Nucleophilic Cleavage of the Sulfur–Sulfur Bond by Phosphorus Nucleophiles. IV. Kinetic Study of the Reduction of Alkyl Disulfides with Triphenylphosphine and Water[1]

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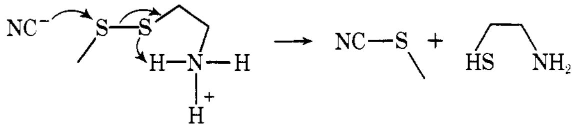
Abstract: A kinetic study of the reaction of a series of symmetrical alkyl disulfides with triphenylphosphine (Ph_3P) in 50% dioxane–H_2O is reported. This study was undertaken to critically examine the possible direct participation of electrophilic and nucleophilic neighboring groups in a reaction which involves cleavage of the S–S bond in the rate-limiting step. The reaction results in reduction of the disulfide to yield the corresponding alkylthiol and triphenylphosphine oxide. Triphenylphosphine sulfide is formed to only a trace extent. In analogy with our previous study of the reaction of symmetrical alkyl disulfides with Ph_3P, the two-step mechanism of Scheme II is suggested. At both low and high pH, the first step, nucleophilic cleavage of the S–S bond by Ph_3P, is rate determining. With the exception of disulfide **6**, the Bronsted plot (Figure 1) for disulfides bearing a single 2 or 3 substituent is linear and affords a slope (β) of −0.98. That all the monosubstituted disulfides fall on the same Bronsted line is strong evidence *against* the direct catalytic participation of the OH, $^+NH_3$, COOH, NH_2, and CO_2 neighboring groups.

That neighboring groups may participate in reactions involving cleavage of the disulfide S–S bond has been suggested in scattered reports for nearly 30 years.[3-10] Studies of the nucleophilic cleavage of cystine, and several of its derivatives, by cyanide[3,4] and sulfite[5] have shown that protonation of neighboring NH_2 and CO_2^- groups results in rate increases of up to 400-fold. These observed effects of neighboring $^+NH_3$ and COOH groups have been ascribed to both electrostatic interactions between the cationic disulfide and the anionic nucleophile,[4,5] and to the direct participation of the $^+NH_3$ group as an intramolecular general acid catalyst,[4,6] eq 1. More recently, the observation that 2-hydroxyethyl 2,4-dinitrophenyl disulfide is cleaved by benzenethiolate anion in xylene three times faster than 2-methoxyethyl 2,4-dinitrophenyl disulfide was attributed to intramolecular general acid catalysis by the OH group.[7] Direct participation of basic groups as intramolecular nucleophilic catalysts has also been suggested. Field and coworkers[8-10] have observed that the thermal disproportionation of unsymmetrical disulfides is more rapid in disulfides containing NH_2, CO_2^-, or SO_2^- groups in the 2 or 3 position. This and other evidence have been interpreted by these authors to indicate the direct nucleophilic participation of the NH_2,[8] CO_2^-,[9,10] and SO_2^- [10] groups in the S–S bond-cleavage step, as for example is illustrated for a neighboring CO_2^- group in eq 2.[11]



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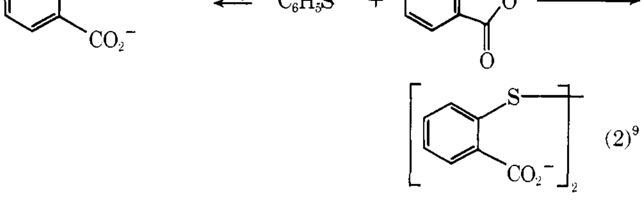


(2)[9]

In none of the previous studies, in which a direct catalytic role was ascribed to a disulfide neighboring group, have alternate explanations for the observed rate effects been convincingly ruled out. The proposed participation of neighboring nucleophiles in the S–S bond-cleavage step of the thermal disproportionation reaction is certainly speculative, since the details of this mechanism are not yet established.[13,14] Moreover, in many of the earlier studies,[3-7] corrections for the polar (inductive and field) effects of the neighboring groups were not made.[15] Since it is now well established that the cleavage of substituted disulfides by nucleophiles is significantly accelerated by electron-withdrawing substituents,[12,17] some of the rate accelerations previously reported may be due simply to the polar effects of the neighboring groups.

