A Kinetic Study of the Nucleophilicity of Substituted Arenethiols in Reaction with *p*-Nitrophenyl Acetate

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The nucleophilic reactivity of a series of substituted arenethiols towards a single ester substrate, *p*-nitrophenyl acetate, has been determined by spectrophotometric measurements of reaction rates in ethanol at 22°. Conversion of a thiol function into a thiolate anion is a prerequisite for nucleophilic attack on the sp^2 carbon atom. The second-order rate constants were correlated with the pK_a values of the corresponding arenethiols *via* the Brönsted equation giving a β value of 0·61; comparison of proton basicity, carbon basicity, and nucleophilicity of the arenethiolate ions has been made. The reaction was found to be little influenced by variation of ionic strength. A three-fold increase of reactivity was observed on changing the solvent from methanol to ethanol. The activation parameters for the reaction of *p*-nitrophenyl acetate and sodium benzenethiolate in ethanol and in methanol as solvents at 22° have been calculated: $\Delta H^‡ = 11{\cdot}22$ kcal mol^{-1}, $\Delta S^‡ = -23{\cdot}15$ cal mol^{-1} K^{-1} in ethanol, $\Delta H^‡ = 12{\cdot}98$ kcal mol^{-1}, $\Delta S^‡ = -19{\cdot}33$ cal mol^{-1} K^{-1} in methanol.

EXPERIMENTAL evidence has been collected which indicates the intermediate formation of acyl-*O*-[1] and acyl-*S*-enzymes[2] involving the hydroxy- or mercapto-group of a serine or cysteine residue in hydrolysis and transfer of acyl groups. The important role played by thiol esters in acyl transfer reactions has prompted much research into the details of the chemistry of these compounds.[3]

However, information on the nucleophilicity of thiols towards an ester sp^2 carbon is relatively scarce and generally concerns the nucleophilic reactivity of aliphatic thiols toward *p*-nitrophenyl acetate[4] (*p*-NPA). Data on the nucleophilic reactivity of aromatic thiols towards an ester carbonyl carbon atom are almost entirely lacking: the only reported study is that of Schonbaum and Bender[5] on the hydrolysis of *p*-NPA catalysed by *o*-mercaptobenzoic acid.

TABLE 1

Rate constants for the reaction of *p*-NPA with benzenethiol in ethanol at 22°

10^5[*p*-NPA]/ M	$10^2[ArSH]_{total}$/ M	$10^2[ArSH]_{free}$/ M	$10^2[ArS^-]$/ M	*I*/M	pH	10^4k_{obs}/ s^{-1} [b,c]	$10^2k_{ArS^-}$/ l mol^{-1} s^{-1}
3·99	2·01	1·02	0·99	0·01	9·22	25·1	25·4
2·03	2·04	1·04	1·00	0·01	9·21	25·5	25·5
4·02	3·03	2·04	0·99	0·01	9·00	25·1	25·4
4·02	1·49	0·49	1·00	0·01	9·54	25·3	25·3
4·02	3·96	2·01	1·95	0·02	9·22	49·5	25·4
4·02	9·92	5·05	4·87	0·05	9·21	115·5	23·7
3·99	1·99	1·00	0·99	0·02 [a]	9·09	25·0	25·3
3·99	1·99	1·00	0·99	0·06 [a]	8·78	23·6	23·8
3·99	2·06	1·12	0·94	0·11 [a]	8·60	21·4	22·8
3·99	2·06	1·12	0·94	0·21 [a]	8·39	20·4	21·7

[a] Ionic strength adjusted by addition of lithium chloride. [b] Defined by d[product]/d*t* = k_{obs}[*p*-NPA]. [c] All k_{obs} values are based on 15—20 kinetic points.

We now report a systematic kinetic study on the effect of solvent, ionic strength, and substituents in the benzene ring of the arenethiol on the reaction of sodium arenethiolate with *p*-NPA. In order to compare the data obtained in the present work with those reported by other authors[4] for similar reactions, the reactions should be carried out in water. The solubility properties of the arenethiols, however, dictated that the kinetics be measured in 95% ethanol, a medium which perhaps approximates more closely than does water to the probably hydrophobic environment of many protein mercapto-groups.[6]

RESULTS AND DISCUSSION

Sodium benzenethiolate reacts with *p*-NPA in 95% ethanol affording sodium *p*-nitrophenolate and *S*-phenyl thioacetate [equation (**1**)]. Control experiments (see Experimental section) established the reliability of the

$$CH_3CO_2C_6H_4NO_2\text{-}p + ArSNa \longrightarrow p\text{-}NO_2C_6H_4ONa + CH_3COSAr \qquad (1)$$

kinetic assays, the absence of side reactions, and the exclusive formation of the *p*-nitrophenolate and the thioester.

Inspection of the kinetic data in Table 1 reveals the

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[1] B. S. Hartley and B. A. Kilby, *Biochem. J.*, 1952, **50**, 672; 1954, **56**, 288; H. Gutfreund and J. M. Sturtevant, *Proc. Nat. Acad. Sci. U.S.A.*, 1956, **42**, 719; B. Zerner and M. L. Bender, *J. Amer. Chem. Soc.*, 1964, **86**, 3669 and references therein; M. L. Bender and F. J. Kezdy, *ibid.*, p. 3704 and references therein.

[2] G. Lowe and A. Williams, *Proc. Chem. Soc.*, 1964, 140; M. L. Bender and L. J. Brubacher, *J. Amer. Chem. Soc.*, 1964, **86**, 5333; G. Lowe and A. Williams, *Biochem. J.*, 1965, **96**, 194; A. W. Lake and G. Lowe, *ibid.*, 1966, **101**, 402; L. J. Brubacher and M. L. Bender, *J. Amer. Chem. Soc.*, 1966, **88**, 5871, 5880.

