V.** Dependence of Selectivity ($r$) and $\Delta r$ for Systems $C_i$ and $C_n$ at 35.0 °C on Mole Fraction ($x$) of Water (w) in Water–EtOH

| $x_w$ | $r_i$ | $r_n$ | $\Delta r$ |
|---|---|---|---|
| 0 | 2.76 (0.08)[a] | −2.79 (0.09) | 5.6 |
| 0.25 | 2.35 (0.05) | −2.71 (0.11) | 5.1 |
| 0.50 | 3.15 (0.04) | −2.96 (0.07) | 6.1 |
| 0.75 | 3.72 (0.07) | −4.40 (0.13) | 8.1 |

[a] Errors given in parentheses are 3 times the standard deviations.

solvent to the corresponding aqueous one, $r_i$ becomes more positive, while $r_n$ varies from small positive values to large negative values except at 70 °C.[22] Figure 5 plots $\Delta r$ for systems $A_i$ and $A_n$ against temperature. In the 20–70 °C range, the $\Delta r$ values in water–EtOH have proved to be far larger than those in dioxane–EtOH.

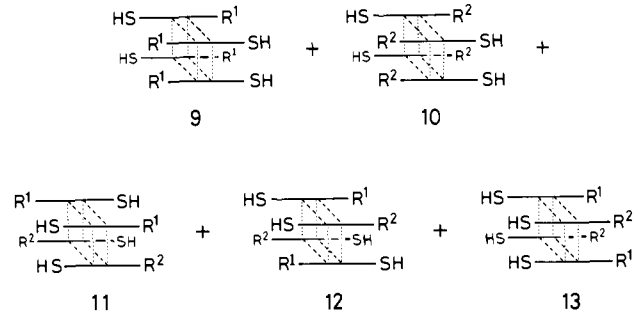
The dependence of $\Delta r$ was further investigated on solvent composition. $\Delta r$ for systems $A_i$ and $A_n$ in water–*i*-PrOH (Table IV) has been found to exceed that in dioxane–*i*-PrOH. For systems $C_i$ and $C_n$ (Table V), there exists a water (w) mole fraction region ($0.50 \leq x_w$) where $\Delta r$ in water–EtOH mixtures exceeds that in pure EtOH; in this case, $\Delta r$ approaches up to 8 at $x_w$ = 0.75.

The above differential selectivity data demonstrate that there are fairly wide temperature and solvent composition ranges where the degree of recognition of alkyl groups becomes far larger in aqueous mixed solvents than in the corresponding nonaqueous ones.

## Discussion

The environment dependence of the selectivity presented here seems not to be so simple. In what follows, we wish to discuss (i) the mechanism of the oxidation, (ii) intermolecular association, (iii) some factors which have possibilities of affecting the selectivity in aqueous media [i.e., heterogeneity of reactions, physicochemical properties of aqueous solutions, and hydrophobic interaction], and (iv) a possible role of aqueous media in the recognition.

**Mechanism of Oxidation.** It has been demonstrated that the product ratio in this type of oxidation is kinetically controlled on the basis of the observations that (i) the product ratios for systems $A_i$ and $A_n$ do not change as the oxidation proceeds and (ii) a thiol–disulfide exchange reaction[23] occurs only slowly under conditions similar to those for oxidation.[24,25] Considering that



9 10 11 12 13

**Figure 7.** Association schemes of tetramers **9–13** formed by dimerization of dimers **6–8**. (---) Hydrogen bonding responsible for the stabilization of dimers; (···) noncovalent weak interactions responsible for the stabilization of tetramers.

the amount of the thiols participating in the thiol–disulfide exchange is reduced as the oxidation proceeds, the exchange reaction does not have so great an influence on the selectivity.

Initial rates ($v_0$), average rates until 5% consumption of thiols, for thiol **1** were about 100 times larger than those for thiol **2** in aqueous MeCN.[26,27] The large reactivity difference between thiols **1** and **2** is not responsible for the observed selectivity in aqueous mixed solvents. This is because $r$ values should become negative regardless of the reaction systems employed, if the selectivity depends upon the reactivity difference; however, $r$ shows large positive values for systems $A_i$ (Tables III and IV), $C_i$ (Table V), and $D_4$ (Figures 3 and 4).[28]

Examples are known where relative rates for oxidation of a 1:1 mixture of **1** and **2** cannot explain the selectivity.[26,29]

**Intermolecular Association.** $^1H$ NMR and IR studies of **1** and **2**, made with the dilution technique, revealed that **1** and **2** formed weak complexes in solution with each other as well as with themselves through two NH···O intermolecular hydrogen bonds.[17a,30] Since the two acylurea bonds in **1** and **2** extend in the opposite direction to each other, association patterns of

(22) The same is true in binary solvent systems containing *i*-PrOH (Table IV) without exception.

(23) It is generally accepted that thiol–disulfide exchange reaction occurs easily if a base is present and that the rate of the exchange increases with temperature: (a) Jocelyn, P. C. *Biochemistry of the SH Group*; Academic: London, 1972; Chapter 5. (b) Fava, A.; Iliceto, A.; Camera, E. *J. Am. Chem. Soc.* **1957**, *79*, 833.

