Model Systems Related to Reactivity of Protein Sulfur Functions. II. The Effect of Hydrophobic Bulk on the Nucleophilicities of Alkyl-Substituted Benzenethiolate Anions toward Disulfide Bonds[1,2]

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The rates of cleavage of the sulfur–sulfur bond of ethyl 2,4-dinitrophenyl disulfide by a series of alkyl-substituted benzenethiolate anions in 95% ethanol at 25° are reported. Nucleophilic attack of ortho-substituted anions at sulfur is influenced by two steric effects: (1) rate acceleration due to inhibition of solvation of the reactant thiolate ion and (2) rate retardation due to steric repulsion between thiolate nucleophile and disulfide substrate. The first known example of net steric acceleration in cleavage of a disulfide bond due to hydrophobic bulk in the nucleophile is reported for *o-tert*-butylbenzenethiolate which is found to be five times as reactive as *p-tert*-butylbenzenethiolate. The effects of hydrophobic bulk in enzymic reactions involving mercaptide cleavage of disulfide bonds are discussed. The rate of sulfur–sulfur bond cleavage by benzenethiolate and *o-tert*-butylbenzenethiolate in reaction with ethyl-, 2-hydroxyethyl-, and 2-methoxyethyl-2′,4′-dinitrophenyl disulfide in 95% ethanol and in xylene at 25° are also reported. In 95% ethanol the order of reactivities for both thiolate anions parallels the inductive effects of H, HO, and CH_3O groups. However, in xylene the order of rates for the 2-hydroxyethyl and the 2-methoxyethyl substrates is reversed. Two explanations of this result are considered: one steric (conformational freezing) and the other electronic (concomitant electrophilic catalysis by hydroxyl of the nucleophilic disulfide cleavage).

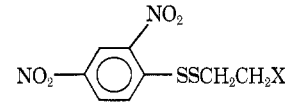
The factors which govern the reactivity of thiols toward disulfides have significance for several areas of biochemistry.[4,5] Among the most important are the use of the disulfide, 5,5′-dithiobis(2-nitrobenzoic acid),[6] as a probing reagent for protein sulfhydryl groups; thiol–disulfide exchanges between small molecule mercaptans and protein disulfide linkages which occur biochemically and are employed for chemical reduction of protein disulfide bonds;[7] intermolecular or intramolecular thiol–disulfide exchange between or within protein molecules such as albumins;[8-10] and reactions of protein thiol functions with disulfide linkages of substrates or cofactors such as proposed for the action of glutathione reductase[11] and lipoyl dehydrogenase.[12]

Although steric hindrance in the vicinity of the nucleophile[4,5] and positioning of catalytically crucial neighboring groups (*e.g.*, ficin[13] and streptococcal proteinase[14]) have been invoked to explain differential reactivities of protein thiol functions, investigations of these factors as they apply to mercaptide–disulfide displacements in model systems are almost entirely lacking.

Fava did observe severe rate retardation ($\sim 10^5$) for the exchange in aqueous solution of [^{35}S]sulfite ion with *tert*-butyl thiosulfate relative to ethyl thiosulfate[15] which is attributed to steric hindrance originating from the thiosulfate substrate. The even larger rate diminution ($\sim 10^6$) reported for the exchange of *tert*-butyl mercaptide ion with *tert*-butyl [^{35}S]disulfide in aqueous alcoholic solvents compared to the analogous *n*-butyl system[16] is probably predominantly due to steric hindrance arising from the disulfide substrate which obscures contributions from hydrophobic bulk in the mercaptide nucleophile.

Chan[17] has suggested participation by a protonated amino group in the cleavage by sulfite ion of postulated intermediate mixed disulfides of cysteine or β-aminoethyl mercaptan with proteinyl sulfur. McPhee[18] reported that introduction of a positive charge into a disulfide molecule greatly increased the rate of sulfitolysis.

We now report kinetic studies of the reactions in 95% ethanol of ethyl 2,4-dinitrophenyl disulfide (**1**) in



1, X = H
2, X = OH
3, X = OCH_3

buffered 95% ethanol with a series of alkyl-substituted benzenethiolate (ArS^-) anions selected for the purpose of assessing the influence of *hydrophobic bulk in the nucleophile*.

Similar measurements were made for the reaction of *each* of ethyl 2,4-dinitrophenyl disulfide (**1**), 2-hydroxyethyl 2′,4′-dinitrophenyl disulfide (**2**), and 2-methoxy-

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ethyl 2′,4′-dinitrophenyl disulfide (**3**) with benzenethiolate and 2-*tert*-butylbenzenethiolate both in buffered (acetic acid–potassium acetate) 95% ethanol and in xylene containing the base 1,4-diazo[2.2.2]bicyclooctane (DABCO) in an effort to establish the occurrence of concomitant *intramolecular* electrophilic catalysis of the nucleophilic displacement. With the possible exception of the work of Chan[17] and McPhee[18] cited above, all previously reported examples of simultaneous electrophilic and nucleophilic catalysis of sulfur–sulfur bond scission have been *intermolecular* in nature, nucleophile, electrophile, and substrate each being initially separate molecular entities.[19]

