Carboxyl-Group Participation in Phosphorothioate Hydrolysis. The Hydrolysis of S-(2-Carboxyphenyl)phosphorothioate

THOMAS H. FIFE AND SHELDON MILSTIEN

Department of Biochemistry, University of Southern California, Los Angeles, California 90033

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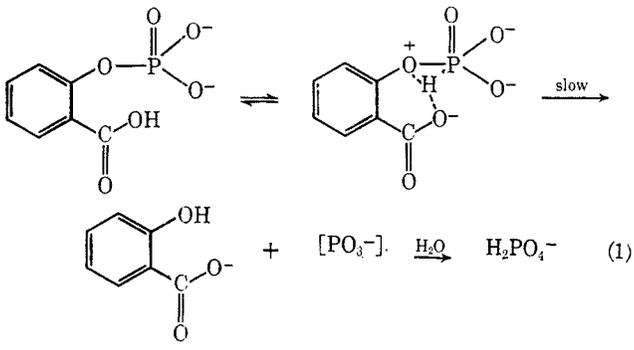
The rates of hydrolysis of S-(2-carboxyphenyl)phosphorothioate have been measured in H_2O and D_2O as a function of pH at 35°. The pH-rate profile has a maximum occurring close to pH 4. The *ortho*-carboxyl-substituted derivative hydrolyzes 5.0 times faster than the *para* derivative at the rate maximum and 6.3 times faster in 5.90 *M* HCl where the compounds are predominantly in the neutral form. A plot of log k_{obsd} for hydrolysis in 5.90 *M* HCl *vs.* the pK_a of the thiol leaving group for a series of S-(4-substituted phenyl)phosphorothioates is linear with a slope of −0.06, but the point for the *ortho*-carboxyl-substituted compound deviates markedly (+1.10 log units). A plot of log *k* for hydrolysis of the monoanionic species of the series of S-monoaryl phosphorothioates *vs.* the pK_a of the leaving group is also linear with a slope of 0.08, but the point of *o*-COOH again shows a positive deviation. The value of k_{H_2O}/k_{D_2O} is 1.7 for hydrolysis of the neutral species of the *o*-COOH derivative and 2.1 for the monoanion. Thus, the *ortho*-carboxyl group is accelerating the reactions of the neutral and monoionized species with possible mechanisms involving partially rate-determining protonation of sulfur. The D_2O solvent isotope effect for hydrolysis of the dianion of S-(2-carboxyphenyl)phosphorothioate is near unity (k_{H_2O}/k_{D_2O} = 1.2), consistent with either preequilibrium zwitterion formation with protonated sulfur or intramolecular nucleophilic catalysis by the carboxylate anion. The presence of an acyl phosphate intermediate which would be formed by nucleophilic attack could not, however, be demonstrated by the hydroxamic acid method. Steric facilitation of the neutral and monoanion reactions is produced by a bulky *ortho* substituent since in both reactions the 2-isopropyl derivative hydrolyzes faster than the unsubstituted compound. The rate of hydrolysis of S-(2-isopropylphenyl)-phosphorothioate dianion, however, was quite slow.

The hydrolysis reactions of *o*-carboxyaryl phosphates[1-4] and *o*-carboxyphenyl phosphoramidate[5] have been extensively studied. Relative rate studies have indicated a direct participation by the *ortho* substituent in the hydrolysis of the *o*-carboxyaryl phosphates. The relatively fast rates for the dianionic species are not due to inductive or resonance effects since the derivatives having *meta*- or *para*-carboxyl groups did not hydrolyze at comparable rates.[2] Chanley and coworkers[2] postulated nucleophilic attack by the *o*-carboxylate anion on phosphorus to give an acyl phosphate intermediate, but Bender and Lawlor[4] subsequently obtained evidence favoring proton transfer from the unionized carboxyl group to the ester oxygen with the most likely mechanism involving a zwitterion which decomposes in the rate determining step to metaphosphate and salicylate (eq 1). The un-ionized carboxyl



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group also accelerates the rate of hydrolysis of the monoanionic and neutral species of *o*-carboxyphenyl phosphoramidate,[5] probably also through a zwitterion intermediate.

The hydrolysis of S-monoarylphosphorothioate monoanion and neutral species very likely involves internal proton transfer to sulfur that is partially rate determining.[6] To assess the effect of a neighboring carboxyl group on phosphorothioate hydrolysis, the rates of hydrolysis of S-(2-carboxyphenyl)phosphorothioate have been measured.

Experimental Section

Materials.—S-(2-Carboxyphenyl)phosphorothioate was prepared by a method analogous to that employed by Chanley and coworkers[2a] for preparation of 2-carboxyphenyl phosphate, using recrystallized *o*-mercaptobenzoic acid (Matheson Coleman and Bell) and phosphorus pentachloride. The product was recrystallized from acetone and benzene and melted at 101–103°. Analysis of both liberated inorganic phosphate by King's method[7] and *o*-mercaptobenzoic acid[8] after complete hydrolysis at pH 4 showed the product to be 98–100% pure.

Anal. Calcd for $C_7H_7O_5PS \cdot H_2O$: C, 33.34; H, 3.57; P, 12.24. Found: C, 33.60; H, 3.59; P, 12.26.