(24) Endo, T.; Kuwahara, A.; Tasai, H.; Murata, T.; Hashimoto, M.; Ishigami, T. *Nature (London)* **1977**, *268*, 74.

(25) Endo, T.; Okubo, A.; Kaneko, Y.; Uehara, M.; Tasai, H.; Sato, A.; Nikki, K.; Nakagawa, N.; Kamei, S. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2224.

(26) Endo, T.; Hashimoto, M.; Orii, T.; Ito, M. M. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 1562.

(27) Endo, T.; Kasahara, H.; Yagi, S.; Tajima, K.; Ito, M. M.; Ogino, M.; Hagino, K. *Chem. Lett.* **1986**, 705.

(28) Triethylamine-catalyzed oxidation of a 1:1 mixture of **1a** and **2** ($R^2$ = Ph, *p*-$MeC_6H_4$, or *p*-$EtC_6H_4$) in aqueous MeCN follows the rate equation $v = k_{1,7}[RSH][Et_3N]^{0.7}$.[26]

(29) Endo, T.; Tajima, K.; Yamashita, M.; Ito, M. M.; Nishida, J.; Ogikubo, T. *J. Chem. Soc., Chem. Commun.* **1986**, 1561.

(30) One reviewer pointed out that the most important macroscopic solvent property which affected the thiol association was perhaps the cohesive energy density.

homodimers (**6** and **8**) are of the head-to-tail type, that of the heterodimer (**7**) being of the head-to-head type (Figure 6). In homodimer **6** or **8**, the distance between the two HS groups is too long for the S–S bond to be formed. Therefore, homodimers **6** and **8** cannot explain the *selective* formation of *symmetrical* disulfides (i.e., $r < 0$) in several cases: (i) system $A_n$ (Tables III and IV); (ii) $R^1$ = *p*-$Me_2NC_6H_4$ and $R^2$ = *n*-$C_4H_9$ to *n*-$C_7H_{15}$;[24] (iii) $R^1$ = *n*-$C_5H_{11}$ and $R^2$ = cyclo-$C_6H_{11}$ and Ph;[27] (iv) system $C_n$ (Table V).

Assuming that a single multimer species in equilibrium with a monomer is a dimer, the degree of association (*f*), obtained by dividing the stoichiometric mole fraction of a solute by the effective mole fraction of the solute,[31] ranges from 1.0 to 2.0.[32] The *f* of 1.95 for **2a**$_i$ (at 0.06 M and 36.0 °C in $CCl_4$) thus suggested the presence of higher aggregates in addition to dimers.[17a]

On the basis of these findings, tetramers [two homotetramers (**9** and **10**) and three heterotetramers (**11**–**13**)] were suggested to be intermediates in this oxidation (Figure 7).[17a,c,33] The finding that oxidation of **1a** and **2** ($R^2$ = Ph) shows a large negative activation entropy[26] of −45 cal $K^{-1}$ $mol^{-1}$ can be understood by the tetrameric intermediates presented here. There is now substantial evidence for the presence of tetramers in solution in chemical and biological systems.[17a,c,34]

Intermolecular association turned out to be the first requirement for molecular recognition.[24,35,36] This view also receives support from the observation that, at higher temperatures (conditions unfavorable for intermolecular association through hydrogen bonding), *r* approaches about 0 (the value statistically expected) for system $A_n$ in aqueous EtOH (Table III).

**Heterogeneity of Reactions.** In binary solvent mixtures of dioxane with EtOH, reaction mixtures are all homogeneous, regardless of temperature (20–70 °C) (systems $A_n$ and $A_i$ in Table III) and solvent composition (system $B_i$ in Table I); in these solvents, the selectivity shows a minimum (Tables I and III). In addition, there are reaction mixtures which are heterogeneous in water–EtOH, regardless of temperature (systems $A_n$ and $A_i$ in Table III) and solvent composition (system $A_i$ in Figure 2 and system $D_4$ in Figure 3); in these aqueous mixed solvents, the selectivity exhibits a maximum (Figures 2 and 3 and Table III) and a minimum (Figures 2 and 3) or increases with increasing temperature (Table III).

In some reaction systems, whether reaction mixtures are homogeneous or heterogeneous depends upon solvent composition and temperature ranges in water–EtOH. For system $B_i$, reaction mixtures are homogeneous in the 1.0–0.5 mole fraction of EtOH region, while the selectivity decreases with increasing mole fraction of EtOH (Table I). For systems $C_n$ and $C_i$, reaction mixtures are homogeneous in the mole fraction of water region ≤ 0.5, whereas the selectivity for systems $C_n$ and $C_i$ shows a small maximum and reaches a minimum, respectively (Table V). For system $D_4$, reaction mixtures are heterogeneous in the 20–40 °C range, while the selectivity passes through a sharp maximum at 35 °C (Figure 4).