We report here a study of the reaction of triphenylphosphine (Ph_3P) with a series of substituted alkyl disulfides. The study was undertaken to critically examine the possible participation of several neighboring groups in a reaction whose mechanism was well established and which involves cleavage of the S–S bond in the rate-limiting step.[1,12] Although a strong thiophile, Ph_3P is a weak proton base,[18] and as a result, the reaction of Ph_3P with disulfides can be studied over a wide pH range.

Results

Stoichiometry. Alkyl disulfides are reductively cleaved when treated at 40° with Ph_3P in 50% dioxane–H_2O in the presence of either dilute sodium hydroxide or dilute hydrochloric acid (eq 3). For all the alkyl disulfides we have studied, uv assay at 290 nm indicated that Ph_3P was quantitatively (97 ± 7%) oxidized. Since the formation of small amounts of triphenylphosphine sulfide (Ph_3PS) would not be detected by the uv assay, and since trisubstituted phosphines are known under certain conditions to promote a desulfurization reaction (eq 4),[19] we chose, in a few representative cases, to examine carefully by gas chromatography (GC) the Ph_3PO and Ph_3PS yields (see Table I). In runs conducted in dilute base, no more than a trace (<1%)

$$\underset{1}{XCH_2S{-}SCH_2X} + Ph_3P \xrightarrow[H^+ \text{ or } OH^-]{\text{dioxane-}H_2O} 2XCH_2SH + Ph_3PO \qquad (3)$$

$$1 + Ph_3P \longrightarrow XCH_2SCH_2X + Ph_3PS \qquad (4)$$

Table I. Products from the Reduction of Substituted Methyl Disulfides by Ph_3P[a]

Substituted methyl disulfide	Buffer	Time, hr	Yield, % Thiol[b,c]	Yield, % Ph_3PO[c,d]	Yield, % Ph_3PS[d,e]	Yield, % Overall[d,f]
2, CH_3	0.05 *M* HCl	21		94.6	0	99.8
2, CH_3	0.02 *M* NaOH	17	72.3			
3, CH_2COOH	0.05 *M* HCl	5	102	100	3.6	90.8
4, CH_2CO_2	0.02 *M* NaOH	25	99.3	105	0	85.8
5, CH_2CONH_2	0.05 *M* HCl	5	96.0	118	3.8	107
6, $CH_2NH_3^+$	0.05 *M* HCl	1.5	102	100	6.8	99.0
6, $CH_2NH_3^+$	0.05 *M* HCl	7		96.5	trace[g]	97.1
7, CH_2NH_2	0.02 *M* NaOH	6	101	98.3	trace[g]	99.6
8, $CH(NH_3^+)COOH$	0.05 *M* HCl	0.7	106			
9, CH_2OH	0.02 *M* NaOH	3	48.7	99.2	trace[g]	96.6
9, CH_2OH	0.02 *M* NaOH	4	46.8			
9, CH_2OH	0.05 *M* HCl	2	50.0			
9, CH_2OH	0.05 *M* HCl	4	44.7			
10, COOH	0.05 *M* HCl	0.5	101	108	4.7	98.6
11, $COOCH_3$	0.05 *M* HCl	0.4	103	97.6	2.4	95.9
$HSCH_2CH_2COOH$[h]	0.05 *M* HCl	3		4.0	4.8	96
$^-SCH_2CH_2CO_2^-$[h]	0.02 *M* NaOH	3		0	0	84

