C41 (1974); H. Bock, H. Alt, and H. Seidl, *J. Am. Chem. Soc.*, **91**, 355 (1969), and literature cited; especially A. G. Brook and C. J. D. Peddle, *J. Organomet. Chem.*, **5**, 106 (1966).
(6) H. Bock, *Angew. Chem.*, **77**, 469 (1965); *Angew. Chem., Int. Ed. Engl.*, **4**, 457 (1965); H. Rau, *Angew. Chem.*, **85**, 248 (1973); *Angew. Chem., Int. Ed. Engl.*, **12**, 224 (1973), and literature quotations given in both.
(7) Recently K. N. Houk, Y.-M. Chang, and P. S. Engel, *J. Am. Chem. Soc.*, **97**, 1824 (1975), reported ionization potentials and $n^+ \rightarrow \pi^*$ transitions of alkyl azo compounds, reviewing previous work.
(8) E. Haselbach and A. Schmelzer, *Helv. Chim. Acta*, **54**, 1575 (1971), and literature quotations given.
(9) M. B. Robin "Higher Electronic States of Polyatomic Molecules", Vol. 2, Academic Press, New York, N.Y., 1975, p 68ff, and references quoted there. Cf. also the addendum on p 327, pointing out that Rydberg transitions generally are not expected to survive in paraffin solution spectra.
(10) G. Bieri, F. Brogli, E. Heilbronner, and E. Kloster-Jensen, *J. Electron Spectrosc.*, **1**, 67 (1972/73).
(11) W. Enssiin, H. Bock, and G. Becker, *J. Am. Chem. Soc.*, **96**, 2757 (1974).
(12) R. L. DeKock and D. R. Lloyd, *Adv. Inorg. Chem. Radiochem.*, **16**, 98 (1974).
(13) M. Veith and H. Bärnighausen, *Acta Crystallogr., Sec. B*, **30**, 1806 (1974).
(14) E. Haselbach and H. Heilbronner, *Helv. Chim. Acta*, **53**, 684 (1970); E. Haselbach, J. A. Hashmall, E. Heilbronner, and V. Hornung, *Angew. Chem.*, **81**, 897 (1969); *Angew. Chem., Int. Ed. Engl.*, **8**, 878 (1970).
(15) H. Bock and B. G. Ramsey, *Angew. Chem.*, **85**, 773 (1973); *Angew. Chem., Int. Ed. Engl.*, **12**, 734 (1973), and literature quoted therein.
(16) R. Hoffmann, *Acc. Chem. Res.*, **4**, 1 (1971).
(17) Cf. E. Heilbronner "Some Aspects of UPS" in Proceedings of the I. International Congress of Quantum Chemistry at Mentone, 1973, R. Daudel and B. Pullmann, Ed., D. Reidel Publishing Co., Dordrecht, Holland, p 211. For the original publication see T. Koopmans, *Physica (Utrecht)*, **1**, 104 (1934).
(18) S. Evans, J. C. Green, P. J. Joachim, and A. F. Orchard, *J. Chem. Soc., Faraday Trans. 2*, 905 (1972); see also M. C. Green, M. F. Lappert, J. B. Pedley, W. Schmidt, and B. T. Wilkins, *J. Organomet. Chem.*, **31**, C55 (1971).
(19) W. Schmidt and B. T. Wilkins, *Angew. Chem.*, **84**, 168 (1972); *Angew. Chem., Int. Ed. Engl.*, **11**, 221 (1972).
(20) Thesis, M. Veith, University of Munich, 1971.
(21) Cf., e.g., T. E. Peacock "Electronic Properties of Aromatic and Heterocyclic Molecules", Academic Press, London, 1965, or J. N. Murrell "The Theory of the Electronic Spectra of Organic Molecules", Methuen & Co, London, 1963.
(22) M. B. Robin, R. R. Hart, and N. A. Kuebler, *J. Am. Chem. Soc.*, **89**, 1564 (1967), and literature quotations given.
(23) J. A. Pople and D. L. Beveridge, "Approximate Molecular Orbital Theory", McGraw-Hill, New York, N.Y., 1970.
(24) M. B. Robin, "Higher Electronic States of Polyatomic Molecules", Vol. 1, Academic Press, New York, N.Y., 1974.
(25) Cf., e.g., C. Sandorfy, P. Ausloos, and M. B. Robin, Ed., "Chemical Spectroscopy and Photochemistry in the Vacuum-Ultraviolet," D. Reidel Publishing Co, Dordrecht, Holland, 1974.
(26) H. Sakurai, Y. Kobayashi, and Y. Nakadaira, *J. Am. Chem. Soc.*, **93**, 5273 (1971).
(27) B. G. Ramsey, *J. Organomet. Chem.*, **67**, C67 (1974).
(28) H. Bock and W. Enssiin, *Angew. Chem.*, **83**, 435 (1971); *Angew. Chem., Int. Ed. Engl.*, **10**, 404 (1971).
(29) Cf., e.g., for boron nitrogen compounds W. Fuss and H. Bock, *J. Chem. Phys.*, **61**, 1613 (1974), or W. Fuss, *Z. Naturforsch. B*, **29**, 514 (1974).
(30) N. Wiberg, W. Joo, and W. Uhlenbrock, *Angew. Chem.*, **80**, 661 (1968); *Angew. Chem., Int. Ed. Engl.*, **7**, 640 (1968).
(31) N. Wiberg and M. Veith, *Chem. Ber.*, **104**, 3191 (1971).
(32) E. Farenhorst and E. C. Kooyman, *Recl. Trav. Chim. Pays-Bas*, **75**, 993 (1953).
(33) J. Kroner, D. Proch, W. Fuss, and H. Bock, *Tetrahedron*, **28**, 1585 (1972).

Reaction of Thiol Anions with Benzene Oxide and Malachite Green

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Abstract: The second-order rate constants for the reactions of a number of thiol anions with benzene oxide (BO) have been determined as have the rate constants and equilibria for the reaction of thiol anions with the acid–base equilibria species of malachite green (MG). β constants for reaction of thiol anion and amines with the nonprotonated species of malachite green are comparable ($\sim$0.3) to the β constants for the reactions of amines and thiol anions with ethylene oxide (EO) and thiol anions with BO. In addition the rate constants for the reaction of thiol anions with BO and EO are comparable. These results are discussed in terms of attack of RS^- directly upon BO and EO in an S_N2 reaction vs. trapping of intimate ion pairs by RS^-. As compared with the ability of ethylene oxide to react with nucleophiles possessing basicities as low as that of water, benzene oxide is attacked by only the more polarizable nucleophile as N_3^-, RS^-, etc. This is shown to be due not to the greater selectivity of benzene oxide toward soft bases, but to the fact that the spontaneous aromatization rate (i.e., water rate) of benzene oxide exceeds the rate of reaction of ethylene oxide with water by ca. 10^3. It is predicted that benzene oxides substituted with highly electron-withdrawing groups will exhibit reactivity to a broad range of nucleophiles. This prediction is predicated on the previously determined fact that aromatization of benzene oxide is associated with a $\rho \simeq -7$. For the purposes of this study, it was desirable to determine the microscopic pK_a values for the thiol species under the conditions of the kinetic and thermodynamic studies (30°, solvent water, $\mu = 1.0$). These constants are reported herein and compared with literature values.