As is evident from the observations mentioned above, there exists no correlation of heterogeneity of reactions with the presence of a maximum (or a minimum) in the selectivity–environment curve. Therefore, the heterogeneity of reactions would not have such a remarkable influence on the observed selectivity.[37]

**Physicochemical Properties of Aqueous Solutions.** Another fascinating aspect of the selectivity–solvent composition profiles (Figures 1 and 2) is the frequent occurrence of the selectivity extrema and inflections at about 0.25, 0.5, and 0.8 mole fraction of organic components. This appears to partly reflect the fact[9b] that in aqueous solutions there are two mole fraction regions ($x_w$ = ca. 0.2 and 0.8) in which bulk properties of aqueous solutions undergo dramatic changes.

The viscosity of aqueous EtOH rises to a maximum at the same mole fraction ($x_{EA} = 0.25$)[11] where the maximum selectivity occurs (Figures 2 and 3).[38] On the other hand, water proton resonances and dielectric constants ($\epsilon$) for aqueous mixed solutions have no correlation with the selectivity. This is because (i) the water proton chemical shifts for water–dioxane mixtures[39] vary linearly with solvent composition, while the selectivity in the mixtures passes through both a sharp maximum and a sharp minimum (Figure 1), and (ii) the $\epsilon$–$x$ curve for water–EtOH mixtures (20 °C)[40] is smooth over all the solvent composition range whereas the selectivity reaches both a maximum and a minimum (Figure 2).

**Hydrophobic Interaction.** When the oxidation is performed in aqueous mixed solvents, hydrophobic interaction[41] may operate between the recognition sites ($R^1$ and $R^2$), thereby affecting the selectivity.

The strength of the hydrophobic interaction (hydrophobicity) is reported to increase progressively with increasing mole fraction of water in aqueous dioxane ($x_w \leq 0.88$ at 25 °C) and in aqueous EtOH ($x_w \leq 0.85$ at 30 °C).[42] The *r*–*x* curve in water–EtOH is smooth for system $C_n$ (Table V); for systems $A_i$ (Figure 2) and $D_4$ (Figure 3), however, the selectivity in this solvent system exhibits both a maximum and a minimum. Also in aqueous dioxane, there are extrema in the *r*–*x* profile (Figure 1).

Hydrophobicity represented by $\Delta G°$ for the transfer of a solute from the pure liquid to dilute aqueous solution[12] increases progressively with increasing temperature. On the other hand, the temperature dependence of *r* in aqueous EtOH shows a maximum for each of systems $A_i$ (Table III) and $D_4$ (Figure 4). The hydrophobicity parameters ($\pi$[43] and MH[44]) increase with increasing number of carbon atoms in the alkyl groups, whereas *r* in aqueous

(31) Davies, M.; Thomas, D. K. *J. Phys. Chem.* **1956**, *60*, 763.

(32) In monomer–dimer equilibrium, the constant for dimerization (*K*) is given as $K = f(f-1)/[c(f-2)^2]$, where *c* represents the stoichiometric concentration. Thus, *f* of 1.95 (0.06 M) corresponds to *K* of $1.24 \times 10^4$ $M^{-1}$; *f* of 1.3 and 1.5 (0.01 M) correspond to *K* of 80 and 300, respectively.

(33) Each tetramer would afford the corresponding disulfide(s) selectively when treated with oxygen: (i) heterotetramers **11** and **12**, and probably **13**, would exclusively give unsymmetrical disulfide **4**, and (ii) homotetramers **9** and **10** would exclusively give symmetrical disulfides **3** and **5**, respectively. Relative concentrations of tetramers, which are considered to control the selectivity, depend primarily upon the reaction systems A–D employed.

(34) According to cryoscopic studies of $[(t\text{-BuC}{\equiv}\text{CLi})_4(\text{THF})_4]$, a dimer–tetramer equilibrium was shown to exist at −108 °C: Bauer, W.; Seebach, D. *Helv. Chim. Acta* **1984**, *67*, 1972.

(35) Associating thiols (**1** and **2**) each have been shown to participate in *two* NH···O intermolecular hydrogen bonds.[17] The corresponding thiols having amide linkages [$HSCH_2C(=O)NH—C_6H_5$ (**14**) and $HSCH_2CH_2NHC(=O)—R^3$ (**15**)] each would participate mainly in a *single* NH···O intermolecular hydrogen bond; this is because *f* (in benzene at 0.01 M and 36.0 °C) for **1** and **2** ranges from 1.3 to 1.5, whereas that for **14** and **15** is less than 1.05.[32,36] We have found that *r* (in aqueous MeCN at 35 °C) for **1a** and **2** ($R^2$ = *i*-Pr to *i*-$C_6H_{13}$) changes from −0.87 to 2.4 with a sharp maximum at $R^2$ = *i*-$C_5H_{11}$,[24] while that for a pair of "nonassociating" thiols, **14** and **15**, alters only slightly from −0.06 to −0.09 ($R^3$ = *i*-Pr to *i*-$C_6H_{13}$) [Endo, T.; Takeda, Y.; Orii, T.; Kuwahara, A.; Ohta, M.; Sakai, M.; Okada, R.; Hashimoto, M. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2687]. This indicates the significance of intermolecular association through two hydrogen bonds in specific molecular recognition. Seeman et al. reported that with two hydrogen bonds fidelity of base pair recognition might be achieved, whereas a single hydrogen bond was inadequate for uniquely identifying any particular base pair [Seeman, N. C.; Rosenberg, J. M.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 804].

