mmol) of allyl ethyl sulfide and 20 mg of anhydrous cupric sulfate was sealed in a Pyrex tube and heated at 110° for 3 min. Analysis by infrared spectrum revealed that the reaction was over. Gas chromatography showed one major peak and one minor peak. Spectral analyses indicated that the major peak is the insertion product of the carbene into the carbon–sulfur bond, and the minor peak is the addition product.

Registry No.—IB-In, 35906-23-1; IB-r, 35906-24-2; IIA-In, 35906-25-3; IIB-In, 35906-26-4; IIIB-In, 35906-27-5; IIIB-E, 35906-28-6; VIIA-In, 35906-29-7; XII, 35906-30-0; XIII, 35906-31-1; XIII B-r, 35906-32-2; XIV, 35906-33-3; XV-a, 35906-34-4; XV-i, 35905-68-1; XVI-i, 35905-69-2; XVII-a, 35905-70-5; XVII-i, 35905-71-6; XVIII-a, 35905-72-7; XVIII-i, 35905-73-8; XIX-a, 35905-74-9; XIX-i, 35905-75-0; XX-i, 35905-76-1; XXI-a, 35905-77-2; XXI-i, 35905-78-3; phenyldiazomethane, 766-91-6; diphenyldiazomethane, 833-40-9.

Model Systems Related to Reactivity of Protein Sulfur Functions. I. The Effect of Hydrophobic Bulk on Acid Strengths of Alkyl-Substituted Benzenethiols and on Nucleophilicities of the Benzenethiolate Anions toward *N*-Ethylmaleimide[1]

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Apparent acid dissociation constants of a series of alkyl-substituted benzenethiols in 95% ethanol are measured by the method of fractional neutralization. Thiol pK_a's correlate well with the pK_a's of the corresponding phenols. Electronic and steric substituent effects are separated (1) by comparison of para- and ortho-substituted isomers and (2) by a Hammett linear-free-energy correlation. The substantially lower acidity of 2-*tert*-butylbenzenethiol compared to the other benzenethiols studied is attributed to steric inhibition of solvation of the thiolate anion rather than to direct steric interactions between the *tert*-butyl group and the adjacent sulfur. The rates of addition of the alkyl-substituted benzenethiols to *N*-ethylmaleimide in 95% ethanol at 25° are reported. The rate of attack of ortho-alkyl-substituted benzenethiolate anion upon the olefinic double bond of *N*-ethylmaleimide is sensitive to the size or bulk of the alkyl group. Two effects are identified: (1) inhibition of solvation of the thiolate anion, which increases its nucleophilicity (rate accelerating), and (2) steric interference between the thiolate nucleophile and the olefin transition state (rate retarding). The first known example of net steric acceleration in a nucleophilic addition to an activated double bond is reported for *o-tert*-butylbenzenethiolate which is found to be an order of magnitude more reactive than the other alkylbenzenethiols studied. The implications of the results as regards hydrophobic bulk effects in enzymatic reactions involving mercaptide functions are discussed.

The specific modification of only the most reactive protein sulfhydryl groups with activated double bond reagents such as *N*-ethylmaleimide (NEM) and acrylonitrile has encouraged their use as probes of SH environment and catalytic involvement.[2] Although it is generally supposed that such factors as location in the three-dimensional protein structure, microscopic environment or neighboring group effect, and interaction with other functional groups determine the rates of SH addition across the double bond of NEM,[3] few studies to evaluate the relative importance of the various factors are available.[4] Furthermore, SH group acid strengths affect the differential nucleophilic reactivities of protein sulfhydryl groups with SH modification reagents. While thiol acid strength as a function of electrical substituent effects has been the focus of previous investigations,[5,6] the effects of hydrophobic bulk on thiol dissociation has remained relatively unexplored. The findings from several protein studies, which indicate that SH groups frequently lie in interior hydrophobic locations,[7,8] suggested that investigation of thiol acid strength and of thiolate nucleophilicity in reaction with NEM as a function of nearby hydrophobic bulk should contribute to the understanding of protein SH group reactivities. Thus, we now report pK_a and kinetic studies with a series of alkyl-substituted benzenethiols selected to assess steric effects on acidity and rates of addition to NEM.

Results

The series of thiols investigated included benzenethiol and its alkyl substituted derivatives: 4-methyl; 3-methyl; 2-methyl; 2,6-dimethyl; 4-*tert*-butyl; and 2-*tert*-butyl.

The solubility properties of the aromatic thiols dictated that the pK_a measurements be performed in 95% ethanol solvent, a medium which approximates more closely than does water the probable hydrophobic environment of many protein SH groups. The absolute pH values obtained in such a medium by the potentiometric methods are not subject to simple

(1) This investigation was supported in part by Grant No. GM 11094 from the Institute of General Medical Sciences, U. S. Public Health Service, and by Contract AT(04-3)-34, Project 102, of the U. S. Atomic Energy Commission; P. D. Boyer, principal investigator.

(2) L. Cohen in "Annual Review of Biochemistry," Vol. 37, P. D. Boyer, Ed., Annual Reviews, Inc., Palo Alto, Calif., 1968, p 695.

(3) J. F. Riordan and B. L. Vallee in "Methods in Enzymology," Vol. 11, C. H. W. Hirs, Ed., Academic Press, New York, N. Y., 1967, p 451.

(4) Quantitative estimates of the influence of some polar and steric reaction parameters on the rates of addition of cysteine derivatives to acrylonitrile in water have been reported: M. Friedman, J. F. Cavins, and J. S. Wall, *J. Amer. Chem. Soc.*, **87**, 3671 (1965).

(5) M. M. Kreevoy, E. T. Harper, R. E. Duvall, H. S. Wilgus, III, and L. T. Ditsch, *ibid.*, **82**, 4899 (1960).

(6) J. P. Danehy and C. J. Noel, *ibid.*, **82**, 2511 (1960).

(7) R. Cecil, "The Protons," Vol. 1, 2nd ed, H. Neurath, Ed., Academic Press, New York, N. Y., 1963, p 380.