Results

The mechanism of the cleavage of disulfides by thiolate in a hydroxylic solvent SOH is depicted by eq 1 and 2.[16,20-23] The analysis of the kinetic data based upon this mechanism is described in the Experimental Section. Other reactions do not complicate the kinetic

$$\mathrm{ArSH} + \mathrm{SOH} \underset{\text{fast}}{\overset{K_a,\ \text{fast}}{\rightleftharpoons}} \mathrm{ArS^-} + \mathrm{SOH_2^+} \qquad (1)$$

$$\mathrm{ArS^-} + \mathrm{NO_2C_6H_3(NO_2)SSCH_2CH_2X} \underset{k_b}{\overset{k_{anion},\ \text{slow}}{\rightleftharpoons}} \mathrm{NO_2C_6H_3(NO_2)S^-} + \mathrm{ArSSCH_2CH_2X} \qquad (2)$$

analysis. Subsequent reaction of the product disulfide formed according to eq 2 with arenethiolate (eq 3) is

$$\mathrm{ArS^-} + \mathrm{ArSSCH_2CH_2X} \longrightarrow \mathrm{ArSSAr} + {}^-\mathrm{SCH_2CH_2X} \qquad (3)$$

expected to be negligibly slow since in all reactions studied $^-SCH_2CH_2X$ is a poor leaving group relative to arenethiolate.[20b,24,25] Indeed, analysis of reaction products showed the absence of symmetrical disulfide ArSSAr.

The observed rate constants for the reactions of alkyl-substituted benzenethiols (ArSH) with ethyl 2,4-dinitrophenyl disulfide (**1**) are summarized in Table I. In all cases, the fit of the data to the integrated expression for $k_{2,obsd}$ (integral of eq 15) is good with a standard deviation of points from the line of about 1% or less of the $k_{2,obsd}$ values. The rate constants for reaction of thiolate (ArS^-) with **1** (eq 2), $k_{2,anion}$, calculated from $k_{2,obsd}$ using eq 16, are also reported in Table I. The standard deviations for runs with a given alkylbenzenethiolate are from 0.5–5.6% of the average $k_{2,anion}$ values.

The $k_{2,anion}$ values for all ArS^- species are comparable within a factor of two except for 2-*tert*-butylbenzenethiolate anion for which the $k_{2,anion}$ value is 8.5 times that for unsubstituted benzenethiolate. Since the rate-determining step (eq 2) involves nucleophilic attack of thiolate upon one of the sulfur atoms of the disulfide bond, the differences in $k_{2,anion}$ reflect the substituent effects upon the nucleophile.

The kinetic data for the reactions of benzenethiol and of 2-*tert*-butylbenzenethiol with 2-hydroxyethyl 2′,4′-dinitrophenyl disulfide (**2**) and with 2-methoxyethyl 2′,4′-dinitrophenyl disulfide (**3**) are reported in Table II for the reaction in 95% ethanol and in Table III for the reaction (in the presence of DABCO) in xylene. Since acid strengths were not available for the xylene medium, the observed rate constants for the molecular thiol species are reported. Thus, comparisons are made of observed k values for a *given thiol* in a given solvent with each of the various disulfide molecules. Whereas in 95% ethanol the rates for the methoxy compound **3** are 1.3–1.5 times as great as for hydroxy compound **2**, in xylene the superiority is reversed with the rates for **2** found to be 2 to 3 times as fast as for **3**.

Discussion

Alkyl Substitution of the Nucleophile.—An objective of the present investigation is to examine the effects of steric bulk introduced into the *nucleophile* upon the reaction of benzenethiolate anions with aryl alkyl disulfides. Studies of the effects of alkyl bulk in *charged* nucleophiles have not been reported previously. Rate retardation is an anticipated result since nonbonding repulsions will occur between a hindered nucleophile and the substrate in the transition state for sulfur–sulfur bond cleavage (eq 2). However, steric bulk in the anion will also inhibit solvent stabilization of the reactant thiolate, thereby increasing the rate of thiolate attack on sulfur. The current work was designed to examine the relative importance of these opposite effects of alkyl substituents.

To distinguish between the different electronic and steric substituent effects, a Brønsted correlation between rates of thiolate nucleophilic attack and thiolate basicity (or equivalently, thiol acidity) for the unhindered benzenethiols is employed here. If structural variation of the thiolate anion affects its nucleophilicity and its basicity comparably, the Brønsted plot produces a straight line whose slope (the Brønsted parameter β) measures the sensitivity of the disulfide cleavage reaction to the thiolate nucleophilicity. A Brønsted plot (Figure 1) of log $k_{2,anion}$ *vs.* pK_a for four unhindered benzenethiols of Table I yields a β value of 0.66 (standard deviation 0.03, correlation coefficient 0.99937).[26] Although the range of pK_a values used to determine this estimate of β is quite narrow, the value obtained is comparable to previously reported β values of 0.53 for the reaction of a series of aliphatic thiols with thiamine *n*-propyl disulfide[20c] and 0.73 for similar aliphatic thiols in reaction with cystine.[27] The qualitative conclusions of the following discussion hold even in the unlikely event that the β of this work is inaccurate by ± 0.15.