S-(4-Carboxyphenyl)phosphorothioate.—All attempts at reacting *p*-mercaptobenzoic acid (mp 214–216°, lit.[9] mp 217°), prepared by the procedure given for *o*-mercaptobenzoic acid,[10] with phosphorus pentachloride yielded only an oil which could not be crystallized. Attempts at purification *via* the cyclohexylamine salt gave only cyclohexylammonium phosphate with little or no product. An attempt was made, therefore, to synthesize the compound from *p*-carboxybenzenesulfenyl chloride and dibenzyl phosphite in chloroform solution by procedures developed for the synthesis of other S-(4-substituted phenyl)phosphorothioates.[6] The final product after crystallization as the cyclohexylammonium salt was found to contain some inorganic phosphate. This was determined by quantitatively measuring the amount of the thiophenol after complete hydrolysis at pH 4 and after enzymatic hydrolysis by *E. coli* alkaline phosphatase.[11] The spectrum of the liberated *p*-mercaptobenzoic acid was identical with that of an authentic sample. The compound was too labile to permit purification by conventional chromatographic

(1) J. Arai, *J. Biochem.* (Tokyo), **20**, 465 (1934).

(2) (a) J. D. Chanley, E. M. Gindler, and H. Sobotka, *J. Amer. Chem. Soc.*, **74**, 4347 (1952); (b) J. D. Chanley and E. M. Gindler, *ibid.*, **75**, 4035 (1953); (c) J. D. Chanley and E. Feageson, *ibid.*, **77**, 4002 (1955).

(3) F. R. Atherton, Special Publication No. 8, The Chemical Society of London, 1957, p 77.

(4) M. L. Bender and J. M. Lawlor, *J. Amer. Chem. Soc.*, **85**, 3010 (1963).

(5) S. J. Benkovic and P. A. Benkovic, *ibid.*, **89**, 4714 (1967).

(6) S. Milstien and T. H. Fife, *ibid.*, **89**, 5820 (1967).

(7) O. Lindberg and L. Ernster, "Methods of Biochemical Analysis," Vol. III, D. Glick, Ed., Interscience Publishers, Inc., New York, N. Y., 1956, p 1.

(8) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

(9) D. Bramley and N. H. Chamberlain, *J. Chem. Soc.*, 376 (1942).

(10) C. F. H. Allen and D. D. MacKay "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Ed., John Wiley & Sons, Inc., New York, N. Y., 1943, p 580.

(11) S-Monoarylthiolphosphates are excellent substrates for alkaline phosphatase: S. Milstien, and T. H. Fife, unpublished data.

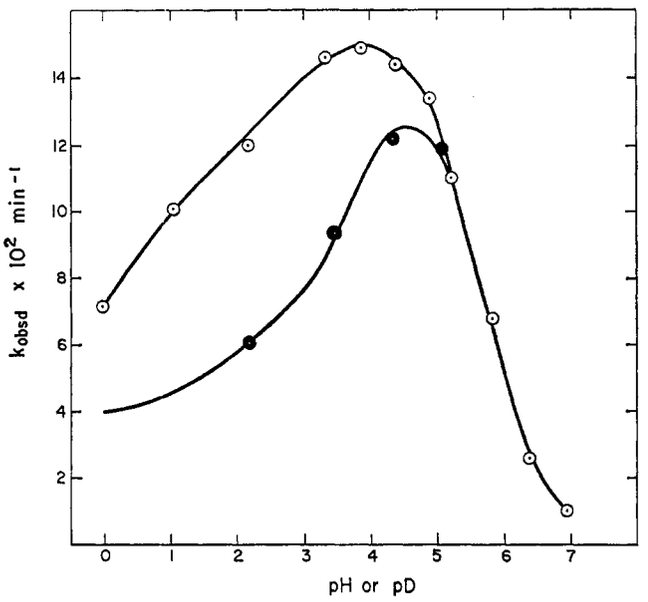


Figure 1.—Plots of k_{obsd} *vs.* pH or pD for hydrolysis of S-(2-carboxyphenyl)phosphorothioate in H_2O, ⊙, and D_2O, ●.

techniques; therefore, elemental analysis was not possible. The observed kinetics should be completely unaffected by the presence of inorganic phosphate since buffer catalysis is not observed with these compounds, and it is felt that the kinetic data are quite accurate. The conclusions arrived at in this paper are not dependent upon inclusion of these data, but the data are of interest for purposes of comparison.

S-(2-Isopropylphenyl)phosphorothioate was prepared by treating *o*-isopropylbenzenesulfenyl chloride [bp 60° (5 mm)] with dibenzyl phosphite in the presence of triethylamine in ether by the same procedure employed for synthesis of *para*-substituted derivatives[6] and then debenzylating by hydrogenation using a Pd–$BaSO_4$ catalyst in tetrahydrofuran as the solvent. The O,O-dibenzyl thiophosphate triester crystallized upon standing at 4° and melted at 39° after recrystallization from ether.

Anal. Calcd for $C_{23}H_{25}O_3PS$: C, 66.97; H, 6.07; P, 7.53. Found: C, 66.76; H, 6.07, P, 7.45.

Examination of the infrared spectrum showed the band due to P–H in the starting material at 4.1 μ to be absent. The dicyclohexylammonium salt of S-(2-isopropylphenyl)phosphorothioate was prepared after debenzylation of the triester.

Anal. Calcd for $C_{21}H_{37}N_2O_3PS$: P, 7.22. Found: P, 7.40.