(36) Endo, T.; Takeda, Y.; Kamada, H.; Kayama, S.; Tasai, H. *Chem. Lett.* **1980**, 417.

(37) There are some examples where the heterogeneity of a reaction is not responsible for the selectivity.[24,25] Moreover, the selectivity in this type of oxidation is not controlled by solubility differences between the thiols (**1** and **2**)[24,25,36] and between the disulfides (**3**–**5**).[24,36]

(38) Studies were made on the relationship between the product ratio in photolysis of azomethane in aqueous *tert*-butyl alcohol and the viscosity of this solvent system: Nodelman, N.; Martin, J. C. *J. Am. Chem. Soc.* **1976**, *98*, 6597.

(39) Kingston, B.; Symons, M. C. R. *J. Chem. Soc., Faraday Trans. 2* **1973**, *69*, 978.

(40) Ref 6a, Vol. 2, Chapter 7.

(41) For reviews on hydrophobic interaction, see: (a) Némethy, G. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 195. (b) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; Chapter 8. (c) Tanford, C. *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*; Wiley: New York, 1973. (d) Ref 6a, Vol. 4, Chapter 1. (e) Ben-Naim, A. *Hydrophobic Interactions*; Plenum: New York, 1980.

(42) Reference 41e, Chapter 3.

(43) Hansch, C.; Steward, A. R.; Iwasa, J.; Deutsch, E. W. *Mol. Pharmacol.* **1965**, *1*, 205 and references cited therein.

(44) Menger, F. M.; Venkataram, U. V. *J. Am. Chem. Soc.* **1986**, *108*, 2980.

EtOH ($R^2$ = $n$-$C_3H_7$ to $n$-$C_5H_{11}$) reaches a maximum at $R^2$ = $n$-$C_4H_9$ (Table II).

The above discussions suggest that (i) the hydrophobic interaction would certainly not be the sole factor and (ii) the observed selectivities are the result of a combination of a number of possible effects with their individual contributions depending on the solvent composition.

**A Possible Role of Aqueous Media in Recognition.** As typically shown in Figures 3 and 4, the selectivity becomes much larger in aqueous media than in the corresponding nonaqueous ones, especially in a *narrow* solvent composition range. This suggests that a role of aqueous media can rather be ascribed to a role of *water* in aqueous media. It is thus assumed that water in aqueous mixed solvents would "amplify" the degree of recognition of nonpolar groups in the narrow composition range.

Engberts et al.[45] reported that, as mole fraction ($x$) of dioxane (DI) was increased, the relative rates of general-base-catalyzed solvolysis of (arylsulfonyl)methyl perchlorates in water–dioxane rose to a maximum ($x_{DI}$ = ca. 0.2) and then fell to values less than that in pure water (ca. $0.5 < x_{DI} \leq 0.8$), whereas in EtOH–dioxane they decreased progressively with increasing mole fraction of dioxane ($0 \leq x_{DI} <$ ca. 0.3). This appears to be consistent with the above hypothesis on the role of water.

We have demonstrated that higher selectivity is achieved when the nonpolar group of a propanol in a mixed solvent resembles a given nonpolar group of one of the reacting molecules in three-dimensional shape: $r$ for system $A_i$ ($R^2$ = $i$-$C_5H_{11}$) is 1.6 and 0.75 in $i$-PrOH–water and $n$-PrOH–water mixtures ($x_w$ = 0.50), respectively; on the other hand, $r$ for system $A_n$ ($R^2$ = $n$-$C_5H_{11}$) is −1.7 and −2.5 in $n$-PrOH–water and $i$-PrOH–water mixtures ($x_w$ = 0.50), respectively.[46] This suggests that specific interactions occur between a nonpolar group of an organic cosolvent (i.e., a Pr group) and a nonpolar group of a solute (i.e., $R^2$). Moreover, this solvent shape effect on the selectivity is produced more markedly in aqueous propanols than in the corresponding nonaqueous ones.[47]

Stillinger pointed out that all the properties of water and aqueous solutions ultimately must be explained in terms of the intermolecular forces that were present.[6b] It has been clarified that $r$ ($R^1$ = Ph) for $R^2$ in aqueous mixed solvents depends upon the strength[48] of weak interactions between the recognition sites [$R^1$ (=Ph) and $R^2$].[29] Considering this finding,[49] one possible explanation of the amplification effect of water in aqueous media is that water molecules influence specific weak interactions between nonpolar groups $R^1$ and $R^2$.

## Experimental Section

**General Procedures.** $^1$H NMR spectra were recorded with a JEOL GX-270 spectrometer. Chemical shifts ($\delta$) are reported downfield from internal $SiMe_4$. The mass spectra were taken on a Hitachi RMU-6M spectrometer and exact mass data on a JEOL JMS-DX303 mass spectrometer. Melting points were determined on a Yamato oil-immersion apparatus and are uncorrected. HPLC separations were conducted on a Waters Model 204 system including a UV detector attached to a Waters 740 data module (integrator).