(8) For example, in human hemoglobin the cysteinyl residue at G11 of the α subunit is inaccessibly located in the interior.[9] Cysteine G14 of the β chain is in the hydrophobic contact area between the α_1 and β_1 subunits, and only the sulfhydryl group at F9 protrudes into the medium.[9]

(9) M. F. Perutz, H. Muirhead, J. M. Cox, and L. C. G. Goaman, *Nature (London)*, **217**, 131 (1968); M. F. Perutz, *J. Mol. Biol.*, **13**, 646 (1965).

TABLE I
RELATIVE ACID DISSOCIATION CONSTANTS OF ARENETHIOLS (ArSH) IN 95% ETHANOL AT 25.0 ± 0.2°

ArSH[a]	Number of runs	pK_a, apparent av ± std dev	$K_{a,PhSH}/K_{a,ArSH}$
PhSH	8	9.58 ± 0.06	1.0
4-MePhSH	4	9.95 ± 0.01	2.4
3-MePhSH	4	9.80 ± 0.01	1.6
2-MePhSH	5	10.22 ± 0.03	4.5
2,6-DiMePhSH	6	10.77 ± 0.02	15.6
4-*tert*-BuPhSH	4	9.90 ± 0.02	2.1
2-*tert*-BuPhSH	8	11.64 ± 0.13	115

[a] Registry numbers are, respectively, 108-98-5, 106-45-6, 108-40-7, 137-06-4, 118-72-9, 2396-68-1, 19728-41-7.

interpretation owing to the indeterminate potential at the junction: solution X (95% alcohol)/KCl bridge (aqueous). However, Bates and coworkers[10] found that the liquid-junction potential is *constant* for different buffers in alcoholic solvents of *fixed* composition, so that the contribution of junction potential to measured pH is not reflected in the *relative* pK_a values.

The apparent acid dissociation constant data of the thiols as determined by the neutralization method are summarized in Table I. There is good agreement between the 30 and 50% neutralization values.

The spectrophotometric pK_a for 2-methylbenzenethiol is 10.44. The 0.2 discrepancy between this value and that obtained by the neutralization method may result in whole or in part from neglect of hydrolysis correction in the latter value.

Correction of the measured pH values for liquid-junction potential and medium salt effects according to the method of Bates, *et al.*,[10] would raise all of the pK_a values about 0.7 unit. As has been observed previously for carboxylic acids[11] and thiols,[12] replacement of an aqueous environment by one of higher hydrocarbon content (lower dielectric constant) such as 95% ethanol markedly weakens the acid strength.

Spectrophotometric determinations of the kinetics of the reaction of each of the alkyl-substituted benzenethiols with NEM were performed in 95% ethanol at a series of buffered pH's. Control experiments as described in the Experimental Section established the reliability of the kinetic assays, the assigned stoichiometry, the absence of side reactions, and the exclusive formation of the addition products, α-arylthio-*N*-ethylsuccinimides.

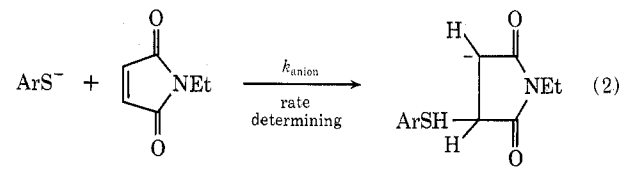
The kinetic data are summarized in Table II. The variation of k_{obsd} and constancy of k_{anion} over the range of pH investigated indicates that the thiolate anion is the sole reactive species in the rate-determining step which is consistent with the mechanism of eq 1–3. If the neutral thiol molecule were to show significant nucleophilic reactivity in the addition to NEM, the observed rate constant would be given by eq 4 and a plot of $k_{obsd}(H^+)$ *vs.* (H^+) would give a significant positive slope equal to k_{thiol}. Such a plot of the data reveals a zero slope within the limits of experimental error. Thus, k_{thiol} must be less than the experimental error of the data ($\leq 7\%$ of k_{anion}).

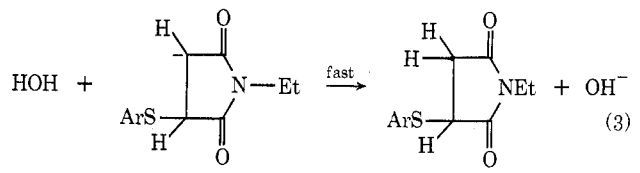
(10) R. G. Bates, M. Paabo, and R. A. Robinson, *J. Phys. Chem.*, **67**, 1833 (1963).

(11) E. Grunwald and B. J. Berkowitz, *J. Amer. Chem. Soc.*, **73**, 4939 (1951).

(12) B. Dmuchovsky, F. B. Zienty, and W. A. Vredenburgh, *J. Org. Chem.*, **31**, 865 (1966).

$$ArSH + OH^- \overset{K_a}{\rightleftharpoons} ArS^- + HOH \quad (1)$$





$$k_{obsd} = \frac{k_{anion}K_a}{(H^+)} + k_{thiol} \quad (4)$$

For all runs the fit of the data to an integrated second-order line gave a standard deviation of points from the line of ~1% or less of the k values. The standard deviations for the k_{obsd} values obtained at a given pH are ≤7% of the average values, and for the k_{anion} values for a given thiol over the entire range of 1.5 pH units are 2.2–7.2% of the average k_{anion} values.

The small (10%) rate increase observed with threefold increase in buffer concentration signifies that general acid catalysis by acetic acid and ionic salt effects are relatively unimportant.

The k_{anion} values for all ArS^- are comparable (within a factor of 2) except for 2-*tert*-butylbenzenethiolate anion for which the k_{anion} value is 17 times that for the unsubstituted benzenethiolate anion.

Discussion

Acidity.—For most chemical reactions of ortho-substituted reactants, the ortho effects observed are not primarily due to steric effects[13] except for groups of considerable bulk such as *tert*-butyl. Thus, it is predicted and borne out by the present acidity measurements reported in Table I that the *tert*-butyl compound is a considerably weaker acid than the other benzenethiols which are unsubstituted or substituted with the smaller methyl group in the ortho position.