[a] In 50% dioxane–H_2O, 40°, ionic strength = 0.10 (KCl), $Na_2EDTA = 5 \times 10^{-4}$ *M*. Yields (%) are reproducible to ±5%. [b] $[Ph_3P]_0 = 1.3 \times 10^{-4}$ *M*, $[\text{disulfide}]_0 = 3.3 \times 10^{-4}$ *M*, by uv with *N*-ethylmaleimide. [c] % Yield is based on the stoichiometry of eq 3. [d] $[Ph_3P]_0 = 3.3 \times 10^{-4}$ *M*, $[\text{disulfide}]_0 = 1.6 \times 10^{-4}$ *M*, by GC. [e] % of original Ph_3P converted to Ph_3PS. [f] Total recovery of original Ph_3P as Ph_3P, Ph_3PO, and Ph_3PS. [g] Trace means a detectable amount of Ph_3PS, but less than 1%. [h] Control experiment, $[Ph_3P]_0 = 2.2 \times 10^{-4}$ *M*, $[RSH]_0 = 3.4 \times 10^{-4}$ *M*, % of original Ph_3P converted to Ph_3PO and Ph_3PS. Other control experiments are noted in the Experimental Section.

of Ph_3PS was detected. In the runs in dilute acid, up to 7% conversion to Ph_3PS was detected. Control experiments (Table I, last two entries) indicate that a large part of the Ph_3PS formed in the acid runs arises from further reaction of the product thiol with Ph_3P. The desulfurization reaction, thus, if occurring at all, occurs to only a trace extent.

The yield of thiol from the reduction of alkyl disulfides with Ph_3P under acidic conditions has been studied polarographically by Humphrey.[20] For a representative set of disulfides we determined the thiol yield under both acidic and basic conditions by spectrophotometric titration of the thiol produced using *N*-ethylmaleimide.[21] Since unreacted Ph_3P interfered with this analysis, a 2.5-fold excess of disulfide was employed. For all but two of the disulfides studied the thiol yield was within experimental error of 100% (Table I). The yield of ethanethiol from **2** was consistently low, and this is attributed to the long reaction times and the volatility of the product thiol. 2,2′-Dithiodiethanol (**9**) consistently gave thiol yields 50% of theoretical. Grayson and Farley[22] have reported that in the absence of solvent Ph_3P reacts with **9** to afford ethylene sulfide, 2-mercaptoethanol, and triphenylphosphine oxide, and they suggested the mechanism of Scheme I. Under our experimental conditions, ethylene sulfide could be detected from the reaction of **9** with Ph_3P, but no attempt was made to quantify its yield since it was assumed to undergo competing polymerization.[23] If in fact the consistent 50% yield of 2-mercaptoethanol we observe does indicate that disulfide **9** reacts predominantly via the process of Scheme I, it is interesting that this rearrangement competes so effectively even at high pH with the capture of phosphonium ion **15** by OH^-.

Kinetics. The reaction of eq 3 was monitored under pseudo-first-order conditions of disulfide in great excess over Ph_3P by observing the decrease in absorbance at 290 nm which accompanies the conversion of Ph_3P to Ph_3PO. In dilute HCl or NaOH buffers, the reactions were cleanly first order (correlation coefficients greater than 0.9995), and the observed pseudo-first-order rate constants (k_{obsd}) were directly proportional to the initial disulfide concentration. At these pH extremes the second-order rate constants ($k_1 = k_{obsd}/[RSSR]_0$) were reasonably pH independent and are summarized in Table II. Where possible, kinetic determinations were made in both HCl and NaOH buffers. This was not possible for disulfides **5, 10, 11, 12,** and **13** as they were not stable in dilute base. The decomposition of disulfide **10** in 0.02 *M* NaOH has been reported previously.[24] The decomposition of disulfides **4, 7, 9,** and **14** in strong base has also been reported,[24] but the decomposition rate is at least an order of magnitude slower than the reaction of these disulfides with Ph_3P. At intermediate pH the reaction is much slower and kinetically more complex. Under these conditions, the pseudo-first-order rate plots showed extensive downward curvature. This type of nonlinear behavior was observed previously in the reaction of Ph_3P with diaryl and aryl alkyl disulfides.[2,12]