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TABLE I
SECOND-ORDER RATE CONSTANTS FOR REACTIONS OF ALKYL-SUBSTITUTED BENZENETHIOLS WITH ETHYL 2,4-DINITROPHENYL DISULFIDE IN 95% ETHANOL

ArSH (Registry no.)	$(ArSH)_0 \times 10^4$ (M)	% reaction completion on which k is based	k_{obsd}[a-c] (M^{-1} sec^{-1})	$k_{2,anion}$[a,c] $\times 10^{-4}$ (M^{-1} sec^{-1})
PhSH	2.986	93	152.9	7.08
(108-98-5)	1.493	88	151.1	7.00
	0.9953	75	152.3	7.07
				Av[d] 7.05 ± 0.03
4-MePhSH	1.071	67	115.2	12.65
(106-45-6)	0.5355	56	108.1	11.87
				Av[d] 12.26 ± 0.39
4-*tert*-BuPhSH	2.620	87	117.2	11.53
(2396-68-1)	2.059	86	121.7	11.98
	1.310	82	115.7	11.39
				Av[d] 11.63 ± 0.25
3-MePhSH	1.527	75	129.6	10.01
(108-40-7)	1.018	62	127.0	9.82
	0.7634	60	126.0	9.74
				Av[d] 9.86 ± 0.41
2-MePhSH	3.065	83	53.7	11.04
(137-06-4)	1.532	69	47.3	9.71
	1.226	69	48.6	9.98
				Av[d] 10.24 ± 0.5
2,6-DiMePhSH	15.82	87	12.8	9.25
(118-72-9)	7.909	85	13.0	9.40
	5.273	84	13.3	9.63
				Av[d] 9.43 ± 0.16
2-*tert*-BuPhSH	40.08	99	11.4	61.3
(19728-41-7)	20.04	95	11.0	59.2
	10.02	90	11.1	59.7
				Av[d] 60.1 ± 0.90
2,4,6-Tri-*tert*-BuPhSH	46.76	24	0.134[e,f]	12.2[e,g]
(961-39-7)	35.52	16	0.139[e,f]	12.7[e,g]
				Av[d] 12.4 ± 0.4

[a] Temperature, 25.0 ± 0.2°. [b] pH 6.91 measured as described in Experimental Section. [c] All initial disulfide concentrations were 4.57×10^{-5} M unless otherwise specified. [d] Average ± standard deviation. [e] Initial disulfide concentration was 6.11×10^{-5} M. [f] Back-reaction not taken into account. [g] Based on value for pK_a of 12.87 estimated as described in Experimental Section.

TABLE II
SECOND-ORDER RATE CONSTANTS FOR REACTIONS OF ARSH WITH 2-SUBSTITUTED ETHYL 2′,4′-DINITROPHENYL DISULFIDES IN 95% ETHANOL

ArSH	Disulfide	$(ArSH)_0 \times 10^5$ (M)	% reaction completion on which k is based	$k_{2,obsd}$[a-c] (M^{-1} sec^{-1})
PhSH	2	16.06	94	343
		8.45	79	323
		5.35	68	334
				Av[d] 333 ± 10
PhSH	3	16.90	95	435
		8.45	85	427
		5.63	74	418
				Av[d] 427 ± 9
2-*tert*-BuPhSH	2	10.02	63	25.2
		5.01	75	27.8
				Av[d] 26.5 ± 1.8
2-*tert*-BuPhSH	3	10.02	64	39.6
		5.01	62	38.9
				Av[d] 39.2 ± 0.5

[a] Temperature, 25.0 ± 0.2°. [b] pH 6.75 measured as described in Experimental Section. [c] All initial disulfide concentrations were 4.58×10^{-5} M. [d] Average ± standard deviation.

The free energy of activation for thiolate cleavage of a disulfide bond may be separated into three major components as in eq 4: (1) the free-energy change resulting from the adjustment of solvation on going from reactants to the transition state, (2) the free-energy change due to changes in electronic interactions and bond reorganization, and (3) the free-energy change due to differences in nonbonding repulsions upon going from separated reactants to the transition state.

$$\Delta F^{\ddagger} = \Delta F^{\ddagger}_{solvation} + \Delta F^{\ddagger}_{electronic} + \Delta F^{\ddagger}_{steric} \quad (4)$$

The effect of substitution on $\Delta F^{\ddagger}$, $\delta_R \Delta F^{\ddagger}$, is given by the difference in substituent effects on the transition state (TS) and upon the ground state (GS) as shown in eq 5. If only the thiolate substituent is varied while

$$\delta_R \Delta F^{\ddagger} = \delta_R F^{TS} - \delta_R F^{GS} \quad (5)$$

the disulfide substrate remains the same, $\delta_R F^{GS}$ becomes $\delta_R F^{anion}$.

In the first paper of this series[1b] it was shown that, for the ortho-substituted benzenethiols of interest, hindered thiols were less acidic than related unhindered thiols owing to steric inhibition of solvation of ortho-substituted thiolate anions. It was argued that, to a good approximation, nonbonding repulsions within the thiolate anion itself could be neglected. Under this condition eq 6 holds approximately. Since substituent

$$\delta_R F^{anion} \approx \delta_R F^{anion}_{solvation} + \delta_R F^{anion}_{electronic} \quad (6)$$

effects on the free energy of the *parent* (un-ionized) alkylbenzenethiol are expected to be small compared to the effects on the thiolate anion, the relative pK_a's of

TABLE III

THIRD-ORDER RATE CONSTANTS FOR REACTIONS OF BENZENETHIOLS WITH 2-SUBSTITUTED ETHYL 2′,4′-DINITROPHENYL DISULFIDE IN XYLENE