The acid strengths of meta- and para-substituted benzenethiols and phenols are known to parallel each other.[14,15] We have found that this correlation continues to hold when extended to ortho substitution of a bulky group such as *tert*-butyl.[16] *o-tert*-Butylbenzenethiol is 55 times a weaker acid than is the para isomer as measured in 95% ethanol in the present work; the factor is 65 for the corresponding phenols where the

(13) M. Charton, *J. Amer. Chem. Soc.*, **91**, 615, 619, 624, 6649 (1969).

(14) G. Schwarzenbach and E. Rudin, *Helv. Chim. Acta*, **22**, 360 (1939).

(15) A linear least-squares regression was applied to the pK_a data of Schwarzenbach and Rudin[12] for 18 pairs of phenols and benzenethiols. A good straight line was obtained with slope $d(pK_a^{ArOH})/d(pK_a^{ArSH}) = 1.004$, standard deviation 0.016, and correlation coefficient 0.9978.

(16) A linear least-squares regression was employed to correlate the acidities of the benzenethiols of Table I and the corresponding phenols of Table III.[17] The straight line obtained had a slope of 0.983, a standard deviation of 0.033, and a correlation coefficient of 0.997. When the 2-*tert*-butyl derivatives were excluded from the correlation, the slope was 0.889, standard deviation 0.041, and correlation coefficient 0.996.

(17) (a) C. H. Rochester, *J. Chem. Soc.*, 676, 4603 (1965). (b) C. H. Rochester, *J. Chem. Soc. B*, 121 (1966). (c) C. H. Rochester, *Trans. Faraday Soc.*, **62**, 355 (1966). (d) C. H. Rochester and B. Rossall, *J. Chem. Soc. B*, 743 (1967). (e) C. H. Rochester, *Trans. Faraday Soc.*, **65**, 1004 (1969).

acid strengths shown in Table II were measured spectrophotometrically at 25° in methanol (Table III).[17,18]

The comparison between ortho-alkylated benzenethiols and the corresponding phenols illuminates the cause of the acid weakening effect of an *o-tert*-butyl group. Although sulfur is a much larger and more polarizable atom than oxygen, substitution of a *tert*-butyl group in the ortho position has similar effects on the acid strengths of both benzenethiol and phenol. The acid weakening effect in the thiol is evidently not due to severe steric repulsion between the sulfur and adjacent alkyl group since a similar effect is observed for the smaller oxygen atom. This conclusion is further supported by an examination of space-filling CPK molecular models which provide reasonable estimates of van der Waals radii. The models show that the sulfur and the *tert*-butyl groups, although in close proximity, can be accommodated in adjacent positions on the benzene ring without distortion. Any repulsion which may exist in the actual molecule can be relieved without loss of sulfur-ring π resonance by a bending of the sulfur–carbon bond *in the plane of the ring* away from the *tert*-butyl group. It is concluded that steric inhibition of resonance in the *o-tert*-butylbenzenethiolate anion does *not* explain the decrease in acidity of the corresponding thiol.

Rather, the large diminution of acid strength for *o-tert*-butylbenzenethiol is attributed to steric inhibition of solvation[21,22] by the adjacent hydrocarbon bulk in the ortho anion. The substitution of a large alkyl group of low effective dielectric constant and of poor hydrogen-bonding ability displaces solvent of higher dielectric constant and good hydrogen-bonding capacity. The exclusion of polar solvent molecules by the alkyl group from a region near a charged group, such as the thiolate anion, increases the free energy of the anion by reduction of solvation stabilization of the charge.

Steric inhibition of solvation of the unionized benzenethiols (*i.e.*, decreased hydrogen bonding of the –SH function with solvent) increases their acidities by raising the ground-state energies. This effect on acidity is in the opposite direction to the corresponding effect on the anions. However, solvation of the full negative charge of the anion is far more important energetically than solvation of the neutral species. Therefore, to a first approximation the effects of steric inhibition of solvation on the neutral thiol molecules may be ignored.

An estimate of the steric effects of *o*-methyl and *o-tert*-butyl substituents can be obtained by two methods. The first and simpler method is a comparison of the acidities of the *o*- and *p*-alkylbenzenethiols. Since the electron-donating capacities of the para alkyl groups are slightly greater than the corresponding ortho groups,[23] the amount of acid weakening observed for the para isomers may be considered as the maximum owing to destabilization of the anion by the *electronic* effects of a single ortho alkyl substituent. Further decrease in acidities of the ortho isomers beyond that attributable to electron release by the ortho alkyl groups (see Table I) is only a factor of 2 for the *o*-methyl compound, but reaches the considerable magnitude of 55, equivalent to a free-energy increment of 2.4 kcal/mol, for the *tert*-butyl species.

The second approach to assessment of steric inhibition to solvation for ortho substituents involves a Hammett σ^- plot[23,26] of the pK_a's of substituted benzenethiols as shown in Figure 1.[27]

The deviations of ortho-substituted benzenethiols from the line defined by the para and meta derivatives provides a measure of the importance of steric inhibition of solvation. The correlation for 14 unhindered aromatic thiols[28] has a slope of $\rho = 2.422 \pm 0.140$, correlation coefficient 0.981.[29] The deviations in pK_a units are 0.42 for 2-methyl, 0.68 for 2,6-dimethyl, and 1.98 for 2-*tert*-butyl substituents. These deviations correspond to free energies for the ortho "steric inhibition of solvation" effect of 0.6 kcal/mol for a single methyl, 0.9 kcal/mol for two methyls, and 2.7 kcal/mol for a *tert*-butyl group. The latter value agrees well with the simple estimate made above (2.4 kcal/mol) for the contribution of the effect of steric inhibition of solvation to the energy of the *o-tert*-butylbenzenethiolate anion.

Thus, the steric inhibition of solvation effect is negligible or minor for *o*-methyl substituents but attains a significant magnitude for a single *o-tert*-butyl group in this system. The large destabilization observed for this bulky group probably reflects the loss of one or more hydrogen bonds between the solvent and the thiolate anion.