Scheme I

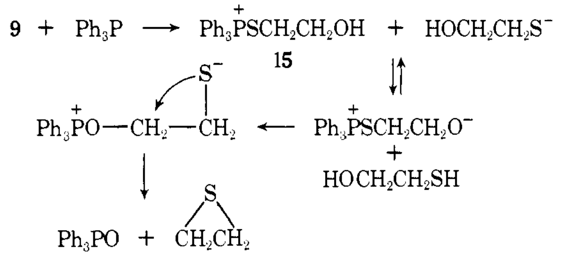
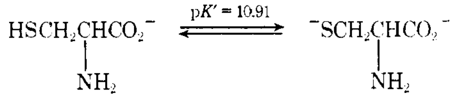


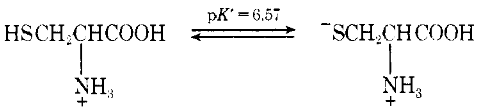
Table II also summarizes the pK_a's of the corresponding thiols which were determined by spectrophotometric titrations[25] under conditions identical with those of the kinetic measurements. The equilibrium constants for eq 5–10 are

$$HSCH_2CH_2\overset{+}{N}H_3 \underset{}{\overset{pK' = 8.95}{\rightleftarrows}} {}^-SCH_2CH_2\overset{+}{N}H_3 \qquad (5)$$

$$HSCH_2CH_2NH_2 \overset{pK' = 10.88}{\rightleftarrows} {}^-SCH_2CH_2NH_2 \qquad (6)$$



$$HSCH_2\underset{NH_2}{\underset{|}{C}}HCO_2^- \overset{pK' = 10.91}{\rightleftarrows} {}^-SCH_2\underset{NH_2}{\underset{|}{C}}HCO_2^- \qquad (7)$$



$$HSCH_2\underset{\underset{+}{N}H_3}{\underset{|}{C}}HCOOH \overset{pK' = 6.57}{\rightleftarrows} {}^-SCH_2\underset{\underset{+}{N}H_3}{\underset{|}{C}}HCOOH \qquad (8)$$

$$HSCH_2COOH \overset{pK' = 9.50}{\rightleftarrows} {}^-SCH_2COOH \qquad (9)$$

$$HSCH_2CH_2COOH \overset{pK' = 10.76}{\rightleftarrows} {}^-SCH_2CH_2COOH \qquad (10)$$

not, however, directly measurable by this technique. The microscopic ionization constants (K')[26,27] corresponding to eq 5-7 can be determined by the method first reported by Benesch and Benesch,[28] in which both the apparent macroscopic proton dissociation constants (measured titrimetrically) and the thiolate anion concentration as a function of pH (measured spectrophotometrically) are determined.[26] Application of this method (see Table III) affords the constants shown. The equilibrium constants of eq 9 and 10 cannot be accurately determined by this technique,[29] but they can be estimated by applying Wegscheider's principle of equivalence.[26,27] In this approximation, an ester group is used as a model for an undissociated carboxylic acid group. The equilibrium constant of eq 8 can be estimated by a combination[26,27] of these two methods (see Table IV).

Discussion

In aprotic solvents, alkyl disulfides do not react with Ph_3P even after several hours at 140°.[31] The reduction of alkyl disulfides by Ph_3P in protic solvents was first noted by Humphrey, who reported that thiols were formed in 80-90% yield when an acidic methanol solution of an alkyl disulfide was treated at reflux with Ph_3P.[20] Our studies in aqueous dioxane confirm the stoichiomery (eq 3) suggested by Humphrey for the acid promoted reaction and also indicate that an identical redox reaction occurs in dilute base. In accord with our previous investigations of the reaction of aryl[12] and aryl alkyl[1] disulfides with Ph_3P, we suggest the two-step mechanism of Scheme II. Such a mechanism