ArSH	Disulfide	$(ArSH)_0 \times 10^4$ (M)	$(DABCO)_0 \times 10^3$ (M)	% reaction completion on which k is based	$k_{3,obsd}$[a,b] (M^{-2} sec^{-1})
PhSH	1	15.96	7.40	35	530
		7.98	3.70	16	518
					Av[c] 524 ± 8
PhSH	2	15.96	7.40	70	3073
		7.98	3.70	49	2946
		7.98	3.70	42	3093
					Av[c] 3044 ± 80
PhSH	3	15.96	7.40	42	1019
		7.98	3.70	23	1003
					Av[c] 1011 ± 11
2-*tert*-BuPhSH	1	50.91	120.1	64	87.8
		25.51	57.1	57	80.9
		25.45	60.0	41	74.4
					Av[c] 81.0 ± 6.7
2-*tert*-BuPhSH	2	25.45	60.0	62	245.1
		12.75	57.1	58	255.0
					Av[c] 250.0 ± 7.0
2-*tert*-BuPhSH	3	25.51	115.9	65	120.8
		25.45	60.0	57	123.8
		12.75	57.9	48	105.5
					Av[c] 116.7 ± 9.3

[a] Temperature, 25.0 ± 0.2°. [b] All initial disulfide concentrations were 4.58×10^{-5} M. [c] Average ± standard deviation.

the substituted benzenethiols provide reasonable relative measures of $\delta_R F^{anion}$. Therefore, $\delta_R F^{GS}$ (eq 5) will be approximately linearly related to the pK_a of the thiol. Any significant deviations of log $k_{2,anion}$ from linearity with pK_a for hindered benzenethiolates in a Brønsted plot may consequently be ascribed to substituent effects on the transition state, *i.e.*, on $\delta_R F^{TS}$. Similar interpretations have been reported for the addition of alkylbenzenethiols to *N*-ethylmaleimide.[2b]

The rate-determining step (eq 2) involves formation of a new bond between the thiolate sulfur and one of the disulfide sulfur atoms. Consequently, for hindered (ortho substituted) benzenethiolates the alkyl bulk proximate to thiolate sulfur is expected to cause deviations from the extrapolated linear Brønsted plot of Figure 1 owing principally to steric repulsions between the nucleophile and the substrate (*i.e.*, $\delta_R F^{TS}_{steric}$). Inductive effects may partially cancel between GS and TS and, in any case, should parallel pK_a rather than contribute to deviations from linearity. Therefore, such deviations will be interpreted as measures of steric repulsion.

The results for reaction of *o*-methyl-substituted benzenethiolates with **1** as plotted in Figure 1 do suggest a small rate retardation of a factor of 1.8 due to steric repulsion between the nucleophile and the disulfide. Consistent with this interpretation, the retardation is more pronounced for two *o*-methyl groups which give a rate retardation from the predicted rate by a factor of ~8.

In the case of *o*-*tert*-butyl substitution, the predominance of the effect in which steric hindrance to solvation of the thiolate anion increases its nucleophilicity leads to a marked acceleration of the rate of cleavage by a factor of 5 relative to the para isomer (Table I). This is the first reported example of rate acceleration in a nucleophilic substitution attributable to decreased solvation resulting from steric bulk in the

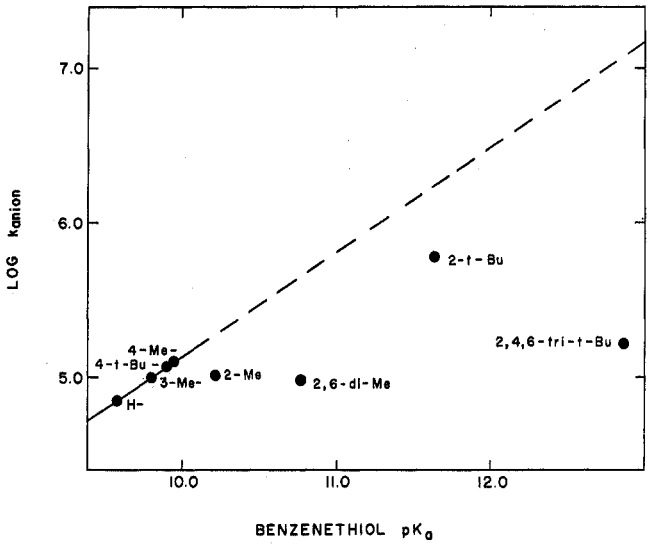


Figure 1.—Brønsted plot for reaction of alkyl-substituted benzenethiolate with ethyl 2,4-dinitrophenyl disulfide.

nucleophile. The rate increment predicted solely for this effect is (from Figure 1) a factor of 15 over the rate for 4-*tert*-butylbenzenethiolate, but this increment is reduced to the observed relative rate as a consequence of a decelerating factor of ~3 apparently caused by steric crowding in the transition state. Furthermore, increased steric repulsion between reactants which results upon introduction of a second *o*-*tert*-butyl group reduces the net rate to a value comparable to that of the methyl-substituted benzenethiols (compare $k_{2,anion}$ for 2,4,6-tri-*tert*-butylbenzenethiolate, Table I). Again, the Brønsted plot of Figure 1 provides an estimate of the contribution of steric repulsion between the nucleophile and the substrate to the net rate. The steric retardation factor of 3 for 2-*tert*-butylbenzenethiol compares with the corresponding factors of 1.8 and 8

for 2-methyl- and 2,6-dimethylbenzenethiolates, respectively. The two *o-tert*-butyl groups of 2,4,6-tri-*tert*-butylbenzenethiolate cause a rate retardation below that predicted from basicity alone by a factor of 72. In this case, severe steric repulsion between reactants in the transition state outweighs the increased thiolate nucleophilicity owing to anion desolvation.