Nucleophilicity.—The addition of ortho-substituted benzenethiolate anions to NEM may be subject to two opposing steric effects. The steric inhibition of solvation effect involves interference by the adjacent alkyl hydrocarbon bulk with protic solvent solvation of the anionic sulfur. Therefore, operation of this effect on the thiolate addition to NEM raises the energy of the thiolate ground state, lowers the desolvation component of the activation energy, and increases the rate constant, k_{anion}. A second effect decreases the rate of

(18) The ordering of substituent effects on acidities of a series of related phenols for a transfer from a given protic solvent to a related protic solvent should remain invariant.[17d,19,20] Thus, the fact that benzenethiol and phenol dissociation constants used in the correlation treatment were measured in different solvents (95% ethanol and methanol, respectively) does not invalidate the conclusion that the effect of *o-tert*-butyl substitution produces similar effects upon benzenethiol and phenol ionizations.

(19) L. A. Cohen and W. M. Jones, *J. Amer. Chem. Soc.*, **85**, 3397 (1963).

(20) B. W. Clare, D. Cook, E. C. F. Ko, Y. C. Mac, and A. J. Parker, *ibid.*, **88**, 1911 (1966).

(21) P. D. Bartlett, *J. Chem. Educ.*, **30**, 22 (1953); P. D. Bartlett, M. Roha, and R. M. Stiles, *J. Amer. Chem. Soc.*, **76**, 2349 (1954).

(22) G. S. Hammond and D. H. Hogle, *ibid.*, **77**, 338 (1955).

(23) Hammett sigma constants (σ^-) for para and meta substituents are tabulated by Ritchie and Sager;[24] the para values are −0.17 for methyl and −0.20 for *tert*-butyl. The ortho σ^- constants, apparently free from steric effects, are −0.13 for methyl and −0.08 for *tert*-butyl.[25]

(24) C. D. Ritchie and W. F. Sager in "Progress in Physical Organic Chemistry," Vol. 2, S. G. Cohen, A. Streitsieser, Jr., and R. W. Taft, Ed., Interscience, New York, N. Y., 1964, p 323.

(25) M. T. Tribble and J. G. Traynham, *J. Amer. Chem. Soc.*, **91**, 379 (1969).

(26) The σ^- constant for 2,6-dimethyl substitution was assumed to be twice the value for *o*-methyl, *i.e.*, −0.26.

(27) R. W. Taft, "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Chapter 13, Wiley, New York, N. Y., 1956.

(28) The pK_a values reported by Schwarzenbach and Rudin[14] in 95% ethanol at 20–22° were adjusted to the pK scale for values in Table I by addition of 0.25 pK unit. The value for 4-*tert*-butyl from this work (Table I) was included in the plot.

(29) Jaffé[30] obtained $\rho = 2.847 \pm 0.150$ for 12 benzenethiol pK_a's (Schwarzenbach and Rudin[14]) correlated with Hammett's original unadjusted σ (not σ^-). Bordwell and Andersen[31] obtained $\rho = 2.578$ for 12 arylthiol pK_a's (in 48% ethanol at 25°) when correlated with σ.

(30) H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).

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

TABLE II
SECOND-ORDER RATE CONSTANTS FOR REACTIONS OF *N*-ETHYLMALEIMIDE AND ARYLTHIOLS IN 95% ETHANOL AT 25.0 ± 0.2°

ArSH	Run no.	Initial concn × 10: $(ArSH)_0$	Initial concn × 10: $(NEM)_0$	pH[a-c]	% reaction completion on which k is based	k_{obsd}[d,e] (M^{-1} sec^{-1})	k_{anion}[d,f] × 10^{-4} (M^{-1} sec^{-1}) ± std dev
PhSH	1	19.64	5.56	5.43	33	1.20	1.67
	2	9.82	11.14	5.43	18	1.29	1.81
	3	9.78	10.63	6.24	60	8.59	1.86
	4	9.81	10.47	6.91	87	43.0	2.00
	5	9.81	10.47	6.91	70	45.3	2.10
	6	5.89	12.56	6.91	76	41.8	1.93
						Av	1.90 ± 0.14
4-MePhSH	7	8.56	9.10	5.43	33	1.27	4.22
	8	43.60	8.11	6.24	95	7.80	4.01
	9	11.11	8.47	6.24	50	7.81	4.01
	10	2.29	12.49	6.24	79	7.79	4.01
	11	4.45	7.37	6.91	60	38.1	4.19
	12	2.67	8.85	6.91	68	37.7	4.13
						Av	4.10 ± 0.09
	13	18.28	11.71	6.29[g]	64	9.64	4.41
	14	9.14	11.71	6.29[g]	59	10.1	4.64
						Av	4.53 ± 0.12
4-*tert*-BuPhSH	17	17.30	5.50	5.43	44	1.15	3.42
	18	15.00	5.05	6.24	68	8.60	3.96
	19	7.50	10.10	6.24	47	8.29	3.82
	20	7.13	10.43	6.91	67	39.3	3.87
	21	4.23	12.51	6.91	83	41.6	4.10
						Av	3.83 ± 0.23
3-MePhSH	22	21.09	5.57	5.43	74	1.20	2.81
	23	10.54	11.14	5.43	13	1.28	2.99
	24	18.89	5.16	6.24	74	7.29	2.63
	25	9.44	10.33	6.24	32	8.02	2.90
	26	5.37	12.52	6.91	73	37.3	2.88
						Av	2.84 ± 0.12
2-MePhSH	27	71.62	10.81	5.43	53	0.452	2.81
	28	67.88	10.66	5.43	24	0.441	2.75
	29	33.94	10.66	5.43	46	0.449	2.79
	30	33.21	10.44	6.24	66	3.20	3.07
	31	33.14	10.57	6.24	82	3.20	3.07
	32	16.57	10.57	6.24	55	3.04	2.91
	33	21.60	5.00	6.91	80	15.2	3.12
	34	10.81	10.00	6.91	64	15.2	3.12
						Av	2.96 ± 0.15
2,6-DiMePhSH	37	79.76	11.27	5.43	36	0.141	3.09
	38	39.88	11.27	5.43	28	0.139	3.04
	39	74.63	11.43	6.24	76	0.809	2.74
	40	37.31	11.43	6.24	54	0.816	2.75
	41	45.38	11.84	6.91	78	4.11	2.97
	42	45.38	11.84	6.91	85	4.23	3.02
	43	22.69	11.84	6.91	63	4.23	3.07
						Av	2.96 ± 0.14
2-*tert*-BuPhSH	46	13.25	13.25	6.91	60	6.26	33.6
	47	13.25	13.25	6.91	48	5.88	31.6
	48	13.25	13.25	6.91	35	6.44	34.5
	49	10.00	10.00	6.91	80	6.43	34.5
	50	25.00	10.00	6.91	72	6.19	33.2
	51	30.00	7.00	6.91	61	5.95	31.9
						Av	33.2 ± 1.14