As part of the normal metabolic paths of catabolism and detoxification, both biotic and exobiotic aromatic structures are converted to arene oxides. However, these normal metabolic processes may result in the assassination of the subject. Thus, arene oxide moieties formed from exobiotic aromatic hydrocarbons have been implicated as causative agents in mutagenesis, carcinogenesis, and tissue necrosis.[2,3] The pathological effects of arene oxides have been attributed to their acting as alkylating agents.[4-7] The enzyme[8] and nonenzyme mediated 1,2-addition of the sulfhydryl group of glutathione to arene oxides appears as a major means of protection from the cytotoxic effects of these agents. Thus, both the cytotoxic effects of arene oxides and the detoxification of these substances involve nucleophilic addition to the oxide moiety. The importance of understanding the mechanisms of nucleophilic addition to arene oxides is obvious. Important studies dealing with the formation of products and their stereochemistry have been published[9-16] but, with the possible exception of the hydration of 8,9-indane oxide,[16] no kinetic investigations of the nucleophilic addition to arene oxide have appeared.[17] The present investigation deals with the kinetics of addition of thiol anions to benzene oxides. The magnitude of the rate constants and their sensitivity to the proton basicity of the

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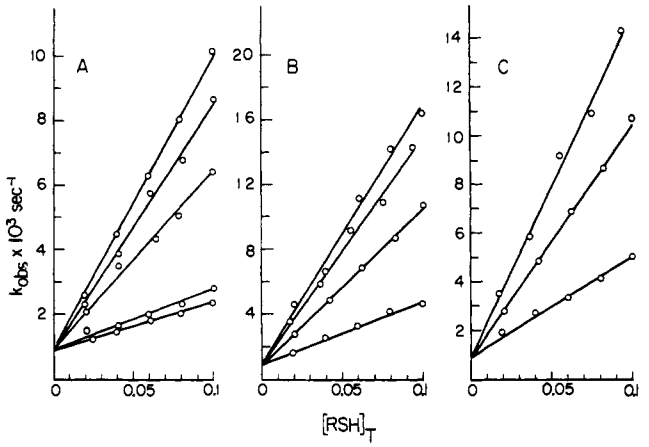


Figure 1. Plots of the concentration of total thiol vs. k_{obsd} for the disappearance of benzene oxide from solution: (A) cysteine ethyl ester at pH values of 7.46, 7.64, 8.59, 9.00, and 9.17; (B) glutathione at pH values of 8.39, 8.75, 9.01, and 9.17; (C) thioglycolate at pH 9.21, 9.81, and 10.44.

thiol anion is compared when the substrate is benzene oxide, ethylene oxide, and the delocalized carbonium ion of malachite green. For the present investigation and those that will follow, we desired a series of thiols whose varied microscopic pK_a's were known at a given temperature, at low total thiol concentration, and at a high constant ionic strength. The microscopic pK_a values of seven thiols are reported at 30°, solvent H_2O, μ = 0.1.

Experimental Section

Materials. Benzene oxide was prepared by literature procedures.[18] The hydrochlorides of L-cysteine ethyl ester and β-mercaptoethylamine (Sigma) were recrystallized from ethanol-ether. 2-Mercaptoethanol (Sigma), sodium thioglycolate (Sigma), D,L-homocysteine (National Biochemical), glutathione (National Biochemical), L-cysteine hydrochloride (Calbio-Chem) and malachite green (as oxalate from MCB) were employed without further purification. Doubly glass-distilled water was employed in all the various studies.

Microscopic pK_a's of Thiols at 30°. The pK_a values of the thiols (5×10^{-5} *M*) were determined spectrally [30.0 ± 0.1°; μ = 1.0 (KCl) in H_2O] employing a titration cell especially designed for the Cary 15 spectrophotometer.[19] Trace heavy metal ion contaminants were removed from the aqueous KCl solvent by shaking with a 0.01% solution of dithiazone in CCl_4. The aqueous layer was then extracted several times with CCl_4 and remaining CCl_4 removed by passing argon through the solution. All operations carried out for removal of trace metal ions and the handling of thiol solutions were performed in Nalgene plastic wear. The titration cell[19] was constructed of polypropylene with quartz windows. The solution in the cell was magnetically stirred and pH monitored continuously using a Radiometer 26 pH meter and a EA 125 combined Metrohm microelectrode. An ABU 1 C Radiometer autoburet charged with 0.1 *M* KOH was employed for the titrations. All solutions were deoxygenated by bubbling argon through them for 1 hr. Argon was continuously passed through the titration cell. The argon itself was deoxygenated by passage through two vanadous ion traps.

Reaction of Thiols with Benzene Oxide. The disappearance of benzene oxide was followed at 320 nm by use of a Gilford Model 2000 spectrophotometer (30 ± 0.1°, μ = 1.0 with KCl, solvent H_2O). In a typical experiment, a solution of mercaptan in 1 *M* KCl was brought to the desired pH with 1 *M* KOH and an aliquot taken and diluted with 1 *M* KCl to provide the desired concentration (0.02 to 0.1 *M*). About 3 ml of the solution was then placed in a cuvette and allowed to temperature equilibrate for 15 min at which time the reaction was initiated by addition of 20 λ of a solution of benzene oxide in peroxide free dioxane to provide a solution ca. 10^{-4} *M* in substrate. Reactions were carried out at five thiol concentrations at each of three pH values for those thiols possessing no other ionizable groups in the pH range of interest. For thiols possessing other interferring ionizable groups, kinetic studies were carried out at each of five thiol concentrations at four or more different pH values (Figure 1). The observed pseudo-first-order rate constants were obtained using a linear least-squares program and a Hewlett Packard Model 9820A desk top computer.

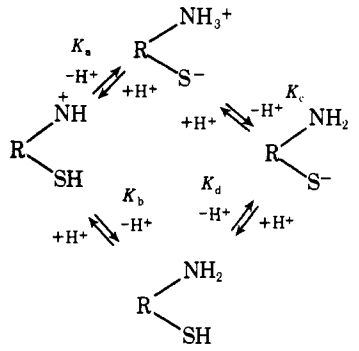
Reaction of Thiols with Malachite Green (MG). These reactions were followed spectrophotometrically (30 ± 1°, μ = 1.0 with KCl, solvent H_2O) by monitoring the decrease in absorbance at 618 nm, employing either a Cary 15 spectrophotometer equipped with an auto-titration and pH-stat cell[19] flushed with vanadous ion scrubbed argon or a Durrum stopped-flow spectrophotometer under nitrogen. Stock solutions of MG in CH_3CN were prepared, and the concentration of MG was determined by addition of 0.5 ml of the stock solution to 25 ml of H_2O and observing the absorbance at 618 nm ($\epsilon_1 = 2.14 \times 10^5\ M^{-1}$ between pH 2.7 and 7.0, length of Cary cell ≃ 3.51 cm). In a typical kinetic experiment, a 250-ml solution of thiol was prepared. A 25-ml aliquot was then pipetted into the polyethylene optical cell and the pH adjusted with either 1 *M* KOH or 1 *M* HCl. After temperature equilibration, the reaction was initiated by addition of 0.5 ml of the CH_3CN solution of MG. In those instances for which stopped-flow measurements were required, either the thiol served as its own buffer or the thiol solution was buffered by 0.05 *M* acetate-acetic acid. A solution of thiol (twice the desired concentration) in 1.0 *M* KCl was brought to the desired pH and then placed in one of the stopped-flow syringes. The other syringe was filled with 1.0 *M* KCl solution containing twice the desired concentration of MG. The actual pH of the reaction solution was determined by mixing equal volumes of the MG and thiol solution outside the stopped-flow apparatus. In all cases the pH was within 0.02 pH units of that of the original thiol solution.

Results

Thiol pK_a Values. Although the pK_a values for biologically important thiols have been determined by various investigators, we desired a set of pK_a's determined in water at a single temperature (30°). For a discussion of operational methodology and a derivation of the necessary equations, one should consult the now classic study of Benesch and Benesch.[20] In the absence of substituent groups whose pK_a values lie in the range of the pK_a of the thiol function, K_{RSH} is provided by eq 1

$$K_{RSH} = a_H(OD)_n/[(OD)_f - (OD)_n] \qquad (1)$$

where K_{RSH} = acid dissociation constant of RSH, a_H = hydrogen ion activity as determined by the glass electrode, $(OD)_f$ = optical density of RS^- obtained at high pH, and $(OD)_n$ = OD at measured pH. Values of K_{RSH} were obtained by computer iteration which generated sigmoid plots of percent $[RS^-]$ vs. pH. In all cases, the individual points (10-20) precisely fitted the titration curves (not shown). In those instances where an ionizable $-NH_3^+$ function was present, the microscopic K_a values (eq 2) were obtained by



(2)

assuming, after Benesch and Benesch,[20] that ε of the $-S^-$ (~240 nm) function remains invariant to the state of ionization of the $-NH_3^+$ function (eq 3).

