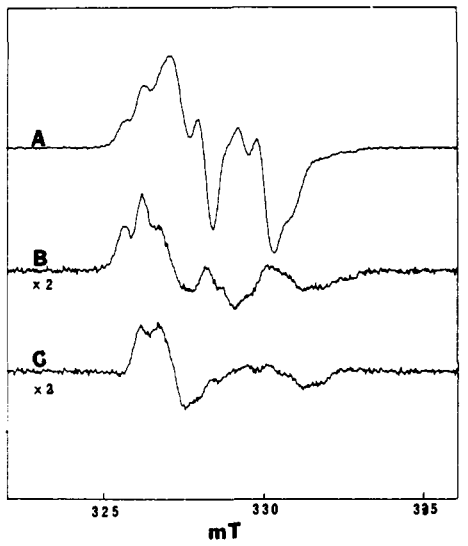
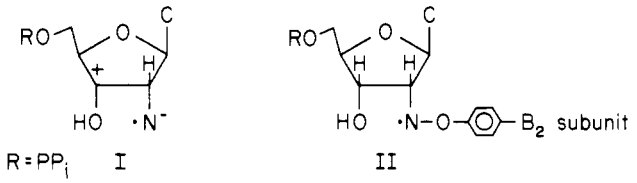


Figure 3. EPR spectra of RDPR with [2′-^{15}N]N_3UDP: (A) 7 min after the addition of [2′-^{15}N]N_3UDP, (B) after subtraction of the remnant tryosine radical from A, (C) after subtraction of the remaining $^{14}N_3$UDP signal (Figure 1C) from the spectrum in B. Spectrum conditions are as in Figure 1.

structure of this radical signal can be interpreted as arising from anisotropic interactions with an $I = 1$ nucleus (large triplet) and an $I = {}^1/_2$ nucleus (smaller doublets).

To test the hypothesis that this new radical is localized on RDPR generated structures such as I or II, specifically on the



nitrogen attached to the 2′-carbon of N_3CDP, we synthesized [2′-^{2}H]N_3UDP[7] and [2′-^{15}N]N_3UDP.[8] The results of the EPR experiments are indicated in Figures 2 and 3. The enzyme incubated for 7 min with [2′-^{2}H]N_3UDP has spectra (Figure 2A, B) identical with those of the corresponding protonated material (Figure 1B, C).

The inability to affect the hyperfine splitting by deuteration of the 2′-carbon strongly suggests that the radical is *not* attached to that carbon. On the other hand, the spectrum of the enzyme incubated with [2′-^{15}N]N_3UDP is consistent with the radical being localized on the nitrogen. Subtraction of the remnant tyrosyl radical spectrum from Figure 3A results in a spectrum (Figure 3B) composed of two radical signals having hyperfine interactions with either ^{15}N or ^{14}N.[8] Subtraction of the ^{14}N-coupled signal (e.g., the spectrum of Figure 1C) results in Figure 3C, which is consistent with ^{15}N hyperfine interactions.

These studies clearly indicate that the new radical species generated is located on a single nitrogen of the intermediate generated by RDPR action on substrate analogue N_3UDP. The hyperfine splitting is not caused by the 2′-H since replacement of the proton with deuterium has no effect on this splitting pattern. Deuterated N_3UDP with labels in the 1′- and 3′-position are being synthesized. Results from experiments with these compounds may then allow us to speculate on the structure of this new radical species and whether it is related to cleavage of the 3′-carbon–hydrogen bond.[3]

Note Added in Proof. [1′-^{2}H]N_3UDP and [3′-^{2}H]N_3UDP upon interaction with RDPR show EPR spectra identical with that in Figure 1C.

(7) Synthetic details are available upon request.
(8) In this synthesis the isotopic label is distributed equally between the nitrogens proximal and distal to the 2′-carbon.

Acknowledgment. We are indebted to Dr. H. Beinert for the use of his EPR facility and to R. Hansen for his technical assistance. This research was supported by Grants GM 29595 from the United States Public Health Service (J.S.) and GM 12394 (H.B.).

Neighboring Group Participation in Organic Redox Reactions. 9. Facilitation of a Disulfide Oxidative Cleavage Reaction

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We wish to report that one of the neighboring tertiary amine groups in bis(3-(dimethylamino)propyl) disulfide (**1**) accelerates the rate of oxidative cleavage of the disulfide by aqueous I_2 by a factor of $\sim 10^6$ over that found with cystine.[1] In addition to the tremendous acceleration, the kinetics of the oxidative cleavage of **1** differ greatly from the kinetics of the aqueous iodine oxidation of cystine[1] and of the bis quaternary ammonium salt $[(CH_3)_3N(CH_2)_3S]_2{\cdot}2I^-$ (**2**) and indicate that intramolecular nucleophilic assistance is concomitant with electrophilic cleavage of the disulfide bond.[2a] The formation and cleavage of the disulfide bond is important in many areas of chemistry[2a,b] and biochemistry.[2c,d] Although unusually facile reactions of disulfides with electrophiles may be due to neighboring group participation,[3] systematic studies of the effects of neighboring groups are few in number. When the kinetics of the electrophilic cleavage of several amino acid disulfides by Ag(I) and by Hg(II)[4] were reported, the differences in reactivity were discussed in terms of electrostatic effects and hydrogen bonding.