[a] All pH measurements are uncorrected for junction potential (95% ethanol–water). [b] pH values are averages of five determinations; standard deviations are 0.02–0.03. [c] All buffers are 0.01 M in total acetic acid plus potassium acetate concentration unless otherwise specified. [d] Defined by equation $d(product)/dt = k_{obsd}(NEM)(ArSH) = k_{anion}(NEM)(ArS^-)$. [e] All k_{obsd} values are based on 5–13 kinetic points and for most runs on ≥7 points. [f] The pK_a values in 95% ethanol used in calculation of k_{anion} are reported in Table I. [g] Total concentration of acetic acid plus potassium acetate is 0.03 M.

anion attack on the double bond (rate constant k_{anion}) owing to steric crowding in the transition state. Thus, for the NEM reactions, the influences of the steric inhibition of solvation effect on the ground state and of steric crowding in the transition state are opposed.

Since in the transition state for the rate-determining step of eq 2, the anionic charge is dispersed over the conjugated NEM system as well as the benzenethiol system, steric hindrance to solvation of the transition state is relatively unimportant compared with that of the ground-state thiolate anion.

Whereas the predominance of the decelerating crowd-

ing effect is usually observed, as in the case of the decreased rate of tertiary alkyl thiolate addition to acrylonitrile relative to primary alkyl thiolate,[4] the remarkable feature of the current results (Table II) is that, for the *o-tert*-butylbenzenethiolate anion, the accelerating effect of steric inhibition of solvation prevails to render it 8.7 times as reactive as the para isomer. (The electronic effects would slightly favor the para thiolate.[32]) We may conclude that, although solvent is to some appreciable degree excluded from the vicinity of the anionic sulfur in the hindered ortho species as indicated by its increased basicity, the approach of the thiolate center to the olefinic carbon in NEM as required in the transition state is not prevented to the same degree. Presumably, the partially formed sulfur–carbon bond in the transition state is sufficiently long to accommodate the adjacent steric bulk.

The following estimation of the relative magnitudes of accelerating effect of steric inhibition of solvation and decelerating steric crowding effects employs the Brønsted correlation between the logarithms of the rate constants and the logarithms of the acid strengths (pK_a's). To the extent that the factors which determine the rates and acidities are identical, a linear relationship between the rate and acidity data will be obtained.

A Brønsted treatment by the present author of the pK_a data of Schwarzenbach and Rudin[14] and the kinetic data of Krishnamurthy and Miller[33] for the addition of seven thiols to the triple bond of ethyl phenylpropiolate gave a correlation coefficient of 0.984. Although no quantitative treatments of the data were performed, parallels have also been observed between thiol pK_a's and thiolate nucleophilicities for additions of arylthiolate anions to the double bond of maleic anhydride[12] and reactions of mercaptide ions with ethylene oxide.[6]

Divergence of a given thiolate from the linear Brønsted plot reflects a noncorrespondence in the factors determining the rate and acidity values. A Brønsted plot of the data from the present study in Figure 2 shows a linear relationship for the four isomers which lack ortho substituents; the ortho-substituted species deviate from the line so defined.[34] The steric inhibition of solvation effect influences both the k_{anion} and pK_a values in a comparable manner; so it does not contribute to the deviations. The only positive influence on the rate data which is *not* reflected in the acidity constants is steric crowding in the transition state. This is reasonable since formation of a partial bond between a thiolate sulfur and a carbon in the transition state for addition to NEM involves juxtaposition of two multiatomic molecules wherein steric repulsions can become important. By contrast, the proton is very small; so its capture by thiolate anion in the acid–base equilibrium would not be expected to be subject to strong steric hindrance. The increment in the free energy of activation due to steric crowding is estimated for the ortho-substituted thiolates from their deviations from the linear plot as 0.5 kcal/mol for a single methyl, 1.2 kcal/mol for two methyls, and 0.9 kcal/mol for a *tert*-butyl; the corresponding rate deceleration factors are 2.4, 7.9, and 4.5, respectively.

Correction for the steric crowding rate deceleration effect for the *o-tert*-butylbenzenethiolate addition to NEM, which shows the largest observed *net rate enhancement*, gives a total acceleration from steric inhibition of solvation by a factor of at least 39 for the ortho