Dicyclohexylammonium S-(4-methylphenyl)phosphorothioate was prepared by the same procedure as employed previously for other compounds in the series.[6]

Anal. Calcd for $C_{19}H_{35}N_2O_3PS$: C, 56.70; H, 8.76; N, 6.97; P, 7.70. Found: C, 56.45; H, 9.03; N, 6.94; P, 7.50.

Kinetic Measurements.—The rates of hydrolysis were measured spectrophotometrically employing the same equipment and procedures previously described.[6] S-(2-Carboxyphenyl)phosphorothioate, obtained as the free acid, is soluble in acetonitrile. Stock solutions, therefore, were prepared at 10^{-2} *M*, and 30 μl was added to each cuvette containing 3 ml of buffer solution to make a 1% acetonitrile solution. The rates of hydrolysis were followed by measuring the increase in absorbance at 245 mμ for S-(2-carboxyphenyl)phosphorothioate and at 270 mμ for the 4-COOH derivative. The rates were generally followed until at least 75% of the reaction had been completed, and infinity points were taken at 10 half-lives. Pseudo-first-order rate constants were calculated with an Olivetti-Underwood Programma 101 programmed to calculate a least-squares evaluation of the slope and intercept of a plot of ln $[(OD_\infty - OD_0)/(OD_\infty - OD_t)]$ *vs.* time. All buffers contained 10^{-5} *M* EDTA to eliminate heavy metal ion catalyzed oxidation of the thiols. Buffer solutions were deoxygenated by bubbling in nitrogen for several minutes prior to addition of the substrate.

Determination of Ionization Constants.—The ionization constants of the free thiols were determined spectrophotometrically by the method of Flexser.[12] The pK_a's of the thiophenols at 35° in 1% acetonitrile–water (μ = 1.0 *M*) follow: unsubstituted, 6.43;[6] 4-chloro, 5.70;[6] 4-nitro, 4.42;[6] 4-methyl, 6.50; 4-carboxyl, 5.86; 2-carboxyl, 7.79, and 2-isopropyl, 7.02. *n*-Butyl mercaptan was found to have a pK_a of 10.3 under the same conditions. A plot of pK_a *vs.* σ, the Hammett substituent constant,[13] was linear with a ρ of −2.1. Bordwell and Andersen[14] previously found a ρ of −2.578 for the pK_a's determined in 48% ethanol–water.

Ionization constants for S-(2-carboxyphenyl) phosphorothioate were determined at 25° by the method of half-neutralization. The values of pK_2 and pK_3 in H_2O were 3.21 and 5.69, respectively. Reproducibility was reasonably good (±0.1 pK unit), considering the lability of the compound. The values of these constants in D_2O were 3.48 and 5.90.

Results

In Table I are presented rate constants for the hydrolysis of S-(2-carboxyphenyl)phosphorothioate in H_2O and D_2O at 35° and at various pH or pD values.[15]

TABLE I

RATE CONSTANTS (k_{obsd} × 10^2 MIN^{-1}) FOR HYDROLYSIS OF S-(2-CARBOXYPHENYL)PHOSPHOROTHIOATE, S-(2-ISOPROPYLPHENYL)PHOSPHOROTHIOATE, AND S-(4-METHYLPHENYL)PHOSPHOROTHIOATE AT VARIOUS pH VALUES, 35°, AND μ = 1.0 *M* KCl

pH	pD	Buffer[a]	2-Carboxy	2-Isopropyl	4-Methyl
7.50		Tris		0.323	
6.95		Phosphate	1.01	0.421	0.447
6.40		Acetate	2.58		
6.05		Acetate			0.751
5.82		Acetate	6.77		
5.22		Acetate	11.00		
	5.07	Acetate	11.90		
4.87		Acetate	13.30	4.78	
4.38		Acetate	14.40		2.35
	4.32	Acetate	12.20		
3.87		Acetate	14.90	5.38	2.50
	3.44	Formate	9.37	3.23	
3.33		Acetate	14.60	5.06	2.51
2.30		Formate			2.33
2.17		HCl	12.00		
	2.17	DCl[b]	6.04		
1.03		HCl	10.10	4.53	2.02
0		1 *M* HCl	7.16	1.89	0.836
		2.95 *M* HCl	6.14		
		5.90 *M* HCl	6.40	1.37	
		6 *M* DCl[b]	3.84		

[a] 0.05 *M*. [b] In 99.8% D_2O.

The values of k_{obsd} are shown plotted *vs.* pH or pD in Figure 1. It can be seen that a rate maximum is observed around pH 4. The lines are theoretical and were calculated employing the measured dissociation constants and as the values of the rate constants in H_2O: $k_{neutral}$ = 0.064 min^{-1}; $k_{monoanion}$ = 0.125 min^{-1}; and $k_{dianion}$ = 0.16 min^{-1}. Those in D_2O were as follows: $k_{neutral}$ = 0.0384 min^{-1}; $k_{monoanion}$ = 0.06 min^{-1}; and $k_{dianion}$ = 0.135 min^{-1}. The values of pK_1 used for constructing the theoretical pH-rate profiles were 0.8 in H_2O and 1.3 in D_2O. The values of $k_{neutral}$ were taken

(12) L. A. Flexser, L. P. Hammett, and A. Dingwall, *J. Amer. Chem. Soc.*, **57**, 2103 (1935).