The above results have clear implications for enzymic reactions between mercaptide groups and disulfide linkages. The nucleophilicity of the mercaptide function can be increased to a point by adjacent hydrophobic side chains. However, too severe crowding of the mercaptide will interfere with attack on the disulfide bond. An optimum degree of hydrophobic shielding can produce enhanced rates. These results further support the suggestions[4,5] that differences in hydrophobic environments explain the differential reactivities of protein thiol functions.

Electrophilic Catalysis of Nucleophilic Cleavage of the Disulfide Bond.—The rates of sulfur–sulfur bond cleavage in 2-hydroxyethyl 2′,4′-dinitrophenyl disulfide (**2**) and related ethyl (**1**) and 2-methoxyethyl (**3**) disulfides by thiolate nucleophiles in the dissociating solvent 95% ethanol (Table II) and in the nondissociating solvent *m*-xylene (Table III) were studied to ascertain the effects of an intramolecular hydroxyl group.

In 95% ethanol, the methoxyethyl disulfide **3** is slightly more reactive than the corresponding hydroxyethyl compound **2** with both unhindered (benzene-) and hindered (2-*tert*-butylbenzene-) thiolate nucleophiles by factors of 1.3 and 1.5, respectively (Table IV).

TABLE IV

COMPARISON OF SUBSTITUENT EFFECTS FOR REACTION OF 2-SUBSTITUTED ETHYL 2′,4′-DINITROPHENYL DISULFIDE WITH BENZENETHIOLS IN 95% ETHANOL

			k_{obsd}	
Compd	Ethyl substitutent	σ_I[a]	For PhSH	For 2-*tert*-BuPhSH
1	H	0.00	152	11.2
2	HO	0.05	333	26.5
3	CH_3O	0.07	427	39.5

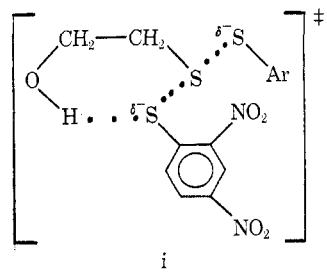
[a] Inductive σ constant for XCH_2 [M. Charton, *J. Org. Chem.*, **29**, 1222 (1964)].

These small differences in rates are due primarily to inductive effects of the oxyalkyl function as shown by the fact that the observed rates for ethyl, 2-hydroxyethyl-, and 2-methoxyethyl systems in this solvent are in the same order as the inductive substituent constants σ_I of CH_3, $HOCH_2$, and CH_3OCH_2 groups.

Indications of intramolecular hydrogen bonding to the leaving group were absent, as was expected, since in a hydrogen-bonding solvent such as 95% ethanol, the substrate intramolecular hydroxyl is solvated and cannot compete with solvation of the leaving group by solvent.

In the aprotic solvent xylene, the reversal of the rates for the oxy compounds was observed. Table III shows that **3** is *slower* than **2** by factors of 3 and 2 for benzenethiol and 2-*tert*-butylbenzenethiol, respectively. Both disulfides **2** and **3** are still cleaved faster than the parent disulfide **1**.

The greater reactivity of the 2-hydroxy compound was expected in the aprotic solvent since this system permits formation of a six-membered-ring transition



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state (i) in which intramolecular hydrogen bonding of the leaving group by the hydroxyl hydrogen can occur. The polar character of the hydrogen can assist the cleavage by electrophilic catalysis and/or the hydrogen bonding can hold the substrate in a favorable conformation for attack by the nucleophilic species.[28] Clearly these rate enhancing effects are precluded in the methoxy substrate **3** since the hydrogen of **2** has been replaced by a methyl group.

Thus, although the electrophilic catalysis interpretation of the results in xylene is not uniquely required by the data, the results are in accord with such an explanation. Except for similar participation posited during a step of sulfonation of protein SH groups,[17] these results provide the first specific experimental support of *the possibility* of concomitant electrophilic and nucleophilic catalysis of disulfide bond cleavage in models for biological systems.[19] Although the rate factor attributable to intramolecular electrophilic catalysis is small for the 2-hydroxyethyl system (a factor of ~3 or 4 when corrected for inductive effects), more favorable or precise positioning in enzymic systems of an electrophilic group (including the possibility of NH_3^+ as well as OH) with respect to the disulfide bond being cleaved may contribute a marked rate enhancement.

Experimental Section

Materials.—The arylthiols used were obtained, purified, and characterized as previously described[1b] except for 2,4,6-tri-*tert*-butylbenzenethiol which was the generous gift of Dr. Wolfgang Rundel. Commercial 1,4-diazo[2.2.2]bicyclooctane (DABCO) was purified by sublimation. Solvent *m*-xylene was reagent grade and was transparent in the region of spectral measurements.

Buffer.—Acetic acid–potassium acetate buffer in 95% ethanol was prepared as previously described.[1b]

Ethyl 2,4-Dinitrophenyl Disulfide (**1**).—Ethyl 2,4-dinitrophenyl disulfide was prepared in quantitative yield from the reaction of ethanethiol with 2,4-dinitrobenzenesulfenyl chloride in glacial acetic acid by the method of Parker and Kharasch.[25] Recrystallization of the product once from acetic acid, then twice from absolute ethanol, gave yellow needles, mp 86.0–86.5°. *Anal.* Calcd for $C_8H_8N_2O_4S_2$: C, 36.93; H, 3.10. Found: C, 36.97; H, 3.00.