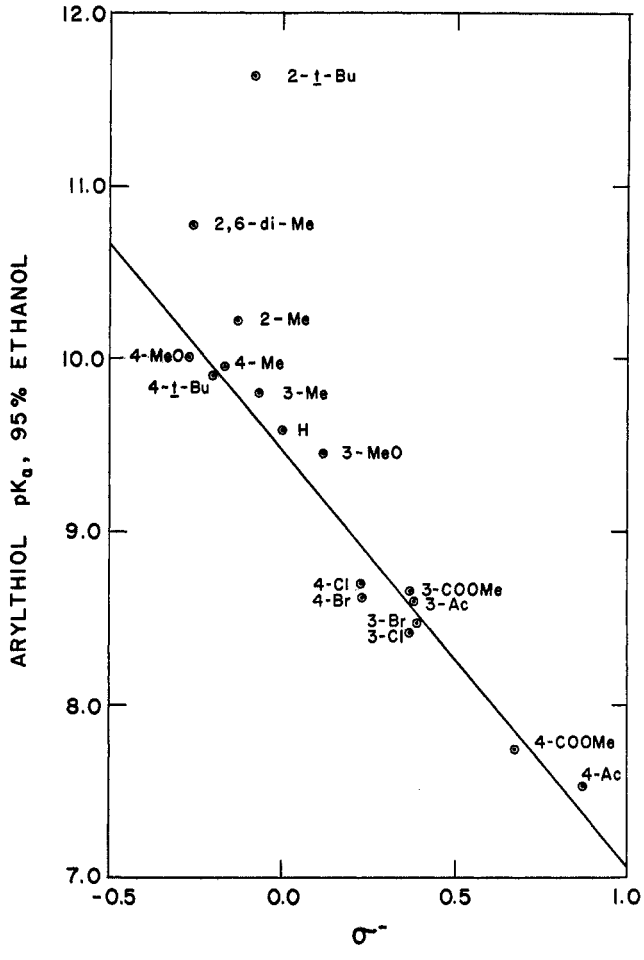


Figure 1.—Hammett σ^- correlation of benzenethiol acidities in 95% ethanol.

TABLE III
ACID DISSOCIATION CONSTANTS OF PHENOLS[a] (ArOH) IN METHANOL AT 25°

ArOH	pK_a
PhOH	14.46
4-MePhOH	14.76
3-MePhOH	14.59
2-MePhOH	15.01
2,6-DiMePhOH	15.48
4-*tert*-BuPhOH	14.65
2-*tert*-BuPhOH	16.46

[a] Reference 17.

(32) The reactive anionic center is slightly more negative for the para isomer; σ^- constants, apparently free from steric effects are, for *o-tert*-butyl, −0.08 and, for *p-tert*-butyl, −0.20.[23]

(33) G. S. Krishnamurthy and S. I. Miller, *J. Amer. Chem. Soc.*, **83**, 3961 (1961).

(34) As a consequence of the adverse physiological reactions experienced by all experimenters exposed to the thiols for continuous periods, the sequence of thiols was not extended beyond those reported in Table II. Thus, the Brønsted straight line of Figure 2 is based only on the data for the four thiols which lack ortho substituents and cover a limited range of about 0.4 pK units. The line obtained has a slope (Brønsted β) of 0.921, standard deviation of 0.070, and correlation coefficient of 0.994. An unlikely error of as much as 30% in β does not change the qualitative conclusion that the steric inhibition of solvation effect dominates over steric repulsions between reactants.

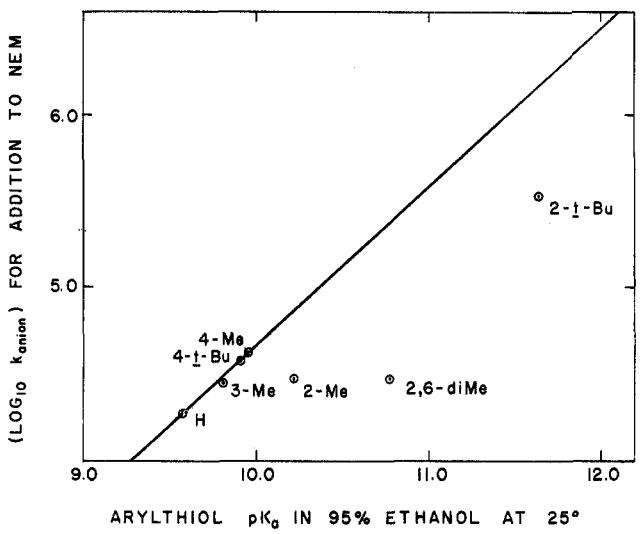


Figure 2.—A Brønsted plot of the arylthiol pK_a data and the arylthiolate–NEM reactivity values at 25° in 95% ethanol

over the para isomer,[35] a ratio which approaches the value of 55 observed for $K_{a,p\text{-}tert\text{-butyl}}/K_{a,o\text{-}tert\text{-butyl}}$.

The above interpretation of our results has implications for those enzymic reactions in which conversion of a thiol function to a thiolate anion is prerequisite to nucleophilic attack on carbon. Differences in the hydrophobic environment of several thiol groups in a single protein or for a single thiol group in two distinct protein conformations can probably produce variations in pK_a of 1–2 units or more. This finding emphasizes that, for cysteinyl residues imbedded in hydrophobic locales, the strategic proximity within the protein of basic functions capable of accepting a proton from the SH function would seem to be essential for the ionization prerequisite to nucleophilic reactivity.

While the present investigation specifically covers only thiolate nucleophilic attack on a trigonal olefinic carbon, it seems reasonable that the conclusions might be extended at least to thiolate reactions at trigonal carbonyl carbons such as in the formation of thiol esters. The thiol function may be situated on the enzyme itself or on a cofactor or substrate. The functionality susceptible to thiolate nucleophilic attack may be located on an external reagent such as NEM, acrylonitrile, or maleic anhydride; on an enzyme molecule which may or may not also harbor the reactive thiol; or on an enzymatic cofactor or substrate.

Our results indicate that optimum reactivity for such systems is attained by the strategic positioning of the thiol: (1) for accessibility to a basic function for proton removal; and (2) in a hydrophobic locale which excluded polar protic solvent molecules and thereby fosters a rate enhancement due to steric inhibition of solvation of the reactant thiolate, but which is sufficiently open and flexible or functionally configured to admit and allow reaction with substrates. Binding of substrate or cofactor or ionization of the thiol group may cause conformational changes which create these conditions for maximum thiol reactivity.

In conclusion, the steric inhibition of solvation effect may be one important contributor to the means by which enzymes under mild conditions in aqueous media so rapidly catalyze reactions which otherwise require organic solvents, elevated temperatures, strong acidic or basic catalysts, or a combination of these.

(35) This estimate does not include correction for electronic effects which favor the para isomer slightly; so the actual magnitude of the steric inhibition of solution effect is probably somewhat larger.

Experimental Section

Solvent.—Ethanol (95%) was purified by distillation of the azeotrope through a 1.5 ft × 1 in. column packed with glass helices, collected under nitrogen, and degassed by bubbling nitrogen through a fritted disk into the solvent for at least 4 hr. The solvent was stored under argon.