Scheme II

$$1 + Ph_3P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\textbf{16}}{Ph_3\overset{+}{P}SCH_2X} + XCH_2S^-$$

$$\textbf{16} + H_2O \xrightarrow{k_2} Ph_3PO + RS^- + 2H^+$$

would be consistent with the kinetics observed in both dilute acid or dilute base if the first step is rate limiting, i.e., $k_2 > k_{-1}[XCH_2S^-]$ and $k_2 > k_1$. As we have discussed in more detail elsewhere,[12] this is the expected result since at high pH the hydrolysis of **16** should be rapid, while at low pH the thiolate anion should be protonated and the k_{-1} step thus suppressed.

In a qualitative sense the results of Table II parallel those of earlier workers.[3-5] Protonation of cystamine (**7**) results in a rate acceleration of 3.6-fold, 3,3′-dithiodipropionic acid (**3**) is 9.7 times more reactive than its dianion **4**, and the protonated form of cystine (**8**) reacts with Ph_3P more than 100 times faster than the corresponding dianion **14**. That these rate differences do not arise, however, from intramolecular catalytic effects, but merely reflect the simple polar effects of the substituents, is apparent when the rate data are plotted in the Bronsted fashion. With the exception of disulfide **6**, the Bronsted plot (Figure 1) for disulfides bearing a single 2 or 3 substituent is linear and affords a slope (β) of -0.98 ± 0.10 (omitting **6**).[32] The large negative value of β indicates that the reaction is markedly accelerated by electron withdrawal. The similar cleavage of symmetrical aryl disulfides exhibited a nearly identical β value of -1.04.[12] That all the 2- and 3-substituted disulfides fall on the same Bronsted line is strong evidence *against* the direct catalytic participation of groups such as OH, $^+NH_3$, COOH, NH_2, and CO_2^-. The points (not shown) for the cystine disulfides **8** and **14** fall considerably below the Bronsted line of Figure 1. This negative deviation is precedented and has been attributed to steric hindrance to the approach of a nucleophile brought about by a second substituent on the 2-carbon.[33] The cause of the negative devia-

Table II. Rate of Cleavage of Substituted Methyl Disulfides by Ph_3P at Low and High pH[a]

Substituted methyl disulfide	$10^3[RSSR]_0$, M	k_1, l. mol^{-1} min^{-1} (no. of runs)[b] NaOH, M 0.01	0.02	0.03	0.04	0.05	1.0	HCl, M 0.02	0.05	0.10	Av k_1, l. mol^{-1} min^{-1}	pK_a Substituted[c] methanethiol
2, CH_3	1.07-5.28	3.41 (3)	3.21 (3)		3.57 (3)		4.01 (3)		4.12 (3)	4.39 (5)	3.75 ± 0.10	11.66
3, CH_2COOH	1.96-4.16								12.1 (3)	11.6 (3)	11.8 ± 0.10	10.76[d]
4, $CH_2CO_2^-$	1.98-4.40			1.28 (3)		1.16 (3)					1.22 ± 0.04	11.36
5, CH_2CONH_2	1.95-4.04								9.66 (6)		9.66 ± 0.37	10.70
6, $CH_2NH_3^+$	1.00-3.84							39.4 (3)	39.7 (3)		39.6 ± 0.6	8.95[d]
7, CH_2NH_2	1.83-4.00		10.9 (3)	10.9 (3)	11.2 (3)						11.0 ± 0.1	10.88[d]
8, $CH(NH_3^+)COOH$	1.00-2.00								133 (3)		133 ± 2.0	6.57[d]
9, CH_2OH	2.32-4.69	15.0 (3)		15.2 (3)				17.3 (3)[e]	17.9 (3)[e]		15.1 ± 0.1	10.71
10, COOH	2.01-4.28							175 (3)	171 (3)	198 (6)	180 ± 5.0	9.50[d]
11, $COOCH_3$	2.10-4.28								380 (3)		380 ± 4.0	9.50
12, CH_2CN	2.42-4.84								62.1 (3)		62.1 ± 1.1	9.91
13, CH_2COOCH_3	2.62-5.24								13.7 (3)		13.7 ± 0.2	10.76
14, $CH(NH_2)CO_2^-$	2.00-4.00	1.00 (3)									1.00 ± 0.03	10.91[d]