**Materials.** Water was purified through a Millipore Milli-Q water purification system followed by distillation. Acetonitrile was purified by distillation from $CaH_2$ and then from $P_2O_5$. Alcohols were purified by distillation from magnesium alkoxides. 1,4-Dioxane was purified by distillation from KOH and then from Na.

Thiols **1** were prepared by reaction of the corresponding thiolesters [MeC(=O)$SCH_2$C(=O)NHC(=O)NH—$R^1$] with cysteamine as described before.[50] In the case of thiols **1b–d**, the reaction mixtures were concentrated in vacuo, washed with water in order to remove the coexisting acetylated cysteamine [MeC(=O)$NHCH_2CH_2SH$] because of high solubilities of the resulting thiols in MeCN used as a solvent, dried in vacuo, and recrystallized from argon-saturated ether–hexane except for **1b** (benzene–hexane). Thiols **1** had the following properties [$^1$H NMR (270 MHz) spectra of **1** were measured in $CDCl_3$ at 0.02 M].[21,25,51]

**1c$_n$**: mp 98–99 °C; $^1$H NMR $\delta$ 0.91 (t, $J$ = 7 Hz, 3 H), 1.30–1.59 (m, 6 H), 2.08 (t, $J$ = 9 Hz, 1 H, SH), 3.26–3.33 (m, 4 H, $SCH_2$ and $NCH_2$), 8.27 (s, 1 H), 9.50 (s, 1 H); MS, $m/e$ 204. Anal. Calcd for $C_8H_{16}N_2O_2S$: C, 47.04; H, 7.89; N, 13.71; S, 15.69. Found: C, 46.86; H, 8.03; N, 13.45; S, 15.66.

**1c$_i$**: mp 116.5–117.5 °C; $^1$H NMR $\delta$ 0.93 (d, $J$ = 6 Hz, 6 H), 1.45 (dt, $J$ = 7 and 7 Hz, 2 H), 1.54–1.72 (m, 1 H), 2.08 (t, $J$ = 9 Hz, 1 H, SH), 3.29–3.36 (m, 4 H, $SCH_2$ and $NCH_2$), 8.24 (s, 1 H), 9.51 (s, 1 H); MS, $m/e$ 204. Anal. Calcd for $C_8H_{16}N_2O_2S$: C, 47.04; H, 7.89; N, 13.71; S 15.69. Found: C, 47.01; H, 7.79; N, 13.52; S, 16.16.

Thiols **2** were prepared by addition of the corresponding acyl isocyanates to freshly sublimed cysteamine in tetrahydrofuran (THF) under argon at 0 °C as described before[25] and had the following properties [$^1$H NMR (270 MHz) spectra of **2** were measured in $CDCl_3$ at 0.02 M].[21]

**2a$_i$**: mp 133.5–134.5 °C (ether–hexane); $^1$H NMR $\delta$ 0.92 (d, $J$ = 6 Hz, 6 H), 1.40 (t, $J$ = 9 Hz, 1 H, SH), 1.52–1.66 (m, 3 H), 2.32 (t, $J$ = 7 Hz, 2 H, $COCH_2$), 2.66–2.74 (m, 2 H, $SCH_2$), 3.45–3.52 (m, 2 H, $NCH_2$), 8.65 (s, 1 H), 8.71 (s, 1 H); MS, $m/e$ 218. Anal. Calcd for $C_9H_{18}N_2O_2S$: C, 49.53; H, 8.31; N, 12.84; S, 14.66. Found: C, 49.29; H, 8.26; N, 12.76; S, 14.81.

**2c**: mp 139.5–140.5 °C (ether–hexane); $^1$H NMR $\delta$ 1.42 (t, $J$ = 9 Hz, 1 H, SH), 1.58–1.98 (m, 8 H), 2.60–2.74 (m, 3 H, COCH and $SCH_2$), 3.46–3.53 (m, 2 H, $NCH_2$), 8.41 (s, 1 H), 8.75 (s, 1 H); MS, $m/e$ 216. Anal. Calcd for $C_9H_{16}N_2O_2S$: C, 49.99; H, 7.46; N, 12.96; S, 14.80. Found: C, 49.87; H, 7.43; N, 12.99; S, 14.92.

**Preparation of Disulfides.** Symmetrical disulfides **3** were easily obtained by treatment of **1** with $O_2$ in the presence of $Et_3N$ in MeCN at room temperature and recrystallized from THF–dichloromethane. Unsymmetrical disulfides **4** were prepared either by repeated recrystallization of the oxidation mixture in cases where $r$ for a given system was larger than about 1 or by reaction of a disulfide [2,4-$(NO_2)_2C_6H_3S$—$SCH_2CH_2NHC(=O)NHC(=O)$—$R^2$] with thiol **1** in the presence of silver acetate in DMF under argon as described before[52] followed by recrystallization of the reaction mixture in cases where $r$ for a given system was smaller than about 1. Disulfides **3** and **4** had the following properties [$^1$H NMR (270 MHz) spectra were measured in $Me_2SO$-$d_6$ at 0.01 M].[21,25]