Arylthiols.—The following thiols were obtained commercially: benzenethiol; 4-, 3-, and 2-methylbenzenethiol; and 4-*tert*-butylbenzenethiol. 2-*tert*-Butylbenzenethiol and 2,6-dimethylenzenethiol were synthesized from the corresponding phenols *via* thermal rearrangements of the *O*-aryl *N*,*N*-dimethylthiocarbamate derivatives as described by Newman and Karnes[36] with the following modifications. Purification of the *O*-aryl dimethylthiocarbamates by chromatography on silica gel with elution by methylene chloride gave better yields (83%) of the *S*-aryl dimethylthiocarbamates obtained from pyrolyses of the *O*-aryl isomers. Whereas Newman and Karnes[36] reported a downfield shift in the nmr bands for the *N*-methyl groups in proceeding from the *O*-aryl dimethylthiocarbamates (doublets at τ 7.1–7.3) to the corresponding *S*-aryl compounds (singlets at τ 7.0–7.1), we observed a shift in the opposite direction for our compounds[37] from doublets at τ 6.6–6.7 for the *O*-aryl compounds to singlets at τ 7.0–7.3 for the *S*-aryl isomers as measured at 60 MHz on a Varian A-60D nmr spectrophotometer.

Preparations of the arenethiols from the *S*-aryl dimethylthiocarbamates were accomplished by refluxing with ethanolic KOH. The 2,6-dimethyl compound required 48 hr for completion of the hydrolysis. The products were worked up by *in vacuo* removal of solvent, addition of water followed by acidification with 6 *N* H_2SO_4, addition of zinc dust, and steam distillation under nitrogen. The thiols were extracted from the steam distillate with ether, the ether extract was dried over $MgSO_4$, the ether was removed by distillation, and the thiols were purified by vapor phase chromatography.

Purification of Arenethiols.—Solid 4-methylbenzenethiol was purified by recrystallization from ethyl alcohol to give a white crystalline solid, mp 44.0–44.8°.

The benzenethiols other than the 4-methyl compound are liquids and were purified by vapor phase chromatography on an Aerograph A90P preparative instrument using a 20 ft × 3/8 in. cyanosilicon-coated (17%) firebrick (60/80 mesh) or Chromosorb W column at column temperatures in the 180–225° range. Helium carrier gas was used, and the collected thiols were stored under argon. All thiols (commercial and synthesized) gave 10–20 impurity peaks in addition to the major band. The thiol fractions collected were shown by recycling to consist of a single peak. Structures and purities were confirmed by nmr spectra and carbon–hydrogen microanalyses. *Anal.* Calcd for benzenethiol, C_6H_6S: C, 65.40; H, 5.49. Found: C, 65.54; H, 5.49. Calcd for methylbenzenethiols, C_7H_8S: C, 67.67; H, 6.50. Found for 2-methylbenzenethiol: C, 67.75; H, 6.57. Found for 3-methylbenzenethiol: C, 67.51; H, 6.62. Found for 4-methylbenzenethiol: C, 67.90; H, 6.58. Calcd for 2,6-dimethylbenzenethiol, $C_8H_{10}S$: C, 69.54; H, 7.30. Found: C, 69.69; H, 7.32. Calcd for *tert*-Butylbenzenethiols, $C_{10}H_{14}S$: C, 72.23; H, 8.49. Found for 2-*tert*-butylbenzenethiol: C, 72.36; H, 8.55. Found for 4-*tert*-butylbenzenethiol: C, 72.26; H, 8.44.

Measurement of Acid Dissociation Constants.—All vessels used for thiols were flushed with *nitrogen* or argon to minimize air oxidation of thiols. All pH measurements were made under argon at 25.0 ± 0.2° using a Copenhagen Radiometer pH meter no. 26 with glass–calomel (aqueous) electrode pair standardized with two aqueous buffers. The base employed for neutralization of thiols was 0.1 *N* KOH in 95% ethanol. Apparent K_a values were determined in 95% ethanol by the method of fractional (30 or 50%) neutralization. The equivalents of thiolate anion

(36) M. S. Newman and H. A. Karnes, *J. Org. Chem.*, **31**, 3980 (1966).

(37) Although the 2-*tert*-butylaryl and 2,6-dimethylaryl compounds for which nmr spectra were measured in this work are not identical with any in the Newman and Karnes[36] series, the latter series did include the 2,3,5,6-tetramethyl, 2,6-di-*tert*-butyl-4-methyl, and 4-*tert*-butyl derivatives.

(ArS^-) formed were assumed equal to the equivalents of OH^- base added; no corrections were made for hydrolytic reversal of the neutralization. The concentration of ArS^- at 50% neutralization was kept constant at 0.1 *M* for all runs to eliminate errors due to differences in ionic strength.

Spectrophotometric determination of the pK_a for 2-methylbenzenethiol was performed as a check on the fractional neutralization method. Since the spectral method gives $(ArS^-)/(ArSH)$ directly, no corrections due to hydrolysis are required. This advantage of the spectral method is offset by the fact that the extinction coefficient (1.9×10^4) for the thiolate anion at 2.68.5 nm (where the difference in extinction coefficients between anion and molecule is maximum) is so high that even with the shortest path cells available (0.1 mm), the solution for OD measurement must be <0.1 as concentrated as that required for pH measurements of optimal stability.

Materials for Kinetic Studies.—Diphenyl disulfide was prepared in quantitative yield from benzenethiol and excess dimethyl sulfoxide by the method of Yiannios and Karabinos.[38]

β-Mercaptoethanol was distilled, bp 153° (749 mm), and stored under argon.

NEM was Aldrich purissima grace, mp 43.8–45.8°, homogeneous to thin layer chromatography on tlc grade silica gel, development with each of chloroform, benzene, acetone, ethyl acetate, or acetonitrile. *Anal.* Calcd for $C_6H_7NO_2$: C, 57.59; H, 5.64. Found: C, 57.53; H, 5.56.