3-Hydroxyethyl 2′,4′-Dinitrophenyl Disulfide (**2**).—The compound 2-hydroxyethyl 2′,4′-dinitrophenyl disulfide was prepared in quantitative yield from 2-hydroxyethanethiol and 2,4-dinitrophenylsulfenyl chloride as previously described[25] except that carbon tetrachloride was used instead of acetic acid solvent to prevent acetylation. Recrystallization four times from aqueous methanol gave yellow needles, mp 108.1–108.6°. *Anal.* Calcd for $C_8H_8N_2O_5S_2$: C, 34.77; H, 2.91. Found: C, 34.81; H, 2.85.

2-Methoxyethyl 2′,4′-Dinitrophenyl Disulfide (**3**).—The compound 2-methoxyethyl 2′,4′-dinitrophenyl disulfide was prepared in quantitative yield from 2-methoxyethanethiol and 2,4-dinitrobenzenesulfenyl chloride by the method described for **2**

(28) For the reaction in the solvent xylene of low dielectric constant (2.4), the nucleophilic species is the ammonium–thiolate ion pair rather than a dissociated thiolate ion. See B. Dmuchovsky, B. D. Vineyard, and B. Zienty, *J. Amer. Chem. Soc.*, **86**, 2874 (1964).

above. Recrystallization three times from absolute ethanol gave yellow needles, mp 78.4–79.0°. *Anal.* Calcd for $C_9H_{10}N_2O_5S_2$: C, 37.24; H, 3.47. Found: C, 37.38; H, 3.48.

2-Methoxyethanethiol.—2-Methoxyethanethiol was prepared in 70% yield by alkaline hydrolysis in methanol of 2-methoxyethyl thioacetate. The latter compound was prepared in 95% yield from 2-methoxyethyl *p*-toluenesulfonate and the potassium salt of thiolacetic acid by the method of Chapman and Owen;[29] its structure was established by ir and nmr spectra.

Kinetics. Spectrophotometric Assay of Product 2,4-Dinitrobenzenethiolate Anion from Thiol–Disulfide Reactions.—The product thiolate was assayed at λ_{max} 425 nm (ϵ 1.90 × 10^4 in 95% ethanol and 1.85 × 10^4 in *m*-xylene). The reactants and product disulfides at the concentrations used do not absorb appreciably at 425 nm. Ground-glass-stoppered cuvettes prefilled with argon were used to minimize facile air oxidation of the chromophoric product to the transparent symmetrical disulfide and to allow rapid mixing so that the first OD measurements could be made at ≤10 sec after zero time. All solution vessels and pipets were preflushed and filled with argon. Solutions of 2,4-dinitrobenzenethiolate anion were shown to maintain a constant optical density over time periods equal to total reaction times.

Control Experiments.—All disulfide reactants were shown to be stable to heating in the reaction solvent and to irradiation with light of wavelength greater than 250 nm at room temperature.

In the xylene reactions it was established that the arenethiols failed to yield any chromophoric product when placed with the disulfide (**1–3**) in the absence of DABCO. Similarly the disulfides plus DABCO did not generate the colored product unless the thiol was present.

Product Analyses.—Thin layer chromatographic analysis on tlc grade silica gel were performed on the products of the thiol–disulfide runs in both 95% ethanol and xylene. In every case development with each of methylene chloride, chloroform, benzene, acetone, ethyl acetate, or acetonitrile showed no mixed disulfide between starting thiol and 2,4-dinitrobenzenethiol or symmetrical disulfide from the starting thiol; the only disulfides present were a small quantity of the starting disulfide and a major proportion of the mixed disulfide of the original thiol and the aliphatic portion of the original disulfide.

That the reaction product was essentially all in the chromophoric 2,4-dinitrobenzenethiolate form at the pH of 6.91 used for the kinetic runs was shown by the failure of optical density to increase when pH was raised above 6.91. This finding is in accord with a pK_a for 2,4-dinitrobenzenethiol estimated to be <4 in 95% ethanol at 25° according to the method of Barlin and Perrin.[30]

Temperature Control.—All reactant solutions, cuvettes, and the spectrophotometer cuvette compartment were thermostated at 25.0 ± 0.2°.

Buffer pH.—All pH measurements were made at 25.0 ± 0.2° using a Copenhagen Radiometer pH meter no. 26 with always the same glass–calomel electrode pair standardized with two aqueous buffers; no corrections were made for the 95% ethanol/water junction potential. It was shown that pH decrease over the course of the reaction was limited to ≤0.04 pH units.