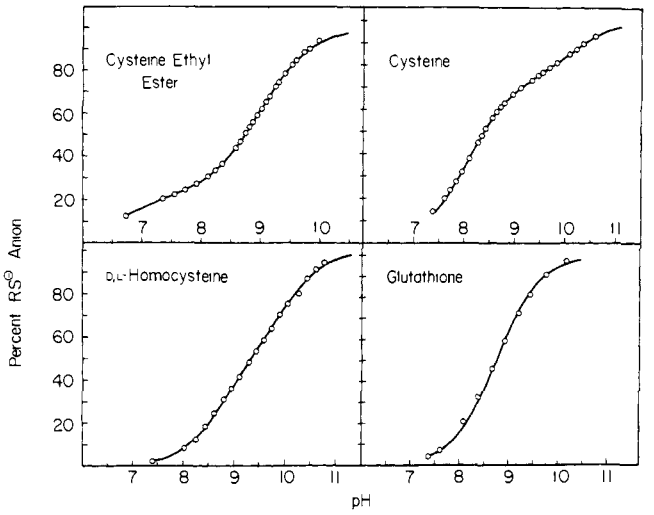


Figure 2. Fit of experimental points of eq 3 for the determination of the microscopic pK_a value of the thiol functions of cysteine ethyl ester, cysteine, and D,L-homocysteine. For glutathione, the experimental points are fitted to an equation for a single sulfhydryl pK_a (eq 1).

$$\frac{[RS^-]}{[RS^-]_{max}} = \frac{K_a/K_b + K_d/a_H}{a_H/K_b + K_a/K_b + K_d/a_H + 1} \quad (3)$$

Equation 3 contains the three unknown quantities K_a, K_b, and K_d and requires three values of $[RS^-]/[RS^-]_{max}$ at three pH's for solution. In practice, 25 values of $[RS^-]/[RS^-]_{max}$ at 25 pH values were determined and a computer program employed which calculated the dissociation constants employing three points at a time, each point being from a different portion of the titration curve. An average value of each of the constants so determined was employed to generate the theoretical curves to fit the experimental points (Figure 2). Examination of Figure 2 reveals that both glutathione and D,L-homocysteine show no break in their thiol titration curves. The glutathione data fit best a single ionization, and attempts to calculate microscopic pK_a values result in identical values for K_a and K_d (insensitivity to the amino group pK_a). On the other hand, the D,L-homocysteine data could only be fit employing eq 3. The assumption of a single thiol ionization constant provided a poor fit to the experimental points. In order to determine the pK_a of the amino group of glutathione, the following procedure was employed. The value of $[RS^-]$ was determined at each pH employing the determined thiol ionization constant and the concentration of neutral amine species $[RNH_2]$ obtained from eq 4.

$$[RNH_2] = \frac{\text{moles of base added}}{\text{volume}} - [RS^-] - ([HO^-] - [HO^-]_{initial}) \quad (4)$$

The generated values of $[RNH_2]$ were then employed to calculate the amine pK_a. The pK_a values determined in this study are provided in Table I. Assignment of the pK_a values to discrete species is provided in Table II.

Reaction of Thiols with Benzene Oxide (BO). All kinetic experiments were carried out at constant pH values and in the presence of total thiol ($[RSH]_T = [RS^-] + [RSH]$) greatly exceeding [BO]. Under these conditions, first-order kinetics prevail (Figure 1). In initial experiments, it was determined that undissociated thiol exhibits no measureable rate of reaction with BO. It has previously been shown that benzene oxide is converted to phenol via specific acid catalyzed and spontaneous pathways.[27] In the alkaline pH range employed in these studies, only the spontaneous (k_0) aromatization of BO is evident. Thus, for thiols possessing a single sulfhydryl ionization constant (β-mercaptoethylamine, 2-mercaptoethanol, and thioglycolic acid), the pseudo-first-order rate constant (k_{obsd}) is provided by eq 5.

$$-\frac{d[BO]}{dt} = k_{obsd}[BO]$$

$$k_{obsd} = k_0 + k_2[RS^-] = k_0 + \frac{k_2K_a[RSH]_T}{K_a + a_H} \quad (5)$$

From eq 5 plots of k_{obsd} at constant pH vs. $[RSH]_T$ provide as slope $k_2K_a/(K_a + a_H)$ and as intercept k_0. Multiplication of the slopes by $K_a/(K_a + a_H)$ provides the value of k_2 at each pH. The average values of k_2, so obtained, are included in Table II. For cysteine, cysteine ethyl ester, and D,L-homocysteine, the rate constants for each thiol anion species were determined separately. For these cases the rate law of eq 6 prevails.

$$k_{obsd} = k_0 + k_{HA}[S^-NH_3^+] + k_A[S^-NH_2] = k_0 + [S^-NH_3^+]\left\{\frac{k_{HA}a_H + k_AK_c}{a_H}\right\} \quad (6)$$

Material balance in $[RSH]_T$ (eq 7)

$$[RSH]_T = \frac{a_H[S^-NH_3^+]}{K_a} + [S^-NH_3^+] + \frac{K_b}{K_a}[S^-NH_3^+] + \frac{K_c[S^-NH_3^+]}{a_H} \quad (7)$$

provides eq 8

$$[S^-NH_3^+] = [RSH]_T\left\{\frac{K_aa_H}{K_aK_c + (K_a + K_b)a_H + a_H^2}\right\} \quad (8)$$

and substitution of eq 8 into eq 6 yields eq 9.

$$k_{obsd} = k_0 + [RSH]_T\left\{\frac{K_a(k_{HA}a_H + k_AK_c)}{K_aK_c + (K_a + K_b)a_H + a_H^2}\right\} = k_0 + [RSH]_TQ(k_{HA}a_H + k_AK_c) \quad (9)$$

Values of k_{HA} and k_A were obtained by plotting k_{obsd} vs. $[RSH]_T$ at constant values of pH and obtaining as slope $Q(k_{HA}a_H + k_AK_c)$ and intercept as k_0. In a secondary plot, the slopes divided by Q were plotted vs. a_H to provide k_{HA} as slope′ and k_AK_c as intercept′.

For glutathione we have determined that the pK_a of the thiol function is not affected by the ionization state of the

Table 1. Comparison of Microscopic pK_a Values Determined for a Number of Thiols

Thiol	pK_A	pK_B	pK_C	pK_D
L-Cysteine ethyl ester	7.3[a]	6.76[a]	8.33[a]	8.87[a]
	7.45[b]	6.77[b]	8.41[b]	9.09[b]
L-Cysteine	8.21[a]	8.65[a]	10.0[a]	9.56[a]
	8.53[b]	8.86[b]	10.36[b]	10.03[b]
	8.50[c]	8.85[c]	10.35[c]	10.00[c]
Cystamine	8.22[a]			
	8.35[b]			
	8.20[c]			
D,L-Homocysteine	9.02[a]	9.04[a]	9.71[a]	9.69[a]
Glutathione	8.72[a]	9.47[a]		
	8.93[e]	9.13[e]	9.28[e]	9.08[e]
2-Mercaptoethanol	9.45[a]			
	9.32[d]			
Thioglycolic acid	9.82[a]			
	10.01[c]			
	10.22[d]			

[a] This study. [b] Benesch and Benesch, ref 20. [c] Elson and Edsall, ref 21. [d] Danehy and Noel, ref 22. [e] Rabenstein, ref 23.

amino group. However, it is possible that the nucleophilicity of the $S^-NH_3^+$ and S^-NH_2 species could be different (eq 10). From a material balance, eq 10 is equivalent to eq 11.