**3b** ($R^1$ = Ph): mp 185–186.5 °C; $^1$H NMR $\delta$ 3.80 (s, 4 H, $SCH_2$), 7.09 (t, $J$ = 7 Hz, 2 H), 7.32 (dd, $J$ = 7 and 8 Hz, 4 H), 7.50 (d, $J$ = 8 Hz, 4 H), 10.30 (s, 2 H), 10.82 (s, 2 H); MS, $m/e$ 418. Anal. Calcd for $C_{18}H_{18}N_4O_4S_2$: C, 51.67; H, 4.34; N, 13.39; S, 15.30. Found: C, 51.68; H, 4.34; N, 13.48; S, 15.37.

**3c$_n$** ($R^1$ = $n$-$C_5H_{11}$): mp 192.5–194.5 °C; $^1$H NMR $\delta$ 0.86 (t, $J$ = 7 Hz, 6 H), 1.20–1.51 (m, 12 H), 3.11–3.18 (m, 4 H, $NCH_2$), 3.65 (s, 4 H, $SCH_2$), 8.16 (s, 2 H), 10.41 (s, 2 H); MS, $m/e$ 406. Anal. Calcd for $C_{16}H_{30}N_4O_4S_2$: C, 47.28; H, 7.44; N, 13.79; S, 15.75. Found: C, 47.18; H, 7.48; N, 13.72; S, 15.88.

**3c$_i$** ($R^1$ = $i$-$C_5H_{11}$): mp 168.5–170.0 °C; $^1$H NMR $\delta$ 0.88 (d, $J$ = 6 Hz, 12 H), 1.35 (dt, $J$ = 7 and 7 Hz, 4 H), 1.46–1.64 (m, 2 H), 3.14–3.21 (m, 4 H, $NCH_2$), 3.64 (s, 4 H, $SCH_2$), 8.14 (s, 2 H), 10.40 (s, 2 H); MS, $m/e$ 406. Anal. Calcd for $C_{16}H_{30}N_4O_4S_2$: C, 47.28; H, 7.44; N, 13.79; S, 15.75. Found: C, 47.24; H, 7.43; N, 13.77; S, 15.92.

**4aa$_n$** ($R^1$ = $p$-$Me_2NC_6H_4$ and $R^2$ = $n$-$C_5H_{11}$): mp 172–173 °C ($CH_2Cl_2$); $^1$H NMR $\delta$ 0.85 (t, $J$ = 7 Hz, 3 H), 1.19–1.30 (m, 4 H), 1.45–1.56 (m, 2 H), 2.26 (t, $J$ = 7 Hz, 2 H, $COCH_2CH_2$), 2.85 [s, 6 H, $N(CH_3)_2$], 2.88–2.93 (m, 2 H, $SCH_2CH_2N$), 3.42–3.52 (m, 2 H, $SCH_2CH_2N$), 3.68–3.77 (m, 2 H, $SCH_2CO$), 6.70 (d, $J$ = 9 Hz, 2 H), 7.32 (d, $J$ = 9 Hz, 2 H), 8.57 (s, 1 H), 10.04 (s, 1 H), 10.29 (s, 1 H), 10.69 (s, 1 H). Exact mass for $^{12}C_{20}{}^1H_{31}{}^{14}N_5{}^{16}O_4{}^{32}S_2$: calcd, 469.1820; found, 469.1806.

**4aa$_i$** ($R^1$ = $p$-$Me_2NC_6H_4$ and $R^2$ = $i$-$C_5H_{11}$): mp 174.5–175.5 °C (MeCN); $^1$H NMR $\delta$ 0.84 (d, $J$ = 6 Hz, 6 H), 1.36–1.57 (m, 3 H), 2.27 (t, $J$ = 7 Hz, 2 H, $COCH_2CH_2$), 2.85 [s, 6 H, $N(CH_3)_2$], 2.88–2.93 (m, 2 H, $SCH_2CH_2N$), 3.42–3.52 (m, 2 H, $SCH_2CH_2N$), 3.68–3.77 (m, 2 H, $SCH_2CO$), 6.70 (d, $J$ = 9 Hz, 2 H), 7.32 (d, $J$ = 9 Hz, 2 H), 8.56 (s, 1 H), 10.04 (s, 1 H), 10.30 (s, 1 H), 10.68 (s, 1 H); MS, $m/e$ 469. Anal. Calcd for $C_{20}H_{31}N_5O_4S_2$: C 51.16; H, 6.66; N, 14.92; S, 13.63.