Calculation of Kinetic Parameters.—The observed second-order rate constants, $k_{2,obsd}$, based on the concentrations of the neutral arenethiol species (ArSH), for reactions with disulfides (DIS) in 95% ethanol to give the products 2,4-dinitrobenzenethiolate ion (P_1) and new mixed alkyl aryl disulfide (P_2), were obtained from the following kinetic analysis which corrects for the back-reaction whose specific rate constant is $k_{2,b}$ (eq 7).

$$\mathrm{ArSH} + \mathrm{DIS} \underset{k_{2,b}}{\overset{k_{2,obsd}}{\rightleftharpoons}} \mathrm{P_1} + \mathrm{P_2} \quad (7)$$

When the equilibrium depicted in eq 7 is attained, and (P_1) is equal to (P_2) at all reaction times as is true for our case, then the forward and reverse reaction rate constants are given by

$$k_{2,obsd}/k_{2,b} = (P_1)_e^2/(ArSH)_e(DIS)_e \quad (8)$$

where the subscript e designates an equilibrium concentration. $(P_1)_e$ is determined from the OD at 425 mμ at reaction completion. The equilibrium concentrations of thiols and disulfides are given by

$$(ArSH)_e = (ArSH)_0 - (P_1)_e \quad (9)$$

and

$$(DIS)_e = (DIS)_0 - (P_1)_e \quad (10)$$

where 0 subscripts indicate initial concentrations. Thus, $k_{2,b}$ can be expressed in terms of $k_{2,obsd}$, the initial and equilibrium concentrations according to eq 11. The assayed rate of forma-

$$k_{2,b} = k_{2,obsd}[(ArSH)_0 - (P_1)_e][(DIS)_0 - (P_1)_e]/(P_1)_e^2 \quad (11)$$

tion of P_1 is given by

$$d(P_1)/dt = k_{2,obsd}(ArSH)(DIS) - k_{2,b}(P_1)^2 \quad (12)$$

Also

$$(ArSH) = (ArSH)_0 - (P_1) \quad (13)$$

$$(DIS) = (DIS)_0 - (P_1) \quad (14)$$

Substituting in eq 12 in terms of equations 11, 13, and 14, we obtain the readily integrable differential expression 15 for the rate of formation of P_1.

$$\frac{1}{k_{2,obsd}}\frac{d(P_1)}{dt} = (ArSH)_0(XDIS)_0 - [(ArSH)_0 + (XDIS)_0](P_1) + [(ArSH)_0(P_1)_e + (XDIS)_0(P_1)_e - (ArSH)_0(XDIS)_0]\frac{(P_1)^2}{(P_1)_e^2} \quad (15)$$

Values of $k_{2,obsd}t$ were calculated for each kinetic point from the integrated expression. A least-squares linear regression applied to $k_{2,obsd}t$ *vs.* the time t gave in all cases good straight lines of slope $k_{2,obsd}$.

The integrated rate expression was programmed in FORTRAN IV for an IBM 360-91 computer.

Kinetic parameters calculated from initial rate data by a method which neglected the back-reaction differed by ≤5% from values corrected for back-reaction. Neglect of back-reaction was necessary in the 2,4,6-tri-*tert*-butylbenzenethiol case where the relatively slow reaction precluded a stable equilibrium OD owing to oxidation of the chromophoric product anion.

The rate constant for the actual nucleophile, thiolate anion, ($k_{2,anion}$) was obtained according to eq 16; the validity of this method has been described previously.[2b]

$$k_{2,anion} = k_{2,obsd}\frac{(H^+)}{K_a} \quad (16)$$

The acidity constant values used were those previously reported[2b] except for 2,4,6-tri-*tert*-butylbenzenethiol for which the low solubility in 95% ethanol precluded pK_a measurements by the method used for the other arenethiols. The pK_a of the 2,4,6-tri-*tert*-butyl derivative was estimated as 12.87 from the value for 2,4,6-tri-*tert*-butylphenol (pK_a = 17.62 in methanol at 25°)[31] since an excellent correlation exists between the pK_a's of alkyl substituted phenols in methanol and the corresponding thiols in 95% ethanol.[32]

$$pK_a^{ArOH} = 0.983\ pK_a^{ArSH} + 4.97 \quad (17)$$

For the reactions conducted in xylene a different kinetic analysis was used. The expected third-order kinetics, first order in each of three different reactants (ArSH, DIS, and DABCO), introduces considerable complexity into integration of the appropriate rate expression. A simpler procedure is to obtain the third-order rate constant $k_{3,obsd}$ from the pseudo-first-order rate constant $k_{1,obsd}$ defined by

$$k_{1,obsd}t = -\ln\frac{(DIS)_0 - (P)}{(DIS)_0} \quad (18)$$

where $(DIS)_0$ is the initial disulfide concentration and (P) is the concentration of product chromophoric anion. A least-squares linear regression is applied to the initial portion of the rate data

(29) J. H. Chapman and L. N. Owen, *J. Chem. Soc.*, 579 (1950).
(30) G. B. Barlin and D. D. Perrin, *Quart. Rev.*, **20**, 75 (1966).
(31) C. H. Rochester and B. Rossall, *Trans. Faraday Soc.*, **65**, 1004 (1969).
(32) See footnote 3 of ref 28.

which yields a value for $k_{1,obsd}$ that is invariant with time within experimental error. Then,

$$k_{3,obsd} = k_{1,obsd}/(DABCO)_0(ARSH)_0 \quad (19)$$

where $(DABCO)_0$ and $(ArSH)_0$ are the initial concentrations of base and thiol, respectively. The procedure is particularly successful for these rate data because both the base and thiol concentrations exceed the disulfide concentrations by a factor of at least 40, and therefore remain essentially constant throughout all runs.

The integrated rate expression was programmed in FORTRAN IV for an IBM 360-91 computer.

Acidity data were not available for the thiols in xylene solution; so rate constants for the anionic species were not determined. Rather comparisons were made of $k_{3,obsd}$ values for a single thiol on the various disulfides (**1**–**3**).