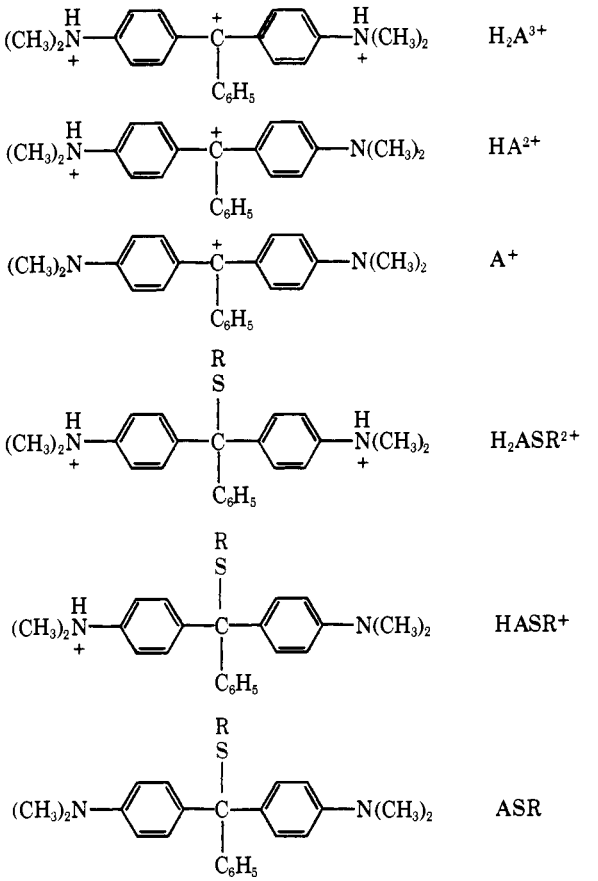
$$k_{obsd} = k_0 + k_{HA}[S^-NH_3^+] + k_A[S^-NH_2] \quad (10)$$

$$k_{obsd} = k_0 + [RSH]_T \left\{\frac{(k_{HA}a_H + k_A K_{a_2})K_{a_1}}{K_{a_1}K_{a_2} + K_{a_1}a_H + a_H^2}\right\}$$

$$= k_0 + [RSH]_T R(k_{HA}a_H + k_A K_{a_2}) \quad (11)$$

Plots of k_{obsd} at constant pH vs. $[RSH]_T$ provide $R(k_{HA}a_H + k_A K_{a_2})$ as slope and k_0 as intercept. In a secondary plot of slope/R vs. a_H, there was obtained k_{HA} as slope′ and $k_A K_{a_2}$ as intercept′.

Reaction of Thiols with Malachite Green (MG). These reactions were carried out under the pseudo-first-order conditions of $[RSH]_T \gg [MG]$. In addition to the thiol species NH_3^+SH, NH_2SH, $NH_3^+S^-$ and NH_2S^-, the species H_2A^{3+}, HA^{2+}, A^+, H_2ASR^{2+}, $HASR^+$, and ASR must be considered:



In the structures H_2ASR^{2+}, $HASR^+$, and ASR, the symbol R refers to the remainder of the thiol nucleophile structure associated with the particular microscopic pK (Table II). The sequence of Scheme I accounts for the equilibrium results of this study. Spectral titration of MG below pH 2.5 is accompanied by a decrease in absorbance at 618 nm and an increase in absorbance at 446 nm which is associated with the prototropic equilibria between the MG species H_2A^{3+} and HA^{2+} ($pK_{a_1} < 1.0$). All equilibrium measurements were restricted to pH values of 2.8 or greater. For this reason it was not essential to take into account the species H_2A^{3+} in our calculations.

Scheme I

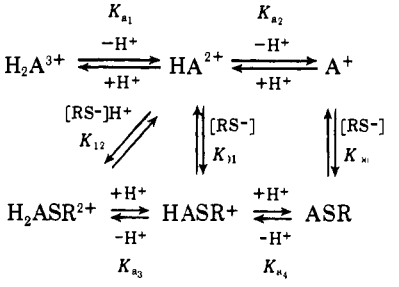


Table II. Microscopic pK_a Values for Thiol Species and Second-Order Rate Constants for Reaction of Thiolate Species with Benzene Oxide (BO)

RSH species	pK_a	k_2, M^{-1} sec^{-1} BO
$HSCH_2CH(NH_3^+)CO_2Et$	7.30	0.055
$HSCH_2CH(NH_3^+)CO_2^-$	8.21	0.075
$HSCH_2CH_2NH_3^+$	8.22	0.085
$HSCH_2HCNHCO(CH_2)_2CH(NH_3^+)CO_2^-$ $CONHCH_2CO_2^-$	8.72	0.114
$HSCH_2HCNHCO(CH_2)_2CH(NH_2)CO_2^-$ $CONHCH_2CO_2^-$	8.72	0.166[a]
$HSCH_2CH(NH_2)CO_2Et$	8.87	0.144
D,L-$HS(CH_2)_2CH(NH_3^+)CO_2^-$	9.02	0.090
$HSCH_2CH_2OH$	9.45	0.172
$HSCH_2CH(NH_2)CO_2^-$	9.56	0.217
D,L-$HS(CH_2)_2CH(NH_2)CO_2^-$	9.69	0.165
$HSCH_2CO_2^-$	9.82	0.184

[a] Ionization of the α-amino group (pK_a = 9.46) provides a moderate increase in rate constant.

Defining the equilibrium constant K_{obsd} as in eq 12

$$K_{obsd} = \frac{[H_2ASR^{2+}] + [HASR^+] + [ASR]}{[HA^{2+} + A^+][RSH]_T} \quad (12)$$

and employing the equilibrium expression of Scheme I, one obtains eq 13

$$K_{obsd} = \frac{[H_2ASR^{2+}]\left\{\dfrac{K_{a_3}K_{a_4} + K_{a_3}a_H + a_H^2}{a_H^2}\right\}}{\left\{\dfrac{[HA^{2+}](K_{a_2} + a_H)}{a_H}\right\}\dfrac{[RS^-]}{U}} \quad (13)$$

where U represents $K_s/(K_s + a_H)$ for thioglycolic acid and cystamine and $K_a a_H/[K_a K_c + a_H(K_a + K_b) + a_H^2]$ for cysteine and cysteine ethyl ester. The employment of $U = K_a a_H/[K_a K_c + a_H(K_a + K_b) + a_H^2]$ was predicated on the fact that, in the pH range employed, the concentration of S^-NH_2 is small compared with that of $S^-NH_3^+$. Rearrangement of eq 13 provided eq 14.

$$\frac{K_{obsd}}{U}(K_{a_2} + a_H) = \frac{[H_2ASR^{2+}](K_{a_3}K_{a_4} + K_{a_3}a_H + a_H^2)}{[HA^{2+}][RS^-]a_H} \quad (14)$$

$$K_{12} = \frac{[H_2ASR^{2+}]}{[HA^{2+}][RS^-]a_H} \quad (15)$$

Substituting the equilibrium expression of eq 15 into eq 14 provides eq 16.

$$\frac{K_{obsd}}{U}\left\{\frac{(K_{a_2} + a_H)}{a_H}\right\} - \frac{K_{12}K_{a_3}K_{a_4}}{a_H} = K_{12}(K_{a_3} + a_H) \quad (16)$$

Equation 16 may also be expressed in the form of eq 17.

$$K_{obsd} = \frac{UK_{12}(K_{a_3}K_{a_4} + K_{a_3}a_H + a_H^2)}{(K_{a_2} + a_H)} \quad (17)$$

The solution of the various constants from experimental

Table III. Equilibrium (Scheme I) and Kinetic (Schemes II and III) Constants Derived from the Reaction of Thiols with Malachite Green (Solvent H_2O, 30°, μ = 1.0)