The rapid reaction of **1**[5] with aqueous I_2 at pH 4–9 yields the sulfonic and sulfinic acids.[6] The procedures used to study the spectrophotometric rates have been described previously.[8] The pseudo-first-order decay of triiodide was monitored over two half-lives, with coefficients of correlation of 0.996–0.999. The data for the I_2 oxidation at pH $\geq$7 were obtained using a stopped-flow spectrophotometer.[8c,9] In Table I the change in rate

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(5) The bis disulfide, **1**, bp 107 °C (10 torr) (Anal. ($C_{10}H_{24}N_2S_2$) C, H, N) was prepared by H_2O_2 oxidation of the thiol **3**[4a] and was derivatized as its bis quaternary salt, $[(CH_3)_3N(CH_2)_3S]_2{\cdot}2I^-$ (**2**) (Anal. ($C_{12}H_{30}N_2S_2I_2$) C, H, N).
(6) The NMR of the product indicated that approximately equimolar quantities of the two acids were formed. The sulfonic acid had been characterized earlier.[7a] The second component (^{1}H NMR δ 3.05 (m, 2), 2.8 (s, 6), 2.45 (m, 2), 2.05 (m, 2); IR (KBr pellet) 1130 (s, br), 950 (s, br) cm^{-1}) has the spectral characteristics of the sulfinic acid[7b] and could be converted to the sulfonic acid with dilute H_2O_2 in less that 1.5 h at room temperature.
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Table I. Rate Constants of Aqueous Iodine Reactions of 1[a]

run	10^4[1]	pH	[KI]	[KCl]	k_{obsd}, s^{-1}
1	5.17	5.85[b]	0.80	0.20	0.0077
2	5.17	5.85	0.60	0.40	0.0113
3	5.17	5.85	0.40	0.60	0.0150
4	5.17	5.85	0.20	0.80	0.0352
5	5.17	5.85	0.10	0.90	0.0867
6	7.71	9.0[c]	0.80	0.20	11.8
7	7.71	9.0	0.40	0.60	29.5
8	7.71	9.0	0.20	0.80	56.6
9	7.71	9.0	0.10	0.90	149
10	5.17	5.66[b]	0.20	0.80	0.0237
11	3.45	5.66	0.20	0.80	0.0156
12	1.72	5.66	0.20	0.80	0.0090

[a] 26.0 °C, all concentrations in molarity, $[I_3^-]_0 = (3\text{-}9) \times 10^{-5}$ M. [b] 0.05 M phosphate. [c] 0.05 M borate.

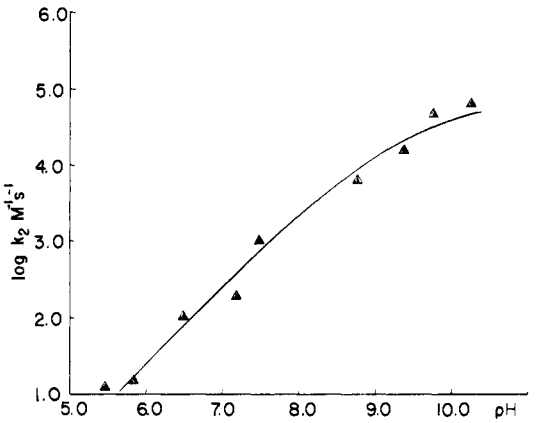


Figure 1. The pH dependence of the I_2 reaction of **1** when [**1**] = (5-8) × 10^{-4} M, [KI] = 0.20 M, [KCl] = 0.80 M, buffer concentrations = 0.0125-0.050 M; the solid curve has been drawn from the equation $k_{obsd} \alpha K_a/(K_a + [H^+])$ where $pK_a = 9.5$.