(45) Menninga, L.; Engberts, J. B. F. N. *J. Phys. Chem.* **1973**, *77*, 1271.
(46) Endo, T.; Takei, H.; Guro, K.; Ito, M. M. *J. Chem. Soc., Chem. Commun.* **1985**, 163 and references cited therein.
(47) Endo, T.; et al., unpublished results.
(48) (a) Endo, T.; Ito, M. M.; Yamada, Y.; Saito, H.; Miyazawa, K.; Nishio, M. *J. Chem. Soc., Chem. Commun.* **1983**, 1430. (b) Ito, M. M.; Kato, J.; Takagi, S.; Nakashiro, E.; Sato, T.; Yamada, Y.; Saito, H.; Namiki, T.; Takamura, I.; Wakatsuki, K.; Suzuki, T.; Endo, T. *J. Am. Chem. Soc.* **1988**, *110*, 5147.
(49) Additionally, two examples have recently been found in which the order of $r$ values for $R^1$ (or $R^2$) is the same as that of the strength of weak $R^1$–$R^2$ interactions for $R^1$ (or $R^2$), $R^1$ or $R^2$ being a Ph group: Endo, T.; Suzuki, T.; Nakajima, Y.; Sakiyama, Y.; Takami, K.; Kato, J. *Chem. Lett.* **1988**, 2037.
(50) Endo, T.; Oda, K.; Mukaiyama, T. *Chem. Lett.* **1974**, 443.
(51) Kato, J.; Ito, M. M.; Hagino, K.; Naitoh, N.; Misaka, M.; Fujishiro, E.; Endo, T. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 1419.
(52) Endo, T.; Tasai, H.; Ishigami, T. *Chem. Lett.* **1975**, 813.

Found: C, 51.07; H, 6.48; N, 14.71; S, 13.45.

**4ba$_i$** ($R^1$ = Ph and $R^2$ = *i*-$C_5H_{11}$): mp 153.5–154.5 °C ($CH_2Cl_2$); $^1H$ NMR $\delta$ 0.84 (d, $J$ = 6 Hz, 6 H), 1.35–1.57 (m, 3 H), 2.27 (t, $J$ = 7 Hz, 2 H, $COCH_2CH_2$), 2.82–2.94 (m, 2 H, $SCH_2CH_2N$), 3.44–3.52 (m, 2 H, $SCH_2CH_2N$), 3.71–3.80 (m, 2 H, $SCH_2CO$), 7.06–7.12 (m, 1 H), 7.29–7.36 (m, 2 H), 7.49–7.55 (m, 2 H), 8.57 (s, 1 H), 10.31 (s, 2 H), 10.82 (s, 1 H); MS, *m/e* 426. Anal. Calcd for $C_{18}H_{26}N_4O_4S_2$: C, 50.70; H, 6.15; N, 13.14; S, 15.01. Found: C, 50.89; H, 6.01; N, 12.85; S, 14.84.

**4c$_n$c** ($R^1$ = *n*-$C_5H_{11}$ and $R^2$ = cyclo-$C_5H_9$): mp 146.0–147.5 °C ($CH_2Cl_2$); $^1H$ NMR $\delta$ 0.86 (t, $J$ = 7 Hz, 3 H), 1.17–1.86 (m, 14 H), 2.71–2.90 (m, 3 H, COCH and $SCH_2CH_2N$), 3.12–3.18 (m, 2 H, $NCH_2CH_2CH_2$), 3.42–3.49 (m, 2 H, $SCH_2CH_2N$), 3.59–3.65 (m, 2 H, $SCH_2CO$), 8.18 (s, 1 H), 8.58 (s, 1 H), 10.31 (s, 1 H), 10.44 (s, 1 H). Exact mass for $^{12}C_{17}{}^1H_{30}{}^{14}N_4{}^{16}O_4{}^{32}S_2$: calcd, 418.1709; found, 418.1720.

**4c$_i$c** ($R^1$ = *i*-$C_5H_{11}$ and $R^2$ = cyclo-$C_5H_9$): mp 150.0–151.5 °C ($CH_2Cl_2$); $^1H$ NMR $\delta$ 0.87 (d, $J$ = 7 Hz, 6 H), 1.31–1.84 (m, 11 H), 2.70–2.90 (m, 3 H, COCH and $SCH_2CH_2N$), 3.14–3.21 (m, 2 H, $NCH_2CH_2CH$), 3.43–3.50 (m, 2 H, $SCH_2CH_2N$), 3.59–3.64 (m, 2 H, $SCH_2CO$), 8.17 (s, 1 H), 8.57 (s, 1 H), 10.30 (s, 1 H), 10.42 (s, 1 H); MS, *m/e* 418. Anal. Calcd for $C_{17}H_{30}N_4O_4S_2$: C, 48.77; H, 7.24; N, 13.39; S, 15.30. Found: C, 48.63; H, 7.23; N, 13.40; S, 15.35.

**Oxidation of a Pair of Thiols.** A mixture of **1** (0.50 mmol) and **2** (0.50 mmol) in 12.5 mL of a solvent was stirred vigorously under oxygen for 15 min in a well-stirred water bath which was thermostated to ±0.1 °C for 20–50 °C and to ±0.5 °C for 70 °C. To this mixture was added $Et_3N$ (0.05 mmol), and vigorous stirring was continued for the time required to complete the oxidation (the oxidation was performed at least twice under the same conditions). When the oxidation was completed, the reaction mixture was evaporated to dryness. The yields of **3** and **4** were determined by use of their absorption at 280 (system A) and 254 nm (systems B–D) after separation of disulfides **3–5** in the mixture by HPLC using $\mu$-Bondapak-CN (system A) and LiChrosorb CN (systems B–D) with *n*-hexane–*i*-PrOH [85:15 (system A), 95:5 (systems B and C), and 94:6 (system D)] as an eluent. The $r$ values given in Figures 1–5 and Tables I–V represent the mean values of two or more experiments and were reproducible within the errors shown therein. The errors in $r$ values are far smaller for systems $A_i$ and $A_n$ because of the larger molar extinction coefficients ($\epsilon$)[25] of the corresponding disulfides.