Mercaptides	Thermodynamic				Kinetic						
	pK_{a_2}	pK_{a_3}	pK_{a_4}	K_{12}	pK_{a_2}	pK_{a_3}	pK_{a_4}	$k_1(HA^{+2})$, M^{-1} sec^{-1}	$k_1(A^+)$, M^{-1} sec^{-1}	$k_{-1}(HASR^+)$, sec^{-1}	$k_{-1}(ASR)$, sec^{-1}
$^-SCH_2COO^-$	2.05	4.5	5.3	1.6×10^{13}	2.8	4.2	4.4	4.9×10^6	2×10^4 [a] 1.6×10^4 [b]	1.4×10^{-2}	6×10^{-2}
$^-SCH_2CH_2NH_3^+$	1.5 ± 0.1	4.3	5.2	5.5×10^{11}	2.0	4.15	5.2	9.7×10^4	7×10^3 [a] 7.3×10^3 [b]	5×10^{-3}	1.3
$^-SCH_2CH_2(NH_3^+)CO_2$	1.5 ± 0.1	4.4	5.2	4.1×10^{11}		4.15	5.4		4.7×10^3 [a]	1.5×10^{-2}	1.95
$^-SCH_2CH_2(NH_3^+)CO_2Et$	1.5 ± 0.1	4.2	5.1	4.1×10^{10}	2.5	4.1	5.0	2.7×10^4	3.2×10^3 [a] 6.8×10^3 [b]	1×10^{-2}	6.3

[a] From intercept of plots of eq 24 at low pH. [b] From stopped-flow kinetics at high pH.

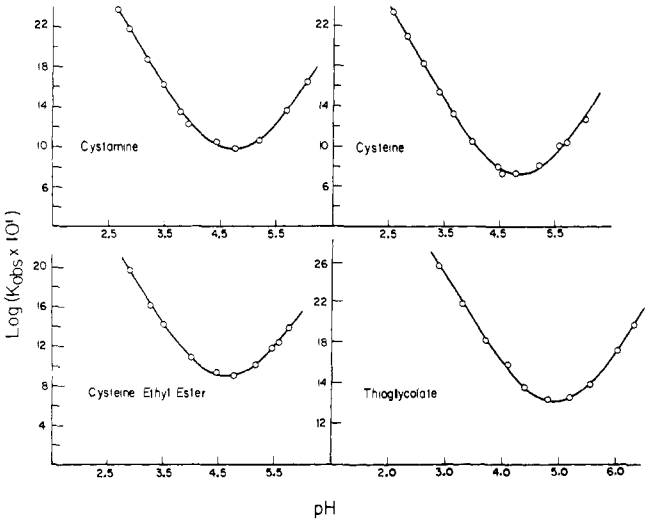
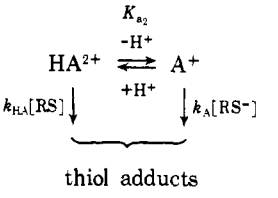


Figure 3. Log K_{obsd} vs. pH profile for reaction of thiol anion species with malachite green. Points are experimental, and the line has been generated from eq 17 employing the constants of Table III.

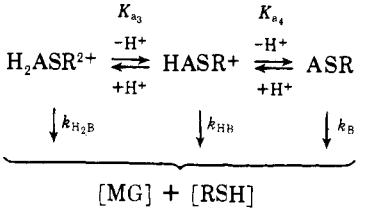
data was carried out by two different procedures which provided consistent results. At low pH the first term of the left hand side of eq 16 is much larger than the second term. Thus, by plotting $K_{obsd}(K_{a_2} + a_H)/Ua_H$ vs. a_H, a straight line should be obtained with slope K_{12} and intercept $K_{a_3}K_{12}$ with the points at higher pH deviating upward from the linear plot. A computer program was written for the purpose of iteration in the value of K_{a_2} to provide the best straight line for the first approximation of K_{12}. The best fit was taken as that with the greatest correlation coefficient (i.e., 0.99 plus). This method was found to be very sensitive to the chosen values of K_{a_2}, with the line curving upward when the value for K_{a_2} is too high and downward when it is too low. The values of K_{a_2} and K_{12} so obtained were then substituted into eq 16 and the left hand side of the equation was plotted vs. a_H and the product $K_{a_3}K_{a_4}$ varied until the best fit to the theoretical line was obtained. The values of $K_{a_3}K_{a_4}$ were then used to recalculate K_{a_2} and so on until the best set of consistent constants were obtained. The second approach was simply to employ eq 17 and chose the best constants as those which fitted log K_{obsd} values to a plot of log K_{obsd} vs. pH. For this purpose, an iteration procedure in K_{12}, K_{a_3}, and K_{a_4} was employed along with the separately determined value of K_{a_2}. The constants obtained from the two procedures were in close agreement. The determined equilibrium constants are given in Table III and plots of log K_{obsd} vs. pH are provided in Figure 3.

The kinetics of the reaction of the thiolate anion species with MG were followed to equilibrium; thus, k_{obsd} is the sum of forward (k_1) and reverse (k_{-1}) rate constants. From

Scheme II



Scheme III



eq 18 and 19 follow eq 20 and 21

$$k_{obsd} = k_1 + k_{-1} \tag{18}$$

$$K' = K_{obsd}[RSH]_T = \frac{k_1}{k_{-1}} \tag{19}$$

$$k_1 = k_{obsd}\left(\frac{K_{obsd}[RSH]_T}{1 + K_{obsd}[RSH]_T}\right) \tag{20}$$

$$k_{-1} = k_{obsd}/(K_{obsd}[RSH]_T + 1) \tag{21}$$

from which k_1 and k_{-1} may be determined at each pH and concentration of $[RSH]_T$. By definition k_1 is the pH and $[RSH]_T$ sensitive forward rate constant (eq 22) which is provided by eq 23 (Scheme II). Rearrangement of eq 23 provides 24.

$$v_f = k_1[MG]_T \tag{22}$$

$$k_1 = \frac{(k_{HA}a_H + k_A K_{a_2})U[RSH]_T}{(K_{a_2} + a_H)} \tag{23}$$

$$\frac{k_1(K_{a_2} + a_H)}{U[RSH]_T} = k_{HA}a_H + k_A K_{a_2} \tag{24}$$

From eq 24 a plot of the left hand term vs. a_H provides a straight line of slope k_{HA} and intercept $k_A K_{a_2}$. In Figure 4 are plotted the values of log k_1 vs. pH. The curves have been generated from eq 23 employing the values of k_A and k_{HA} listed in Table III. The constant k_{-1} pertains to the three paths of reversion of H_2ASR^{2+}, $HASR^+$, and ASR to thiol and MG (Scheme III, eq 25, 26, and 27).

$$v_r = k_{-1}[ASR]_T \tag{25}$$

$$v_r = (k_{H_2B}[H_2ASR^{2+}] + k_{HB}[HASR^+] + k_B[ASR]) \tag{26}$$

$$k_{-1} = \frac{k_B K_{a_3}K_{a_4} + k_{HB}a_H K_{a_3} + k_{H_2B}a_H^2}{K_{a_3}K_{a_4} + K_{a_3}a_H + a_H^2} \tag{27}$$

Solution of eq 27 in terms of the various constants was approached through its fitting to a plot of log k_{-1} vs. pH. The results of these fittings revealed that the term k_{H_2B} must be quite small so that $k_{H_2B}a_H^2$ in the numerator of eq 27 can be ignored. This allows the writing of eq 28.

$$k_{HB}a_H + k_BK_{a_4} = k_{-1}\frac{(K_{a_3}K_{a_4} + K_{a_3}a_H + a_H^2)}{K_{a_3}} \quad (28)$$

A plot of the right hand side of eq 28 vs. a_H provides a straight line of slope k_{HB} and intercept $k_BK_{a_4}$ when the correct values of K_{a_3} and K_{a_4} are employed.