(13) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII; H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).

(14) F. G. Bordwell and H. M. Andersen, *J. Amer. Chem. Soc.*, **75**, 6019 (1953).

(15) The glass electrode correction formula of T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961), was employed in the determination of pD.

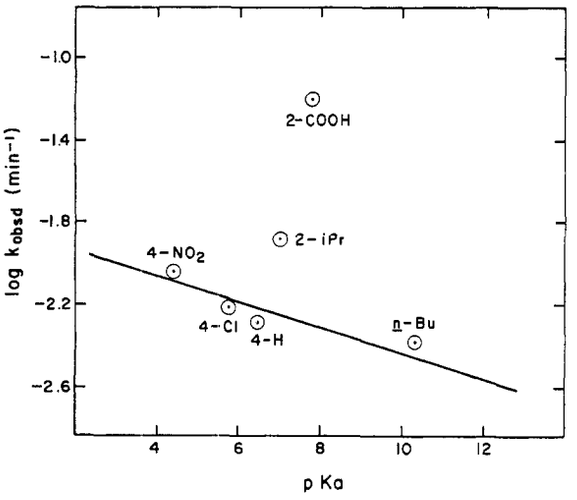


Figure 2.—Plot of log k_{obsd} for hydrolysis of the series of S-(substituted phenyl)phosphorothioates and S-(*n*-butyl)phosphorothioate in 5.90 *M* HCl (4-H, and 4-NO_2 in 6.12 *M* HCl) *vs.* the pK_a of the thiol leaving group.

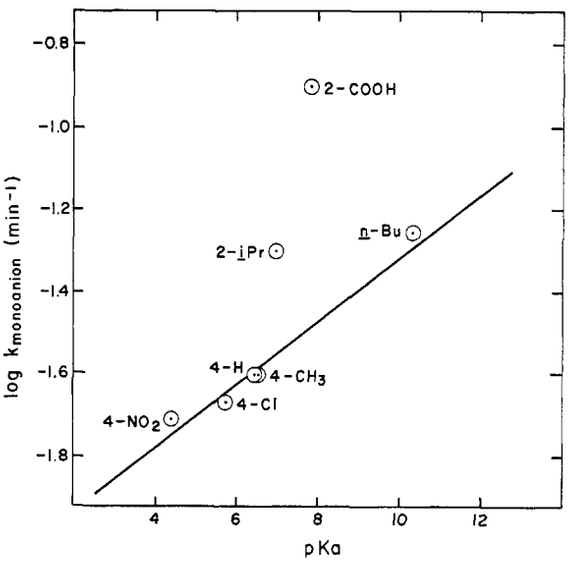


Figure 3.—Plot of log $k_{monoanion}$ for the series of S-(substituted phenyl)phosphorothioates and S-(*n*-butyl)phosphorothioate *vs.* the pK_a of the thiol leaving group.

to be the observed rate constants in 5.90 *M* HCl or 6 *M* DCl. k_{H_2O}/k_{D_2O} ratios follow: neutral species, 1.7; monoanion, 2.1; and dianion, 1.2. In Table I are also presented rate constants for hydrolysis of S-(2-isopropylphenyl)phosphorothioate and S-(4-methylphenyl)-phosphorothioate.

The rate constant for hydrolysis of S-(4-carboxyphenyl)phosphorothioate in 5.90 *M* HCl was 0.0102 min^{-1}, while at the pH rate maximum (pH 4) it was 0.030 min^{-1}. Thus, the *ortho*-carboxyl-substituted derivative hydrolyzes 5.0 times faster than the *para*-carboxyl derivative at the rate maximum and 6.3 times faster in 5.90 *M* HCl where the compounds are predominantly in the neutral form.

A plot of the logarithms of the rate constants for hydrolysis of the neutral species *vs.* the pK_a of the thiol leaving group for the series of S-(4-substituted phenyl)-phosphorothioates is shown in Figure 2. Inclusion of data for S-(*n*-butyl)phosphorothioate[16] allows the correlation to be made over approximately 6 pK units, and it can be seen that the point for the alkyl derivative fits well on the line with the S-arylphosphorothioates. The linear plot has a slope of −0.06. The point for the *ortho*-carboxyl-substituted compound deviates in a positive manner by 1.10 log units. In Figure 3 is shown a plot of the logarithms of the rate constants for hydrolysis of the monoanionic species of the series of phosphorothioates *vs.* the pK_a of the leaving group. The slope is 0.08. Data for hydrolysis of the monoanion of S-(*n*-butyl)phosphorothioate[16,17] has also been included, and again the point for that compound deviates only slightly from the line. The point for *o*-COOH, however, shows a positive deviation of 0.60 log units.

The rates of hydrolysis of S-(2-carboxyphenyl)phosphorothioate were determined at 25, 35, 45, and 55° ± 0.1°. Rate constants at the various temperatures are reported in Table II. Activation parameters are given in Table III. The errors reported in ΔH^* and ΔS^* were calculated from the standard error of plots of ln k_{obsd} *vs.* $1/T$.