[a] In 50% dioxane-H_2O, 40.0 ± 0.1°, ionic strength = 0.10 (KCl), $Na_2EDTA = 5.0 \times 10^{-4}$ M, $[Ph_3P]_0 = (1.3-1.7) \times 10^{-4}$ M, monitored at 290 nm. [b] The number of measurements made at differing $[RSSR]_0$ at this pH. [c] Experimentally determined by spectrophotometric titration under identical conditions. [d] The corresponding microscopic ionization constant, determined (or estimated) as described. [e] The first-order plots exhibit slight curvature. They are not included in the average.

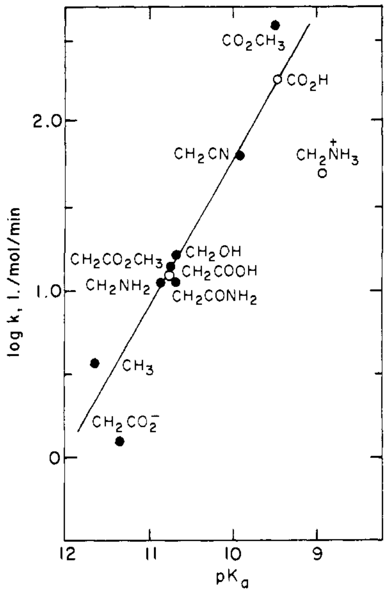


Figure 1. Bronsted plot of log k_1 vs. pK_a. Open circles are used for pK_a values corresponding to microscopic ionization constants.

tion observed for the $^+NH_3$ substituted disulfide **6** is not known.

In summary then, we find *no* evidence for the direct participation of a variety of electrophilic and nucleophilic neighboring groups in the reductive cleavage of substituted alkyl disulfides with Ph_3P. The relative reactivities which are observed are explicable solely in terms of simple polar and steric effects. It is perhaps worth noting that a comparison of our data with that of Cecil and McPhee[5] provides some evidence for the operation of an electrostatic effect[34] in the case of cleavage by the anionic nucleophile sulfite. Although not absolutely required, this is a reasonable explanation for the observation that the spread in reactivity between anionic, neutral, and cationic disulfides (e.g., **8** and **14**) is considerably smaller with the neutral nucleophile Ph_3P than with the anionic nucleophile SO_3^-.[2]

Experimental Section

Materials. The purification of dioxane, water, Ph_3P, and the methods and instruments used for the pH measurements and titrimetric pK_a determinations were described previously.[12] The notation "50% dioxane-H_2O" refers to a water-dioxane solution made by diluting (0.5 *V*) ml of dioxane to *V* ml with water in a volumetric flask.[12] All errors reported are ±1 standard deviation from the mean for a series of measurements. Analytical gas-liquid chromatography (GLC) utilized a Hewlett-Packard Model 700 with a flame ionization detector.

Disulfides **2**, **3**, **6**, **9**, **10**, **13**, and cystine were purchased from Aldrich or Sigma. Disulfides **5**,[35] **11**,[36] and **12**[37] were prepared by standard methods. Methyl mercaptoacetate,[36] 3-mercaptopropionamide,[38] and 3-mercaptopropionitrile[39] were prepared by standard methods. 2-Mercaptoethylamine hydrochloride (Calbiochem) contained considerable amounts of the corresponding disulfide and was purified by conversion to the free base[40] and recrystallization from ethyl acetate. Other thiols were purchased from Aldrich, Mallinckrodt, or Sigma. All disulfides and thiols were purified by recrystallization or fractional distillation and exhibited physical properties in accord with literature values.