Discussion

Thiol pK_a Values. The pK_a values of the thiols of this study have been determined previously under varying experimental conditions. The measurements of this study were made at 30 ± 0.1° and at μ = 1.0 with thiol concentrations of 10^{-4} to 10^{-5} *M*. The Raman spectral determinations of Elson and Edsall[21] were at ca. 25° and employed thiol concentrations of 1 or 2 *M*. Measurements of thiol pK_a values by Danehy and Noel[22] were carried out at 30° with thiol concentrations of about 0.01 *M* without added salt. The nuclear magnetic resonance measurements of Rabenstein[23] were carried out at 25° with μ varying from 0.2 to 0.53 and total thiol at 0.15 *M*. The spectral titrimetric measurements of Benesch and Benesch[20] were carried out at 23°, μ = ca. 0.2 and total thiol of 10^{-4} to 10^{-5} *M*. Comparison of the microscopic pK_a values obtained by us with those previously determined are provided in Table I. Examination of Table I reveals that: (1) for L-cysteine ethyl ester, our values are about 0.1 unit less than those determined by Benesch and Benesch;[20] (2) for L-cysteine and cystamine, our values are 0.32 and 0.1 unit, respectively, less than those determined by Benesch and Benesch[20] and by Elson and Edsall;[21] (3) for glutathione, we find the ionization of the sulfhydryl group to be independent of the ionization of the ammonium group. Attempts to obtain microscopic pK_a values resulted in pK_a = pK_d and pK_b = pK_c (eq 2). Rabenstein,[23] on the other hand, was able to obtain microscopic pK_a values for glutathione. The discrepancy may be due to the high concentration of glutathione employed in Rabenstein's study. The discrepancies between our titration data and those reported previously may be understood in part from the fact that the conditions of thiol concentration, temperature, and ionic strength are not duplicated from study to study.

Equilibrium Constants Determined from the Reactions of Thiols with Malachite Green. Both equilibrium and kinetic methods have been employed to determine the pK_{a_2}, pK_{a_3}, and pK_{a_4} values of Scheme I (Table III). The method of equilibrium determination is sensitive to the assigned pK_a values which should be accurate to ±0.1 or ±0.2 unit. Some discrepancy exists in the pK_{app} values determined by the two methods employed. This is not completely surprising for it is a general occurrence for pK_{app} values obtained via kinetic methods to differ by up to 0.5 or so log unit from the identical constant determined by a thermodynamic technique.[24]

Examination of Table III shows that there is a good agreement in the pK_a values obtained from the reactions of cysteine, cysteine ethyl ester, and cystamine but that the values obtained with thioglycolic acid are significantly different. Considering the equilibrium constants obtained with cysteine, cysteine ethyl ester, and cystamine, the values of pK_{a_2} (1.5 ± 0.1), pK_{a_3} (4.3 ± 0.1), and pK_{a_4} (5.2 ± 0.1) are in good agreement with those obtained by Cigen[25] (1.45, 4.6, and 5.2, respectively). In the present study, RS^- species provides the ligand to the carbonium ion while, in Cigen's studies, HO^- was the ligand. The rather different

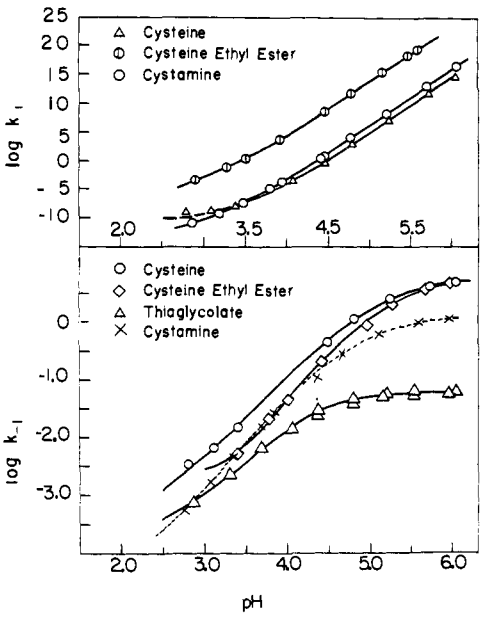
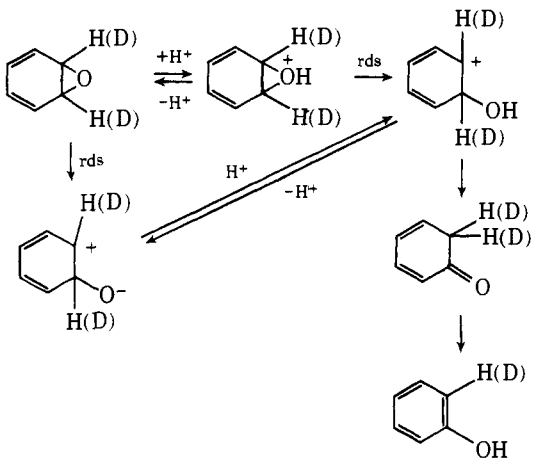


Figure 4. Upper portion: Log k_1 vs. pH for reaction of three thiols with MG. Points are experimental and the curve generated from eq 23 employing the values of $k_{1_A}{}^+$ and $k_{1_{HA}{}^{2+}}$ of Table III. Lower portion: Log k_{-1} vs. pH for dissociation of MG thiol adducts. Points are experimental and the curves generated from eq 28 employing the $k_{-1_{HASR}{}^+}$ and $k_{-1_{ASR}{}^+}$ constants of Table III.

pK_a values obtained when thioglycolate dianion serves as the RS^- ligand require explanation. None appears more profound than the simple noting that thioglycolate possesses no charged ammonium substituent while MG, cysteine, cysteine ethyl ester, and cystamine do. It is possible that the thioglycolate dianion can complex with MG. Spectral and kinetic evidence for the ion pairing of MG with aryl sulfonates in aqueous solution have been obtained by Bunton and Paik.[26] Charge repulsion between MG and the charged ammonium group on other thiol anion species could decrease any tendency toward complex formation. In any event, the rate constant $k_{HA}{}^{2+}$ for thioglycolate may not be used in any linear free energy treatment in which it is compared with rate constants for the other thiols.

Rates of Reaction of Thiol Anions with Benzene Oxide and Malachite Green. Benzene and other simple arene oxides have been reported not to yield nucleophilic addition products on treatment with nonpolarizable nucleophiles as NH_3, NH_2^-, and hydroxide ion.[13] However, nucleophilic addition by hard or nonpolarizable nucleophiles does, albeit grudgingly, apparently occur when the reactions have been carried out in a solvent of low dielectric constant. In a typical experiment, a reaction period of 14 days is required to obtain a 64% yield of *trans*-6-methoxycyclohexa-2,4-diene-1-ol on reaction of BO with 4 equiv of MeO^- in MeOH.[14] The ability of MeO^- to react with the oxide in alcohol may be due to repression of the competing aromatization reaction through destabilization of the intermediate carbocation (vide infra) with a resultant increase in the $\Delta F^{\ddagger}$ for its formation. This speculation receives some support from the observation that both the spontaneous and acid catalyzed aromatization of benzene oxide, naphthalene oxide, and phenanthrene oxide are markedly depressed on decreasing the dielectric of the media.[27] In contrast to the situation with nonpolarizable nucleophiles, attack by soft and polarizable nucleophiles as RS^- and N_3^- is readily established. Thiophenoxide has been shown to provide with BO *trans*-6-phenylthiocyclohexa-2,4-dienol (H_2O solvent, 0°, 64% yield). Further, by use of selectively deuterated BO, the reaction was established to occur solely via 1,2-addition (in

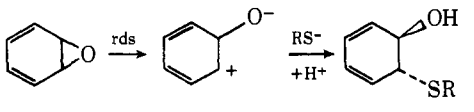
Scheme IV



contrast to the trans-1,2 and trans-1,6 addition of N_3^-). Similar trans-1,2 addition of other thiol anions has been tacitly assumed.[14]