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Reactions of Sulfur Diimides with Ketenes

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The reaction products of sulfur diimides **1** with diphenylketene (**2a**) are temperature dependent. The reaction of diphenylsulfur diimide (**1a**) with **2a** at 6–8° gave the 1,2 cycloadduct **4** and at 80° the 1,1 cycloadduct **6a**. Refluxing **4** in benzene led to **6a** and **2a**. In contrast to **1a**, di-*tert*-butylsulfur diimide (**1b**) and **2a** at 0–2° gave 1,2 cycloadduct **3b**, which readily underwent rearrangement to **6b** under hexane reflux. The reaction of sulfur diimides **1** with alkylketenes gave no 1,2 or 1,3 cycloadducts but the thiobisamine derivatives **23** or **24** or their hydrolysis products. The reaction between diphenylsulfur diimide (**1a**) and dimethylketene (**2c**) gave rise to 2-phenylimino-3,3-dimethyl-1*H*-2,1-benzothiazin-4(3*H*)-one (**27a**) in addition to *N*,*N*′-diphenyl-*N*-(2-methylpropenoyl)-*N*′-isobutanoyl thiobisamine (**24b**).

Some studies on the reaction of sulfur diimides with diphenylketene have recently been reported. In our previous communication,[1] the structure of the product from diphenylsulfur diimide (**1a**) and diphenylketene (**2a**) was assumed to be 1-phenylimino-2,4,4-triphenyl-1,2-thiazetidin-3-one (**3a**) on the basis of ir, mass spectrum, and some chemical properties. An X-ray structure investigation,[2] however, showed that the structure is 2,4,4,5-tetraphenyl-1,2,5-thiadiazolidin-3-one (**6a**) instead of **3a**. The result is in accordance with the reported reaction of di-*p*-ethoxycarbonylphenylsulfur diimide with diphenylketene (**2a**).[3]

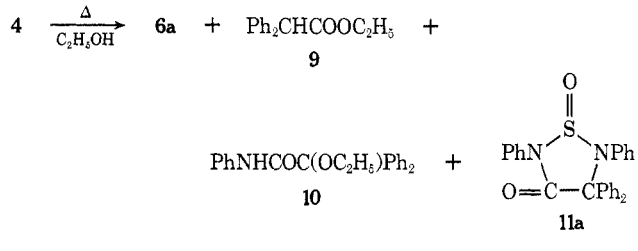
On the other hand, Kresze and Grill[4] isolated from the reaction of di-*p*-toluenesulfonylsulfur diimide with **2a** a 1-imino-1,2-thiazetidin-3-one derivative, which was easily isomerized to the 1,2,5-thiadiazolidin-3-one. Thus, variations in the sulfur diimide resulted in the formation of two types of 1,1 cycloadducts.

We have studied whether or not **6a** is formed *via* **3a** in analogy to Kresze's result.[4] Further, we report the reaction of various alkylketenes with sulfur diimides.

Part A

Results and Discussion

Reaction of Diphenylsulfur Diimide with Diphenylketene.—The reaction between diphenylsulfur diimide (**1a**) and diphenylketene (**2a**) in refluxing benzene gave the 1,3 cycloadduct, 2,4,4,5-tetraphenyl-1,2,5-thiadiazolidin-3-one (**6a**) in 67% yield. The reaction at lower temperature (6–8°), however, afforded only an unstable cycloadduct **4**. The yield of **4** was dependent on the molar ratio of **1a** to **2a** used in the reaction. The reaction using **1a** in double the molar quantity of **2a** gave **4** in 75% yield, while equimolar amounts produced **4** in 32% yield together with recovered **1a** (24%). On the other hand, refluxing an equimolar mixture of **4** and **1a** in benzene led to only **6a** (76%). With refluxing ethanol, **4** gave **6a** (44%), diphenylacetic acid ethyl ester (**9**) (38%), 1-ethoxy-1,1-diphenylacetanilide (**10**) (27%), and 2,4,4,5-tetraphenyl-1,2,5-thiadiazolidin-3-one 1-oxide (**11a**) (11%).



The unstable cycloadduct **4** contains 1 mol of **1a** and 2 mol of **2a** by elemental analysis, although the mass spectrum of **4** does not show any peak above the fragment ion peak at *m*/*e* 408 corresponding to the elimination of **2a** from the molecular ion. The ir spectrum of **4** exhibits carbonyl, carbon–carbon double bond, and ether absorptions at 1685, 1625, and 1275 cm^{-1}, respectively.

The chemical degradation and the physical data above do not clearly establish the structure of **4**. Accordingly, the structure was determined by X-ray analysis to be 2,3,4,6,7-pentahydro-2,4,4,7-tetraphenyl-3-oxo-1,5,2,7-thiaoxadiazepin-6-ylidenediphenylmethane. The molecular structure of **4** is shown in Figure 1.

Its formation would be rationalized in terms of one of two possible paths (path A and path B). As outlined in Scheme I, path A can be accounted for by a sequence of cycloaddition (**3a**), ring opening to the di-

(1) T. Minami, O. Aoki, H. Miki, Y. Ohshiro, and T. Agawa, *Tetrahedron Lett.*, 447 (1969).
(2) N. Yasuoka, N. Kasai, T. Minami, Y. Ohshiro, T. Agawa, and M. Kakudo, *Bull. Chem. Soc. Jap.*, **43**, 1905 (1970).
(3) H. H. Hörhold and H. Eibisch, *Tetrahedron*, **25**, 4277 (1969).
(4) H. Grill and G. Kresze, *Tetrahedron Lett.*, 1427 (1970).