Products. Determination of Ph_3P, Ph_3PO, and Ph_3PS. A 50% dioxane-H_2O solution (150 ml), 0.02 *M* in sodium hydroxide (or 0.05 *M* in hydrochloric acid), 0.1 *M* in potassium chloride, and 5×10^{-4} *M* in EDTA, was deoxygenated for 30 min with oxygen-free nitrogen.[1] Triphenylphosphine (0.50 mmol) and the disulfide (0.25 mmol) were added, and the solution was maintained at 40° under a nitrogen atmosphere for the time indicated in Table I. After the solution was cooled to room temperature, 75 ml of ether was added, the aqueous layer saturated with sodium chloride, and the organic layer separated. The aqueous layer was washed with 75 ml of ether, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. A weighed amount of triphenylmethane was added as an internal standard and the product mixture analyzed by GLC.[41] Peak areas were corrected for detector response by standard methods. Triphenylphosphine sulfide could be detected at the 1% level. The results are presented in Table I.

Table III. Macroscopic and Microscopic Dissociation Constants for 2-Mercaptoethylamine and Cysteine Ethyl Ester[a]

Constant	2-Mercaptoethylamine	Cysteine ethyl ester
pG_1	8.40	6.01
pG_2	11.03	10.48
pK_1'	8.95	6.57
pK_2'	8.54	6.15
pK_3'	10.47	9.92
pK_4'	10.88	10.34

[a] In 50% dioxane–H_2O, 40.0 ± 0.1°, ionic strength = 0.10 (KCl), $Na_2EDTA = 5.0 \times 10^{-4}$ *M*.

Table IV. Macroscopic and Microscopic Dissociation Constants for Cysteine[a,b]

Constant	Value	Constant	Value
pG_1	3.07	pK_{12}'	8.99
pG_2	8.53	pK_{32}'	10.34
pG_3	11.10	pK_{132}'	10.91
pK_1'	3.07	pK_3'	6.15
pK_{21}'	5.48	pK_{13}'	8.72
pK_{31}'	5.64	pK_{23}'	9.92
pK_{231}'	6.21	pK_{123}'	10.64
pK_2'	6.57		

[a] In 50% dioxane–H_2O, 40.0 ± 0.1°, ionic strength = 0.10 (KCl), $Na_2EDTA = 5.0 \times 10^{-4}$ *M*. [b] The microscopic constants are defined in terms of Scheme I of ref 27.

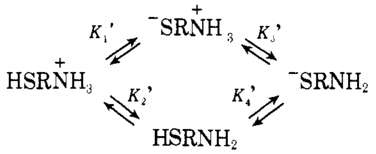
The following control experiments (see also Table I) were conducted under similar conditions. (a) A 50% dioxane-H_2O solution of 3-mercaptopropionamide (0.62 mmol), Ph_3PO (0.57 mmol), and hydrochloric acid (0.05 *M*) was kept at 40° for 65 hr. Assay by GLC indicated that no Ph_3PS or Ph_3P was formed and that 80% of the Ph_3PO was recovered. (b) A 50% dioxane-H_2O solution of Ph_3P (2.5×10^{-3} *M*) and hydrochloric acid (0.05 *M*) was maintained at 40° for 3 hr. Assay by GLC indicated that no Ph_3PO or Ph_3PS was formed and that 70% of the Ph_3P was recovered. The low recovery of Ph_3P is partially attributed to a slow acid-catalyzed reaction of Ph_3P with dioxane.[42] (c) A 50% dioxane-H_2O solution of Ph_3PS (0.22 mmol), 3-mercaptopropionic acid (0.38 mmol), and hydrochloric acid (0.05 *M*) was maintained at 40° for 3 hr. Assay by uv at 260 nm (Ph_3PS, ϵ 5230; Ph_3PO, ϵ 1510) indicated a 107% recovery of Ph_3PS. A similar reaction run in 0.02 *M* sodium hydroxide resulted in a 95% recovery of Ph_3PS.