Acetic acid for buffer preparations was dried by refluxing with acetic anhydride for 15 hr followed by fractionation. Potassium hydroxide was dissolved to 0.1 *N* in 95% ethanol, and the solution filtered prior to use for acetic acid–potassium acetate buffer preparations.

Spectrophotometric Assay of NEM and Related Control Experiments.—NEM was assayed at 302.0 nm (ε 700) (near its band maximum at 297.0 nm); absorption by arylthiols is low at 302.0 nm, usually $\epsilon_{ArSH} < 0.1\epsilon_{NEM}$. Ground-glass stoppered cuvettes prefilled with argon were used to minimize oxygen access and consequent disulfide formation and to allow rapid mixing by cell inversion so that the first OD measurements could be made at ≤10 sec after zero time.

Absence of solvolytic side reactions of NEM was established by control runs on NEM solutions. The constancy of OD at 302.0 nm over the duration of kinetic runs showed that solvolytic ring cleavage to produce *N*-ethylmaleamide or the amide ethyl ester which are transparent at 302.0 nm did not occur. This finding was confirmed by product analyses described later.

The addition products absorb at 302.0 nm ($\epsilon_{product} \cong 0.14$–$0.48 \times \epsilon_{NEM}$), and correction for this absorption was made in calculation of the NEM concentration, $(NEM)_t$, at any time *t*, from the expression $(NEM)_t = F(NEM)_0$ for $(ArSH)_0 > (NEM)_0$ and $(NEM)_t = (NEM)_0 - F(ArSH)_0$ for $(ArSH)_0 < (NEM)_0$ where $F = 1 - [(OD)_0 - (OD)_t]/[(OD)_0 - (OD)_\infty]$. The near coincidence of ε at 302.0 nm for NEM and α-(2-*tert*-butylphenylthio)-*N*-ethylsuccinimide prevented the use of the method described above. Instead the reaction was quenched at specific times by addition of 100 μl of β-mercaptoethanol. The $(NEM)_t$ was determined from the immediate OD decrease observed upon quenching.

Amperometric Assay of Thiol and Related Control Experiments.—Amperometric silver ion titrations of thiol[39] were performed for kinetic runs with benzenethiol to confirm that equimolar quantities of NEM and ArSH were consumed at any time *t* as assumed in the calculations of kinetic constants. The method used a rotating platinum wire indicator electrode, a saturated calomel reference electrode, and a Sargent manual polarograph, Model III, for current measurements. Calibration of the method using known weights of benzenethiol gave agreement within 1%.

Quenching for kinetic runs in which thiol was assayed was accomplished by addition of 50 ml of 1 *N* H_2SO_4 in 95% ethanol. The method was proved effective by prequenching of measured kinetic concentrations of thiol followed by addition of a kinetic concentration of NEM; three runs gave thiol titers which agreed within 1% with the thiol weights.

Diphenyl disulfide was shown not to react with silver ion under the titration conditions. A control experiment in which equimolar quantities of thiol and disulfide were used gave a titer within 1% of the thiol weight. Solutions of thiols delivered by the pipet technique employed for the kinetic runs and allowed to stand in stoppered cuvettes (preflushed with argon) for 15 min prior to silver ion titration gave thiol analyses within 1% of the values by weight. It was therefore concluded that no appreciable disulfide formation occurred during the periods of kinetic measurements.

Kinetic runs followed both by spectrophotometric NEM analyses and titrimetric thiol assays gave an average agreement of 2% for a series of 10 points.

Product Analyses.—Thin layer chromatographic analyses on tlc grade silica gel with each of a range of solvents (methylene chloride, chloroform, benzene, acetone, ethyl acetate, or acetonitrile) gave a single spot when equimolar amounts of reactants were employed. The reactions of NEM with 2-methylbenzenethiol and with 2-*tert*-butylbenzenethiol were carried out on a small preparative scale. The products isolated in quantitative yield without further purification were shown to correspond to the expected α-arylthio-*N*-ethylsuccinimides by nmr spectra and carbon and hydrogen analyses. *Anal.* Calcd for $C_{13}H_{15}NO_2S$ [α-(2-methylphenylthio)-*N*-ethylsuccinimide]: C, 62.64; H, 6.07. Found: C, 62.62; H, 6.04. Calcd for $C_{13}H_{21}NO_2S$ [α-(2-*tert*-butylphenylthio)-*N*-ethylsuccinimide]: C, 65.97; H, 7.27. Found: C, 66.13; H, 7.04.

Temperature Control.—All reactant solutions, cuvettes, and the spectrophotometer cuvette compartment were thermostated at 2.50 ± 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 potentials.

The pH range studied was 1.5 units. Measurements at higher and lower pH's were not feasible owing to solvolytic cleavage of NEM at higher pH and appreciable competition from disulfide formation due to the slower addition at lower pH. It was shown that the pH remained constant throughout the course of the reactions.

Calculation of Kinetic Parameters.—The kinetic parameters calculated are defined

$$d(product)/dt = k_{obsd}(NEM)(ArSH) = k_{anion}(NEM)(ArS^-) \quad (5)$$

The k_{obsd} values were determined from the standard integrated second-order expression and a least-squares regression programmed in FORTRAN IV for an IBM 360-91 computer. As

$$(ArS^-) = K_a(ArSH)/(H^+) \quad (6)$$

where K_a is the apparent acid dissociation constant for thiol

$$k_{anion} = k_{obsd}(H^+)/K_a \quad (7)$$

Since measurements of (H^+) and K_a were made with the same electrodes, they include equal contributions from the 95% alcohol–water junction potential. Therefore, values of k_{anion} calculated according to eq 7 should be accurate.

Registry No.—NEM, 128-53-0; α-(2-methylphenylthio)-*N*-ethylsuccinimide, 35740-35-3; α-(2-*tert*-butylphenylthio)-*N*-ethylsuccinimide, 35740-36-4.

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(38) C. N. Yiannios and J. V. Karabinos, *J. Org. Chem.*, **28**, 3246 (1963).

(39) I. M. Kolthoff and W. E. Harris, *Ind. Eng. Chem., Anal. Ed.*, **18**, 161 (1946).