The most characteristic reactions of benzene oxides, naphthalene oxides, and non-K-region oxides of phenanthrene are specific acid and spontaneous epoxide ring opening to yield a carbonium ion which then rearranges to yield phenols (Scheme IV). Much evidence is available to support Scheme IV and rate-determining carbonium ion formation. This includes: (1) log k_{obsd} vs. pH rate profiles that establish two pathways, one first order in H_3O^+ and the other spontaneous;[27] (2) the marked decrease in rate accompanying substitution (benzene oxide) of electron-withdrawing groups ($\rho \simeq -7$) for both specific acid and spontaneous paths;[28] (3) lack of primary hydrogen/deuterium kinetic isotope effects for the NIH shift;[28] (4) the establishment of oxygen migration (the so-called oxygen-walk);[29] and (5) solvent trapping of intermediate carbonium ion in acidic media (i.e., H_2O or methanol).[30]

The kinetics for the reaction of nucleophiles with arene oxides have not been examined in order to differentiate between the possibilities of: (i) direct nucleophilic attack by RS^- upon epoxide carbon or a tight ion pair;[32] and (ii) the trapping by RS^- of the carbonium ion species generated after the rate-determining step in aromatization (Scheme IV). The trapping of a resonantly stabilized carbonium ion formed along a reaction path has precedence. As an example, one may sight the trapping of the transient oxocarbonium ion formed on hydrolysis of methyl orthobenzoate by hydroxylamine and semicarbazide to yield the corresponding methyl imidate esters.[31] Stereochemical arguments against the mechanism of eq 29 based on the formation of



(29)

trans adduct in the reaction of $C_6H_5S^-$ with BO (loc. cit.) are not completely convincing since shielding in the carbonium ion by the $-O^-$ or -OH substituent to approach by the bulky $C_6H_5S^-$ is most likely. The extent of this shielding to cis addition is difficult to surmise. However, the fact that the disappearance of BO (Figure 1) from solution is first order in $[RS^-]$ species rules out the possibility of the trapping by RS^- of the carbonium ion formed in the rate-determining step of the NIH shift (eq 29). For the aromatization reaction, the possibility of the formation of a tight internal ion pair (O^-C^+), whose partitioning to BO and carbonium ion (OH C^+) is partially rate determining, must be taken into account (eq 30, Figure 4). One might, therefore, consider if it is reasonable that RS^- intercepts the ion-pair species. Sneen[32] has presented arguments in support of tight ion pairs as intermediates in SN2 displacements, and it is conceivable that such a species could be common to both the reaction of RS^- with BO and the aromatization of the latter. A decrease in the dielectric constant accompanying the incorporation of organic solvent would provide less destablization to the ion pair since it is a zwitterion,[33] than to the carbocation (Figure 5). The result would be (as seen[27]) to decrease the rate of the aromatization reaction with little effect on the rate of addition.

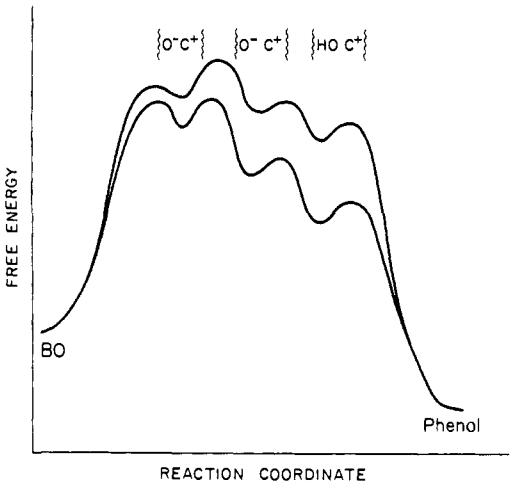
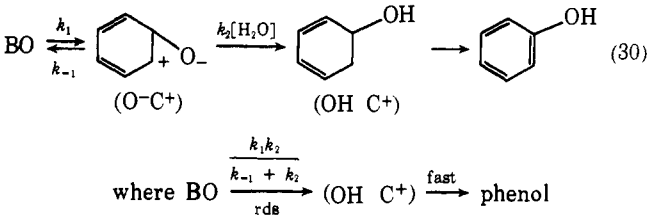


Figure 5. Hypothetical reaction coordinate diagram for the aromatization of benzene oxide in solvents of high (lower curve) and low (upper curve) dielectric constants. The species {O^- C^+} represents an internal ion pair whereas {O^- C^+} and {HO C^+} represent carbonium ion species; the latter being formed from the former by diffusion-controlled proton transfer from water.



(30)

where BO $\xrightarrow[\text{rds}]{\frac{k_1k_2}{k_{-1}+k_2}}$ (OH C^+) $\xrightarrow{\text{fast}}$ phenol

In Figure 6 there is plotted, in the Bronsted fashion, the logarithms of the second-order rate constants for the reaction of RS^- species with A^+, BO, and ethylene oxide[22] vs. the pK_a of RSH. Examination of Figure 6 reveals that the β_{nuc} values for reactions of RS^- with ethylene oxide (20°) and BO and A^+ (30°) are comparable. The small β_{nuc} values indicate that the basicity of the RS^- species exhibited toward the proton has little to do with the nucleophilicity of RS^- toward either substrate. The value of β_{nuc} for the reaction of RS^- species with BO, the delocalized carbonium ion of A^+, and ethylene oxide[34] is comparable to β_{nuc} for reaction of amines[35] with A^+. This feature and the similarity in the rate constants for reaction of RS^- species with BO and ethylene oxide may be interpreted as suggesting similar intimate ion trapping mechanisms. The mechanisms of aliphatic epoxide ring opening has received considerable attention. The weight of evidence supports either direct nucleophilic displacement or displacement through a tight ion pair.[32] Thus, for water attack,[36] the importance of the activity of H_2O in aqueous organic solvents, volumes of activation,[37] entropies of activation, and deuterium solvent kinetic isotope effects supports best an "SN2" reaction. The reaction of HO^-, EtO^-, MeO^-, AcO^-, and Cl^- with aliphatic oxides are all first order in the nucleophile, yield

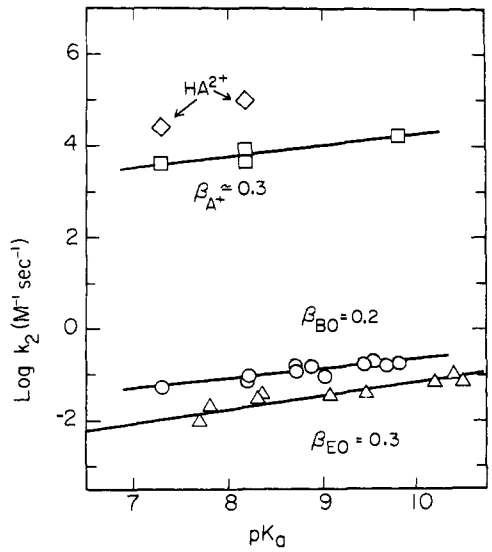


Figure 6. Bronsted plot for reaction of thiol anion species with ethylene oxide (EO) at 20°, benzene oxide (BO) at 30°, and the unprotonated species of malachite green (A^+) at 30°.

products accountable by substitution on primary in preference to secondary carbon, and provide Walden inverted products.[36,38-40] The differentiation between an SN2 reaction and displacement upon an internal ion pair is not readily made. As an example, the near identity of the rate constants for attack of RS^- upon BO (30°) and ethylene oxide (20°) may be explained by either mechanism. The internal ion pair formed from BO would be more stable, due to delocalization of the positive charge, than that from ethylene oxide. However, nucleophilic addition to the resonantly stabilized internal pair formed from BO should be slower than attack upon the localized charge of the internal pair formed from ethylene oxide. The reactions of RS^- with MG serve as an example of nucleophilic addition to a resonantly stabilized carbonium ion. The second-order rate constants are certainly less than one would anticipate for attack on a carbocation. One could argue that the compensation between internal ion pair stability and susceptibility to nucleophilic attack could make the second-order rate constants for reaction of RS^- with BO and ethylene oxide similar. In any event, one must conclude that the mechanism for reaction of RS^- with the epoxides of ethylene and benzene are mechanistically akin.