TABLE II

RATE CONSTANTS ($k_{obsd} \times 10^2$ MIN^{-1}) FOR HYDROLYSIS OF S-(2-CARBOXYPHENYL)PHOSPHOROTHIOATE AT VARIOUS TEMPERATURES

Temp. °C	pH 3.30	5.90 *M* HCl
25	3.92	2.14
35	14.60	6.40
45	50.40	17.60
55	135.2	

TABLE III

ACTIVATION PARAMETERS FOR HYDROLYSIS OF S-(2-CARBOXYPHENYL)PHOSPHOROTHIOATE

pH	ΔH^* kcal/mol	ΔS^* eu[a]
3.30	22.4 ± 0.5	2.5 ± 1.6
5.90 *M* HCl[b]	19.2 ± 0.03	−9.7 ± 0.1

[a] Calculated at 35° with the rate constants having the units sec^{-1}. [b] Neutral species.

An attempt was made to detect a possible acyl phosphate intermediate in the hydrolysis of S-(2-carboxyphenyl)phosphorothioate dianion. A 5×10^{-3} *M* solution of substrate was allowed to hydrolyze in pH 5.20 acetate buffer at 35°. At 20, 40, 60, and 80% of the reaction 1.0-ml aliquots were withdrawn and analyzed by the hydroxamate method of Lipmann and Tuttle.[18] No hydroxamic acid could be detected. A tenfold increase in thiolphosphate concentration was without effect. In a similar experiment the substrate was dissolved in buffered hydroxylamine solution (pH 7.0), and the solution was allowed to stand 24 hr at room temperature. Again no hydroxamic acid could be observed. High concentrations of thiosalicylic acid are partially insoluble in water, and a precipitate was noticed upon addition of HCl during the analysis. To dissolve this precipitate, 3 ml of purified dioxane was added to each sample before addition of the $FeCl_3$ reagent. A very slight color was observed which was due to a ferric–thiosalicylate complex since the color

(16) D. C. Dittmer, O. B. Ramsay, and R. E. Spalding, *J. Org. Chem.*, **28**, 1273 (1963).

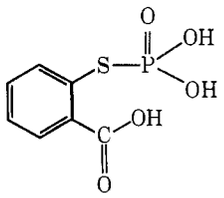
(17) E. B. Herr, Jr., and D. E. Koshland, Jr., *Biochim. Biophys. Acta*, **25**, 219 (1957).

(18) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

could be discharged upon further addition of HCl. Reaction with hydroxylamine is a standard technique for measuring the concentration of aqueous solutions of acyl phosphates.[18,19] Thus, detectable amounts of acyl phosphate are not present.

Discussion

In regard to the kinetics of the hydrolysis of S-(2-carboxyphenyl)phosphorothioate a number of species of different ionization state must be considered. The



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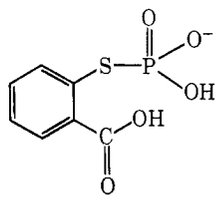

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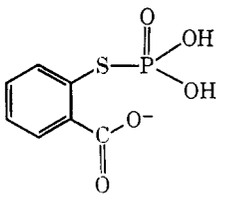

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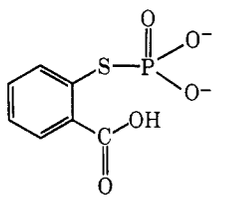

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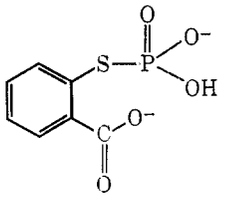

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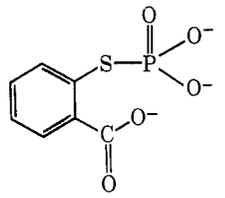
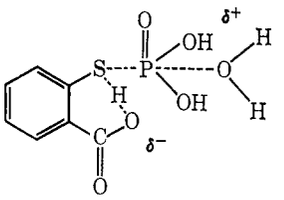


VI

trianionic species VI is quite stable as can be seen in Figure 1 from the lack of reactivity at high pH values.

There is no apparent hydronium ion catalysis of the hydrolysis of S-(2-carboxyphenyl)phosphorothioate or of other S-monoaryl thiolphosphates,[6] the rates being nearly invariant at HCl concentrations from 1 to 6 *M*. In 5.90 *M* HCl the compounds exist almost entirely as the neutral species, form I. The logarithms of the rate constants for neutral species hydrolysis are a linear function of the pK_a of the leaving group (Figure 2) with a slope of -0.06. The point for the *ortho*-carboxyl-substituted compound, however, shows a marked positive deviation indicating that the carboxyl group is accelerating the reaction. While a direct comparison with the corresponding *para*-substituted derivative shows an enhancement in rate by the *ortho*-carboxyl group of 6.3 times, the comparison on the basis of fit to the plot of log k *vs.* pK_a of the leaving group shows an enhancement of nearly 13 times. This is the result of the higher pK_a for the thiol group of thiosalicylic acid compared with *p*-mercaptobenzoic acid. The rate is slower in D_2O than H_2O ($k_{H_2O}/k_{D_2O} = 1.7$, similar to the ratio found for hydrolysis of other S-monoarylphosphorothioate neutral species).[6] Thus, it is likely that the reaction involves proton transfer that is partially rate determining.[20] In view of the negative values of ΔS^* and small susceptibility to electronic effects, it is likely that in addition to proton transfer, nucleophilic attack by a water molecule is occurring in neutral species hydrolysis,[6] as in VII. The value of ΔS^* for hydrolysis of the S-(2-carboxyphenyl)phosphorothioate neutral species, -9.7 eu, is slightly less negative than was observed previously for the other members of the series, but is much more negative than found for monoanion and dianion hydrolysis.[6]



VII

Proton transfer from a neighboring carboxyl group might be more favorable than from a phosphate OH since a six-membered hydrogen-bonded ring can be formed rather than four-membered or six-membered through a solvent molecule. Steric effects by the *ortho* substituent are also influencing the observed rate since S-(2-isopropylphenyl)phosphorothioate hydrolyzes 2.5 times faster than S-phenylphosphorothioate in 6 *M* HCl.