Thiol Determinations. The reactions were conducted as described for the Ph_3PO and Ph_3PS analyses except that 0.5 mmol of disulfide and 0.2 mmol of Ph_3P were used. After the times indicated in Table I, the reaction mixture was allowed to cool to room temperature, and 1.00 ml was pipetted into 4.00 ml of a 1.51×10^{-3} *M* solution of *N*-ethylmaleimide in pH 6 phosphate buffer (0.5 *M*). The absorbance at 300 nm was measured and the thiol concentration determined from working curves constructed with thioglycolic acid. Controls indicated that there was no interference from EDTA, Ph_3PO, and small amounts (up to 8×10^{-5} *M*) of Ph_3P. The results are presented in Table I.

Kinetics. Kinetic measurements were made with a Beckman Model 25 recording spectrophotometer, equipped with an autosampler and temperature controller. All runs were run at 40.0 ± 0.1° and observed at 290 nm. First-order rate constants were calculated as described previously.[12] Under acidic conditions Ph_3P undergoes a slow reaction with dioxane.[42] This reaction (Ph_3P is 10% consumed in 2 hr in 0.05 *M* HCl at 40°) is significantly slower than the reaction of Ph_3P with **1** and thus is believed to introduce little error into the measured rate constants. In a typical run, 3.0 ml of a 50% dioxane–H_2O solution containing disulfide, buffer, and potassium chloride, was pipetted into a capped 1-cm cuvette. After equilibrating for at least 15 min, the reaction was initiated by adding 20 μl of a freshly prepared 0.023 M solution of Ph_3P in dioxane. Absorbance vs. time was recorded on a strip-chart recorder or printed on paper tape using a Beckman Model 3115 Printer. The rate constants were identical with either method of data acquisition.

Spectrophotometric pK_a Measurements. A cuvette containing 3.0 ml of the appropriate buffer (0.01 M) in 50% dioxane–H_2O, ionic strength = 0.10 M (KCl), EDTA = 5×10^{-4} M was equilibrated in the Beckman Model 25 spectrophotometer. A freshly prepared stock solution (20 μl) of the thiol in deoxygenated water or dioxane was added and the absorbance immediately read at 240 nm. Wavelength was not scanned due to the rapid oxidation of some of the thiols in the more basic solutions. The following buffers (pH) were used: dichloroacetate (4.14), glycine (8.78), proline (10.50, 10.68), carbonate (11.14, 11.73), sodium hydroxide (0.01 and 0.02 M). The calculation of pK_a was performed as described.[25]

Determination of Microscopic Dissociation Constants by the Spectrophotometric Method.[26,28] Apparent macroscopic dissociation constants (G_1 and G_2) were determined titrimetrically as described previously.[12] They exhibited standard deviations of 0.02 pK unit or less. Spectrophotometric measurements were made as described above in a series of buffers (8–12 measurements) over the pH range 10.7–12.0. The microscopic constant K_1' is the average of the K_1' values calculated[26] from the values of α_{SH} (thiolate anion mol fraction) measured in each buffer. The microscopic constants (Table III) for 2-mercaptoethylamine and cysteine ethyl ester were calculated exactly as described[26] for the scheme shown in eq 11. A consideration of the errors associated with our measurements of G_1, G_2, and α_{SH} indicates that the maximum error in pK_1' should be ±0.2 pK unit.[43]



(11)

For cysteine the apparent macroscopic dissociation constants (G_1, G_2, and G_3 measured titrimetrically), the values of α_{SH} (measured spectrophotometrically), and the microscopic constants for cysteine ethyl ester (Wegscheider's principle) were utilized to calculate, exactly as described,[26] the microscopic constants shown in Table IV. The microscopic constants for cysteine are defined as described[26] and correspond exactly to the constants of Scheme I of ref 27.

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$$HSRCOOH \overset{K_a'}{\rightleftharpoons} {}^-SRCOOH$$

$$HSRCOOH \overset{K_b'}{\rightleftharpoons} HSRCOO^-$$

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