constants with varying iodide concentrations are listed in runs 1-5 and 6-9. Linear regression of the log k_{obsd} vs. log [I^-] had slopes of -1.15 (pH 5.85) and -1.19 (pH 9.0), both with correlation coefficients of 0.997. Also in Table I (runs 10-12) are the effects of changes in the concentration of **1** on the rate constants. Linear regression of the log k_{obsd} vs. log [**1**] gave a slope of 0.86 and a correlation coefficient of 0.998. Thus, at a given pH, the rate law for the reaction of **1** with I_3^- is $d[I_3^-]/dt = -k'_{obsd}[\mathbf{1}][I_3^-][I^-]^{-1}$. In Figure 1 are plotted the values of log (k'_{obsd}/[**1**]) vs. pH for the reaction of **1**. The solid line was calculated by using $k_{obsd} \alpha K_a/(K_a + [H^+])$ where $pK_a = 9.5$. These data indicate that an unprotonated amine is the nucleophile in the reaction. The overall rate law is $d[I_3^-]/dt = -k'(K_a/(K_a + [H^+]))[\mathbf{1}][I_3^-][I^-]^{-1}$, and in a given run, $k_{obsd} = k'(K_a/(K_a + [H^+])[\mathbf{1}][I^-]^{-1}$ M s^{-1}. By use of $K_{I_3^-}$ for the equilibrium constant for triiodide formation,

$$d[I_3^-]/dt = -k'(K_a/(K_a + [H^+]))[\mathbf{1}][I_2]K_{I_3^-}$$

The rate law for **1** is consistent with the mechanism shown in eq 1-3 where R = $(CH_3)_2N(CH_2)_3$-, the unprotonated amine.

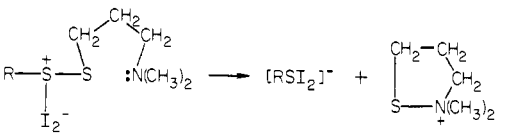
$$I_3^- \rightleftharpoons I_2 + I^- \quad (1)$$

$$I_2 + RSSR \rightleftharpoons RSSR \cdot I_2 \quad (2)$$

$$\underset{\mathbf{3}}{RSSR \cdot I_2} \xrightarrow{slow} [RSI_2]^- + \underset{\mathbf{4}}{[RS]^+} \quad (3)$$

(9) Because **1** reacts with I_2 at a rate unprecedented for a disulfide it was necessary to ensure that the rapid reaction was not due to thiol impurities, which were not detectable by NMR or GC. By use of conditions of run 4, Table I, where $[I_3^-] = 4.7 \times 10^{-5}$ M, the first-order reaction (coefficient of correlation ≥0.999) of I^{3-} goes to completion and has a half-life of 16.5 s. Under the same conditions, but by using solutions 5.27×10^{-4} or 5.27×10^{-5} M in the thiol, the reaction has a half-life of less than 1 s. When more dilute solutions of thiol were used, a slow, incomplete decay of triiodide was observed and the rate of decay was not first order. Thus, a thiol contaminant could not be responsible for the observed reaction in solutions of **1** under the above conditions.

Attack of iodine on unprotonated **1** to give **3** is followed by a rate-determining cleavage of the S-S bond by an intramolecular amine group yielding an N-alkylated sulfenamide **4**. The geometry of the cleavage reaction is suggested by the theoretical model that has been invoked for concomitant electrophilic nucleophilic disulfide cleavage.[10] In this model the electrophile, I_2, attacks in a direction 90° from the S-S bond axis while the nucleophile comes in along an extension of the S-S bond axis.



3 **4**

Surprisingly, iodine reacts 30 times faster with I than with methionine, a reactive acyclic thioether.[11] Compound **1** reacts ~10^6 times faster than its bis quaternary ammonium salt **2**, which is a water-soluble disulfide lacking a neighboring nucleophilic group. The rate constant for the iodine oxidation of **2** is 1×10^{-4} s^{-1} when [**2**] = 5.58×10^{-4} M and [I^-] = 0.10 M at pH 9.2 *or* 8.2 and increases to 2×10^{-4} s^{-1} with a 4-fold decrease in iodide. The insensitivity of the rate constants for **2** to changes in pH or [I^-] also characterized the kinetics of the iodine oxidation of cystine. The slow reaction of **2** with iodine yields the sulfonic acid. The unusual reactivity of **1** has led us to initiate a systematic examination of the effect of other neighboring groups and of other electrophiles on the cleavage of disulfides.

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Experimental and Theoretical Studies on Diamagnetic Susceptibility of Amides and Their N-Substituted Derivatives

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Recently, some interesting findings regarding diamagnetic behavior of the C=O group have been reported[1] in aldehydes, ketones, acids, esters, and acyl chlorides. And since, except for some fragmental reports, no systematic studies appear to have been made on the amides, it is therefore, considered interesting to carry out experimental and theoretical studies on amides and their N-substituted derivatives in which C=O group is considerably affected by different existing structural environments.

The magnetic susceptibilities of amides have been measured by the Gouy method.

In order to analyze different existing structural environments and to establish a correlation of χ_M with the structural factors, χ_Ms of these molecules have been calculated theoretically. The Pascal, Pacault, and Hoarau method,[2] based on atomic suscep-

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