**Acknowledgment.** We express our sincere thanks to Professor Teruaki Mukaiyama for encouragement with this work. This work was partly supported by Grant-in-Aids for Scientific Research 61226012 and 62216019 from the Ministry of Education, Science and Culture, Japan. We thank Mr. Tsukasa Ishiodori for helpful suggestion, Mr. Takashi Suzuki for valuable assistance, and Mr. Akihiko Kusai, JEOL, for measuring high-resolution mass spectra.

# Kinetics and Mechanism of the Decomposition in Aqueous Solutions of 2-(Hydroxyamino)imidazoles

**Judy L. Bolton and Robert A. McClelland***

*Contribution from the Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1.* *Received February 7, 1989*

**Abstract:** A kinetic study is reported of the reaction in aqueous solution whereby 1-X-2-(hydroxyamino)imidazoles **2** are converted into 1-X-2-amino-4,5-dihydro-4,5-dihydroxyimidazolium ions, with substituents X = H (**2a**), $CH_3$ (**2b**), $CH_2CH_2Br$ (**2c**), $CH_2CHOHCH_2OCH_3$ (**2d**), $CH_2CONHCH_2CH_2OH$ (**2e**), and $CH_2CHOHCH_2NC_5H_{10}$ (**2f**). A mechanism is proposed with the neutral form of the imidazole as the kinetically active species, undergoing rate-limiting cleavage of the N–O bond with no catalysis ($OH^-$ as leaving group) and with catalysis by the hydronium ion and by buffer acids. These reactions produce a resonance-stabilized imidazolenitrenium ion **7**, which reacts with water and added nucleophiles leading to products. Observations consistent with the mechanism include the following: (i) The 1,3-dimethyl-2-(hydroxyamino)imidazolium ion, a model of protonated **2** that cannot be converted to the reactive neutral form, is unreactive. (ii) Under conditions where 98% of the products are due to reaction with the added nucleophile glutathione (GSH), there is no change in rate constant. (iii) The rate–pH profiles for **2b–2e** have regions at high pH and low pH where the rate constants are independent of pH, as required by the mechanism. (iv) The acidity constant for **2bH$^+$** obtained through kinetic analysis is the same as that obtained by NMR spectroscopy. (v) Effects of the N-1 substituents are consistent with the formation of an electron-deficient intermediate. (vi) Decreasing solvent polarity results in a decrease in the rate constant of the uncatalyzed N–O heterolysis, with an $m$ value of ~0.5. The proposed mechanism is a heterocyclic analog of the Bamberger rearrangement of *N*-phenylhydroxyamine to *p*-aminophenol. The 2-imidazole system is however more reactive, a feature shown to be predictable on the basis of the $\sigma^+$ value for this group. Through analogy with acetal hydrolysis, the production of a stabilized cationic intermediate is suggested to be responsible for the presence of general acid catalysis. The ratio $k_{GS}$:$k_w$ for reactions of the nitrenium ion **7b** with glutathione anion and water is $5 \times 10^5$ $M^{-1}$. This implies that $k_w$ must be less than $10^4$ $s^{-1}$, since the reaction with the thiol anion cannot occur faster than diffusion. A comparison with $k_w$ values for other nitrenium ions and carbenium ions shows that the imidazolenitrenium ion is an exceptionally long-lived species. Metabolic reduction of 2-nitroimidazole drugs is known to result in covalent binding to DNA as well as in depletion of intracellular glutathione; the possibility that the nitrenium ion is responsible is considered in the context of the results of this investigation.

Nitroimidazoles see use against a variety of anaerobic bacterial and protozoal infections[1] and are currently in clinical trials as radiation sensitizers of hypoxic (oxygen-deficient) tumor cells.[2] Biological investigations of 2-nitroimidazoles **1** (Scheme I), the derivatives more commonly employed in the latter studies, have demonstrated that there are a number of effects that appear to correlate with reductive metabolism, with the implication that some product or intermediate of reduction of the nitro group is the biologically active species.[3]

To evaluate the origins of these effects, we have been examining the chemistry of the 2-(hydroxyamino)imidazoles **2** which result from reduction with four-electron equivalents. Such species can

(1) Grunberg, E.; Titsworth, E. H. *Annu. Rev. Microbiol.* **1973**, *27*, 317–346.

(2) Wardman, P. *Curr. Top. Radiat. Res.* **1977**, *11*, 347–398.

(3) Rauth, A. M. *Int. J. Radiat. Oncol. Biol. Phys.* **1984**, *10*, 1293–1300.

0002-7863/89/1511-8172$01.50/0 