From Figure 1 it can be seen that ionization produces a considerable rate enhancement. Employing the dissociation constants found for S-(2-carboxyphenyl)phosphorothioate it is not possible to construct a theoretical pH-rate profile giving a good fit to the data if it is assumed that a neutral species reaction plus only a monoanion or only a dianion reaction is being observed. At the pH-rate maximum the dianionic species would be predominant, and the fast rate of hydrolysis at pH 4 must be due primarily to that species, but it is apparent that both the monoanion and the dianion are highly reactive and are contributing to the profile. Species II and III and IV and V are, of course, kinetically indistinguishable.

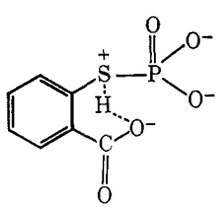
The carboxyl group may be participating in the monoanion reaction in the unionized form (species II) by a proton transfer mechanism. The D_2O solvent isotope effect for monoanion hydrolysis ($k_{H_2O}/k_{D_2O} = 2.1$) is consistent with the occurrence of partially rate determining proton transfer. However, the deviation produced by the *ortho*-carboxyl group in the plot of log $k_{monoanion}$ *vs.* pK_a of the leaving group is not large and steric effects are undoubtedly of some importance. The large solvent isotope effect might give some support for schemes involving III as a reactive monoanion. It has been shown that the D_2O solvent isotope effect for monoanion hydrolysis decreases as the pK_a of the leaving group becomes higher,[6] k_{H_2O}/k_{D_2O} being 1.78 for hydrolysis of S-(4-nitrophenyl)phosphorothioate monoanion and 1.4 with S-phenylphosphorothioate mono-

(19) G. Di Sabato and W. P. Jencks, *J. Amer. Chem. Soc.*, **83**, 4400 (1961).

(20) It should be noted that differences in stretching and bending frequencies of H–S and H–O bonds might give rise to different ratios of k_{H_2O}/k_{D_2O} for hydrolysis of oxygen and thiolphosphate esters even if preequilibrium zwitterion formation was occurring. See, for example, G. E. Lienhard and W. P. Jencks, *J. Amer. Chem. Soc.*, **88**, 3982 (1966). The value of the D_2O solvent isotope effect for hydrolysis of the monoanion of S-phenylphosphorothioate, however, is fairly small ($k_{H_2O}/k_{D_2O} = 1.4$), and this ratio is only 1.2 for hydrolysis of the dianion of S-(2-carboxyphenyl)phosphorothioate; so it seems improbable that such effects could completely explain the larger isotope effects found for other phosphorothioate neutral species and monoanions.[6]

anion. These ratios are in accord with proton transfer becoming more nearly complete in the transition state as P–S bond breaking becomes more difficult. Therefore, in view of the relatively high pK_a of the thiosalicylic acid leaving group, if the monoanion reaction was analogous to that for the other compounds only a small solvent isotope effect would be expected. The fairly large D_2O solvent isotope effect for neutral species hydrolysis, even when the leaving group has a high pK_a, is probably due to proton transfer being partial since P–S bond breaking necessary to reach the transition state would be reduced because of nucleophilic attack by solvent.

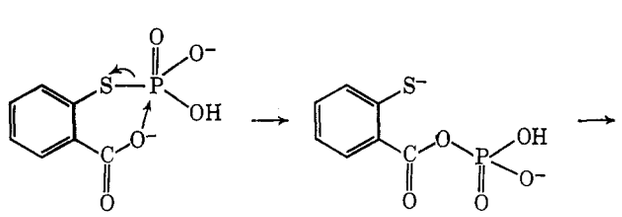
The rate of hydrolysis of the dianionic species of S-(2-carboxyphenyl)phosphorothioate is faster than that of any of the phosphorothioate monoanions studied and is five times faster than that of the corresponding *para*-carboxyl derivative. The dianion rate constant is approximately one-half that of the *p*-NO_2-substituted compound and 14 times *greater* than that of the *p*-Cl derivative.[6] Phosphorothioate dianion reactions are strongly aided by electron withdrawal,[6] and both 4-nitro- and 4-chlorothiophenol are much better leaving groups than thiosalicylic acid. The fast dianion rate cannot then be attributed to inductive effects. The dianionic species hydrolyzes with only a small D_2O solvent isotope effect ($k_{H_2O}/k_{D_2O} = 1.2$). The value of the D_2O solvent isotope effect should be at a maximum when the system O---H---X is symmetrical.[21] If proton transfer is occurring, therefore, it must either be essentially complete, as with species VIII, or else must not have proceeded to an appreciable extent. The relatively high



VIII

pK_a of thiosalicylic acid would make P–S bond breaking extremely difficult without concurrent protonation as shown by the stability of the trianion, VI.