The second-order rate constants for the reaction of amines with ethylene oxide vary from $\sim 10^{-3}$ to $\sim 10^{-5}$ M^{-1} sec^{-1} on change of the pK_a of the related ammonium ions from ~4 to ~11.[41] If the second-order rate constants for reaction of amines with BO are similar to those for ethylene oxide, as is the case with thiol anions, then no reaction would be seen with BO. This is due to the fact that the aromatization of BO would predominate. Though the reactivity of BO and ethylene oxide toward nucleophiles may be similar, the spontaneous disposition of BO is through aromatization while that for ethylene oxide is nucleophilic addition of water. The spontaneous rate for BO is $\sim 10^3$ greater[27] than that for ethylene oxide.[36,37,42,43] Thus, the fact that ethylene oxide is subject to nucleophilic attack by weaker nucleophiles than is BO is simply due to the fact that ethylene oxide remains in solution longer. The second-order rate constants determined for the reaction of RS^- species with BO are just sufficiently large to allow their determination over the competing aromatization reaction (Figure 1). The β_{nuc} value for reaction of amines with ethylene oxide is ca. 0.3. If the same were true for reaction of amines with BO, then one might predict that nucleophilic addition of amines might be seen in the case of electron-deficient benzene oxides. Under these restrictions, the aromatization reaction would be greatly slowed ($\rho \simeq -7$).[28] This possibility is presently under investigation.

Note Added in Proof. The prediction that electron withdrawing substituents should allow nucleophilic attack by amines on BO has been fulfilled in the observation of nucleophilic catalysis of the aromatization of 4-carbo-*tert*-butoxybenzene oxide by amines and the isolation and proof to structure (NMR) for the 3-trans adduct of trimethylamine [see communication by D. M. Johnson and T. C. Bruice, *J. Am. Chem. Soc.*, **97**, 6901 (1975)].

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References and Notes

(1) Department of Chemistry, King's College, University of London.
(2) (a) "World Symposium on Model Studies in Chemical Carcinogenesis" (Baltimore, 1972), Marcel Dekker, New York, N.Y.; (b) O. G. Fahmy and M. J. Fahmy, *Cancer Res.*, **33**, 2354 (1973).
(3) P. L. Grover, P. Sims, E. Habermann, H. Marquardt, T. Kuroki, and C. Heidelberger, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1098 (1971).
(4) I. Y. Wang, R. E. Rasmussen, and T. T. Crocker, *Biochem. Biophys. Res. Commun.*, **49**, 1142 (1972).
(5) A. Borgen, H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, and I. Y. Wang, *J. Med. Chem.*, **16**, 502 (1973).
(6) P. L. Grover and P. Sims, *Biochem. Pharmacol.*, **19**, 2251 (1970).
(7) P. L. Grover and P. Sims, *Biochem. Pharmacol.*, **22**, 661 (1973).
(8) D. M. Jerina, J. W. Daly, P. Zaltzman-Nirenberg, and S. Udenfriend, *Biochem.*, **9**, 147 (1970).
(9) E. Vogel and G. Günther, *Angew. Chem.*, **79**, 429 (1967); *Angew. Chem., Int. Ed. Engl.*, **6**, 385 (1967).
(10) C. H. Foster and G. A. Berchtold, *J. Am. Chem. Soc.*, **93**, 3831 (1971).
(11) K. Reiff, U. Schumacher, G. Stubenrauch, and W. Tochtermann, *Tetrahedron Lett.*, 1553 (1973).
(12) R. M. DeMarinis, C. N. Filer, S. M. Waraszkiewicz, and G. A. Berchtold, *J. Am. Chem. Soc.*, **96**, 1193 (1974).
(13) R. M. DeMarinis and G. A. Berchtold, *J. Am. Chem. Soc.*, **91**, 6525 (1969).
(14) A. M. Jeffrey, H. J. C. Yeh, D. M. Jerina, R. M. DeMarinis, C. H. Foster, D. E. Piccolo, and G. A. Berchtold, *J. Am. Chem. Soc.*, **96**, 6929 (1974).
(15) G. J. Kasperek, T. C. Bruice, H. Yagi, N. Kaubisch, and D. M. Jerina, *J. Am. Chem. Soc.*, **94**, 7876 (1972).
(16) G. J. Kasperek, P. Y. Bruice, T. C. Bruice, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **95**, 6041 (1973).
(17) A portion of the present study has appeared as a communication [D. M. E. Reuben and T. C. Bruice, *J. Chem. Soc., Chem. Commun.*, 113 (1974)].
(18) E. Vogel, W. A. Boll, and H. Gunther, *Tetrahedron Lett.*, 609 (1965).
(19) J. R. Maley and T. C. Bruice, *Anal. Biochem.*, **34**, 275 (1970).
(20) R. E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).
(21) E. L. Elson and J. T. Edsall, *Biochemistry*, **1**, 1 (1962).
(22) J. P. Danehy and C. J. Noel, *J. Am. Chem. Soc.*, **82**, 2511 (1960).
(23) D. L. Rabenstein, *J. Am. Chem. Soc.*, **95**, 2797 (1973).
(24) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms", Vol. I, W. A. Benjamin, New York, N.Y., 1966, Chapter 1.
(25) R. Cigen, *Acta Chem. Scand.*, **15**, 1892 (1961).
(26) C. A. Bunton and C. Paik, personal communication.
(27) G. J. Kasperek and T. C. Bruice, *J. Am. Chem. Soc.*, **94**, 198 (1972).
(28) G. J. Kasperek, T. C. Bruice, H. Yagi, and D. M. Jerina, *J. Chem. Soc., Chem. Commun.*, 784 (1972).
(29) (a) P. Y. Bruice, G. J. Kasperek, T. C. Bruice, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **95**, 1673 (1973); (b) G. J. Kasperek, P. Y. Bruice, T. C. Bruice, H. Yagi, and D. M. Jerina, *ibid.*, **95**, 6041 (1973).
(30) G. J. Kasperek, T. C. Bruice, H. Yagi, N. Kaubisch, and D. J. Jerina, *J. Am. Chem. Soc.*, **94**, 7876 (1972).
(31) J. G. Fullington and E. H. Cordes, *J. Org. Chem.*, **29**, 970 (1964); A. M. Wenthe and E. H. Cordes, *J. Am. Chem. Soc.*, **87**, 3173 (1965); K. Koehler and E. H. Cordes, *ibid.*, **92**, 1579 (1970).
(32) R. A. Sneen, *Acc. Chem. Res.*, **6**, 46 (1973).
(33) G. A. Rogers and T. C. Bruice, *J. Am. Chem. Soc.*, **96**, 2473 (1974).
(34) The Bronsted treatment for the reaction of amines with ethylene oxide will be discussed in a forthcoming paper (P. Y. Bruice, T. C. Bruice, and D. M. Jerina).
(35) J. E. Dixon and T. C. Bruice, *J. Am. Chem. Soc.*, **93**, 3248 (1971).
(36) P. O. I. Virtanen, *Ann. Acad. Sci. Fenn., Ser. A2*, 124 (1963).
(37) J. Koskikallio and E. Whalley, *Can. J. Chem.*, **37**, 783 (1959).
(38) F. A. Long and J. G. Pritchard, *J. Am. Chem. Soc.*, **78**, 2663 (1956).
(39) H. C. Chitwood and T. C. Freure, *J. Am. Chem. Soc.*, **68**, 680 (1946).
(40) W. Reeve and A. Sadle, *J. Am. Chem. Soc.*, **72**, 1251 (1950).
(41) P. O. I. Virtanen and R. Korhonen, *Acta Chem. Scand.*, **27**, 2650 (1973).
(42) J. N. Bronstein, M. Klipatrick, and M. Klipatrick, *J. Am. Chem. Soc.*, **51**, 428 (1929).
(43) H. J. Lechtenstein and G. H. Tarigg, *Trans. Faraday Soc.*, **44**, 905 (1948).