Nucleophilic attack by carboxylate anion at phosphorus to give an acyl phosphate intermediate would be expected to proceed without a substantial D_2O solvent isotope effect, but attempts to demonstrate the presence of acyl phosphate by reaction with hydroxylamine



products (2)

gave negative results. S-(2-Carboxyphenyl)phosphorothioate decomposes much faster than substituted benzoyl phosphates.[17] As a consequence, if an acyl phosphate intermediate was being formed, it should be present in significant amounts. Nucleophilic catalysis, therefore, can be ruled out. Bender and Lawlor[4] found no evidence for nucleophilic attack by the carboxylate anion in the hydrolysis of salicyl phosphate. The D_2O solvent isotope effect near unity for hydrolysis of the dianion of that compound is consistent with proton transfer to a zwitterion intermediate.

The possible explanations for the facilitation of the phosphorothioate dianion rate by the neighboring carboxyl group then are (a) partial proton transfer or preequilibrium zwitterion formation, (b) electrostatic facilitation, or (c) steric acceleration due perhaps to relief of crowding in the ground state by stretching of the P–S bond in the transition state. The general accelerating effect of various *ortho* substituents in other hydrolytic reactions, phenyl β-D-glucopyranoside hydrolysis for example,[22] has been previously observed. Bulky *ortho* substituents, however, slightly reduce the rate of hydrolysis of phenyl phosphate monoanions.[23] In contrast, S-(2-isopropylphenyl)phosphorothioate monoanion was found to hydrolyze slightly faster than S-phenylphosphorothioate monoanion, but the *o*-isopropyl group has a much smaller rate-enhancing effect than *ortho* carboxyl.[24] At higher pH where the dianion would be the predominant species, the rate of hydrolysis of the *o*-isopropyl derivative is quite slow.[24] Thus, it is improbable that the fast dianion rate for S-(2-carboxyphenyl)phosphorothioate can be accounted for completely on the basis of steric effects.

It is of interest that the apparent rate enhancement due to an *ortho*-carboxyl group observed with S-(2-carboxyphenyl)phosphorothioate is less than the factor of 10^2 between the rates of hydrolysis of the *o*- and *p*-carboxyphenyl phosphate dianions involving a zwitterion intermediate.[2,4] If VIII is the reactive dianion, then the smaller rate enhancement by the *ortho*-carboxyl group in comparison with the oxygen esters would have to be attributed to a smaller enhancement in stability of the zwitterionic species when the proton is on sulfur. A still smaller facilitation is seen in phosphoramidate hydrolysis.[5]

It is also of interest that the observed rate constants at the pH-rate maxima are almost identical for S-(2-carboxyethyl)phosphorothioate[25] and S-(*n*-butyl)phosphorothioate.[16,17] The observed carboxyl group effect in the case of the 2-carboxyphenyl derivative is, therefore, very likely a function of correct steric positioning. If, on the other hand, the carboxyl group is free to rotate out of correct alignment, as with the alkyl derivative, little enhancement will be observed. The importance of "steric fit" in chemical and presumably in enzymatic hydrolysis reactions is thus again illustrated.[26]

Registry No.—S-(2-Carboxyphenyl)phosphorothioate, 21317-44-2; S-(2-isopropylphenyl)phosphorothioate,

(21) F. H. Westheimer, *Chem. Rev.*, **61**, 265 (1961).

(22) R. L. Nath and H. N. Rydon, *Biochem. J.*, **57**, 1 (1954).

(23) C. A. Bunton, E. J. Fendler, E. Humeres, and K. Yang, *J. Org. Chem.* **32**, 2806 (1967).

(24) The value of $k_{monoanion}$ for hydrolysis of S-(2-isopropylphenyl) phosphorothioate at 35° is 0.05 min^{-1} while k_{obsd} at pH 7.50 is 0.0032 min^{-1}. In comparison S-phenyl phosphorothioate has $k_{monoanion}$ = 0.025 min^{-1} and k_{obsd} at pH 7.50 = 0.0048 min^{-1} (see ref 6).

(25) S. Akerfeldt, *Acta Chem. Scand.*, **15**, 575 (1961).

(26) T. C. Bruice and U. K. Pandit, *Proc. Nat. Acad. Sci. U. S.*, **46**, 402 (1960); T. C. Bruice and U. K. Pandit, *J. Amer. Chem. Soc.*, **82**, 5858 (1960).

21317-45-3; dicyclohexylammonium S-(4-methylphenyl)phosphorothioate, 21317-46-4.

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The Reaction of Phenols with Ethyl Azodicarboxylate

SIEGFRIED H. SCHROETER

General Electric Research and Development Center, Schenectady, New York

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The thermal reaction of some 2,4-, 2,6-, and 2,4,6-substituted phenols with ethyl azodicarboxylate has been studied. In general, both C–C coupling products derived from the phenoxy radicals as well as coupling products from the phenoxy and the hydrazodicarboxylate radicals are formed. The latter compounds were independently prepared by the acid-catalyzed addition of ethyl azodicarboxylate to the phenols. 2,4,6-Tri-*t*-butylphenol reacts with ethyl azodicarboxylate under surprisingly mild conditions with loss of a 2-*t*-butyl group to yield 1-(2-hydroxy-3,5-di-*t*-butyl)phenyl 1,2-ethyl hydrazodicarboxylate. No reaction is observed between azodicarboxylate and phenols of high oxidation potential such as 2-chloro-, 2,6-dichloro-, 2,6-dinitro-, or 2-chloro-6-phenylphenol.

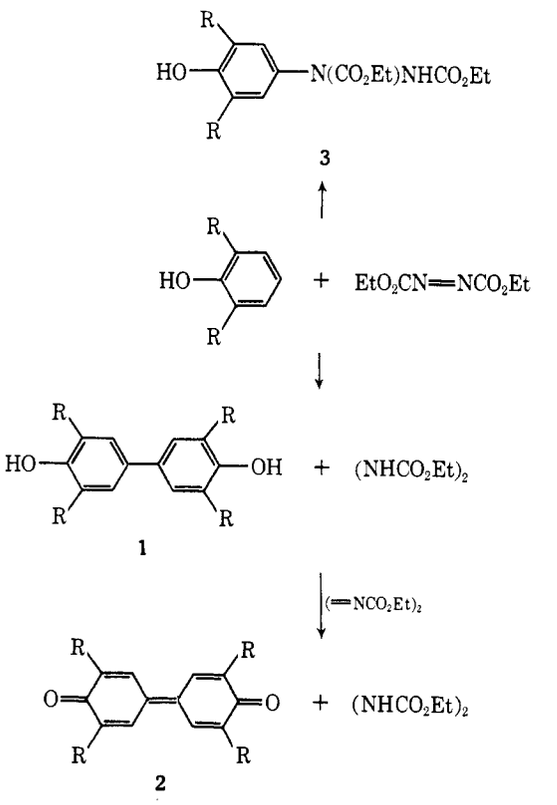
Azo-disubstituted compounds possessing electron-withdrawing substituents, among which azodicarboxylates are the most readily available, are known to show a strong tendency to abstract hydrogen atoms from suitable donors. Alcohols,[1-3] mercaptans,[3] hydrazo compounds,[3] and hydroquinones[4] are readily oxidized by ethyl azodicarboxylate to the corresponding carbonyl compounds, disulfides, azo compounds, and quinones, respectively. Other compounds possessing labile hydrogens such as benzylic hydrocarbons,[5] aldehydes,[6a] or formates[6] react with azodicarboxylates to give the coupling products of the resulting carbon and hydrazo radicals.[7]

$$R'H + RN{=}NR \longrightarrow R'NR\text{–}NHR$$

Azo esters might then be expected to be good oxidizing agents for phenols. However, no report of such reactions has been made, although the reaction of phenols with a large number of oxidizing agents has been studied intensively.[8] Huisgen and coworkers[5] described the use of phenols as inhibitors in the radical addition of azo esters to hydrocarbons, but did not characterize any of the products derived from the phenols. The oxidation of phenols with ethyl azodicarboxylate was therefore examined.

2,6-Disubstituted Phenols.—Phenols of low oxidation potentials such as the 2,6-dialkyl-substituted compounds readily reacted with ethyl azodicarboxylate to yield the C–C coupling products (**1** and/or **2**) derived from the phenoxy radicals as well as the addition products (**3**) of the azo ester to phenols (Scheme I).

SCHEME I



As the low solubility of 3,3′,5,5′-tetramethyldiphenoquinone (**2a**) makes the determination of this compound rather easy, the reaction of 2,6-dimethylphenol was studied in some detail (Table I). Oxidation with azo ester proceeded slowly at room temperature, but with a remarkable rate at 50° (Table I, runs 12–15, 1–3). Equimolar amounts of phenol and azo ester gave a 50% yield of the diphenoquinone and an equimolar amount of ethyl hydrazodicarboxylate when the reaction was carried out at a high concentration of the reactants. With increasing dilution, the amount of diphenoquinone formed decreased (runs 1, 10, 11) and more of the 4,4′-dihydroxybiphenyl (**1a**) and the addition product

(1) G. O. Schenck and H. Formanek, *Angew. Chem.*, **70**, 505 (1958).
(2) R. C. Cookson, I. D. R. Stevens, and C. T. Watts, *Chem. Comm.*, 259 (1965).
(3) F. Yoneda, K. Suzuki, and Y. Nitta, *J. Amer. Chem. Soc.*, **88**, 2328 (1966).
(4) O. Diels and P. Fritsche, *Chem. Ber.*, **44**, 3018 (1911).
(5) R. Huisgen, F. Jakob, W. Siegel, and A. Cadus, *Ann. Chem.*, **590**, 1 (1954).
(6) (a) K. Alder and T. Noble, *Chem. Ber.*, **76**, 54 (1943); (b) R. Huisgen and F. Jakob, *Ann. Chem.*, **590**, 37 (1954).
(7) Addition products may also be formed *via* a concerted mechanism; see W. A. Thaler and B. Franzus, *J. Org. Chem.*, **29**, 2226 (1964), and references cited therein.
(8) (a) V. V. Ershov, A. A. Volod'kin and G. N. Bogdanov, *Russ. Chem. Rev.*, **32**, 75 (1963); (b) H. Musso, *Angew. Chem.*, **75**, 965 (1963); (c) K. U. Ingold, *Chem. Rev.*, **61**, 563 (1961); (d) "Oxidative Coopling of Phenols," W. I. Taylor and A. R. Battersby, Ed., Marcel Dekker, Inc., New York, N. Y., 1967.