[3] M. Florkin and E. H. Stotz, ' Comprehensive Biochemistry,' Elsevier, Amsterdam, vol. 15, 1964; T. C. Bruice and S. J. Benkovic ' Bio-organic Mechanism,' Benjamin, New York, vol. I, 1966; W. P. Jencks, ' Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969; M. L. Bender ' Mechanism of Homogeneous Catalysis from Protons to Proteins,' Wiley–Interscience, New York, 1971.

[4] (*a*) W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, 1960, **82**, 1778; (*b*) J. R. Whittaker, *ibid*, 1962, **84**, 1900; (*c*) J. W. Ogilvie, J. T. Tildon, and B. S. Strauch, *Biochemistry*, 1964, **3**, 754; (*d*) W. Tagaki, T. Amada, Y. Yamashita, and Y. Yano, *J.C.S. Chem. Comm.*, 1972, 1131.

[5] G. R. Schonbaum and M. L. Bender, *J. Amer. Chem. Soc.*, 1960, **82**, 1900.

[6] D. Semenow-Garwood, *J. Org. Chem.*, 1972, **37**, 3797.

following points. (1) At constant thiolate concentration the appearance of *p*-nitrophenol follows first-order kinetics in agreement with equation (2). (2) The

$$d[p\text{-nitrophenol}]/dt = k_{\mathrm{obs}}[p\text{-NPA}] \qquad (2)$$

constancy of k_{obs} with changing $[\mathrm{ArSH}]_{\mathrm{free}}$ and its variation with changing $[\mathrm{ArS^-}]$ indicate that the thiolate anion is the sole reactive species in the rate-determining step and that un-ionized benzenethiol does not participate significantly in the reaction. (3) By dividing the pseudo-first-order rate constant (k_{obs}) by the thiolate concentration $[\mathrm{ArS^-}]$, true second-order rate constants were obtained ($k_{\mathrm{ArS^-}}$). Table 1 shows that $k_{\mathrm{ArS^-}}$ is independent, within experimental error, of the concentration of *p*-NPA, ArSH, and $\mathrm{ArS^-}$ and of pH. The small (*ca.* 7%) rate decrease observed with five-fold increase in buffer concentration signifies that general acid catalysis by ArSH and ionic salt effects are relatively unimportant. This was confirmed by kinetic runs at constant buffer concentration and at different ionic strengths adjusted to the required value by addition of lithium chloride. The constancy of $k_{\mathrm{ArS^-}}$ at different pH values and the zero value of the intercept at zero reagent concentration in the plot of k_{obs} against $[\mathrm{ArS^-}]$ confirms that the ethanol solvent does not significantly compete with benzenethiolate in the reaction.

In Table 2 the rate constants for the reaction of *p*-NPA with the substituted sodium arenethiolates are summarized. Electron-donating substituents increase the reaction rate, whereas electron-withdrawing substituents have the opposite effect.

TABLE 2

Rate constants for the reaction of *p*-NPA with X-substituted arenethiols in ethanol at 22° and ionic strength 0·01M

$[p\text{-NPA}] = 4 \times 10^{-5}\mathrm{M}$; $[\mathrm{ArSH}]_{\mathrm{total}} = 2 \times 10^{-2}\mathrm{M}$; $[\mathrm{ArSH}]_{\mathrm{free}} = 10^{-2}\mathrm{M}$; $[\mathrm{ArS^-}] = 10^{-2}\mathrm{M}$

X	$10^2k_{\mathrm{ArS^-}}$/ l mol^{-1} s^{-1}	$2 + \log k_{\mathrm{ArS^-}}$	pK_a [a]
1 *m*-Cl	4·9	0·69	8·09
2 *m*-Br	5·0	0·70	8·20
3 *m*-F	6·4	0·81	8·34 [b]
4 *p*-Br	8·2	0·91	8·33
5 *p*-Cl	10·2	1·01	8·41
6 *p*-F	23·8	1·38	8·88
7 *m*-OMe	20·6	1·31	9·14
8 H	25·4	1·40	9·28
9 *m*-Me	33·0	1·52	9·52
10 *p*-Me	48·3	1·68	9·60
11 *p*-OMe	99·9	2·00	9·71

[a] Values taken from ref. 10*c*. [b] Directly determined by a method similar to that described in ref. 10*c*.

It is customary to correlate rate data by means of the Brönsted equation. The Brönsted equation has been widely employed in numerous general base and general acid catalysed processes;[7] however it has also been shown to be of great importance, particularly within a given reaction series, in the field of nucleophilic reactivity.[4,8] In Figure 1 the rate constants of the reaction are plotted logarithmically against the apparent acid dissociation constants in ethanol (pK_a) of the corresponding arenethiols. Inspection of Figure 1 shows that the arenethiolates as attaching nucleophiles can be correlated by the Brönsted equation with β 0·61 (*r* 0·999). This value was calculated from the least squares regression line for the *meta*-substituents which all lie on a straight line.

It is of interest to compare the β value observed by us with those obtained in an analogous manner from nucleophilicity–basicity correlations in reactions involving *p*-NPA and other types of bases. Bruice and Lapinski [8a] have demonstrated that logarithmic plots of reaction rates *versus* pK_a of a series of oxygen and nitrogen bases toward *p*-NPA in 28·5% aqueous ethanol at 30° give separate lines of slope 0·8; Ogilvie *et al.*[4c] have reported a Brönsted β value of 0·38 for the reaction of alkanethiols and *p*-NPA in aqueous solution at 25°. The β value we obtained, although higher than that expected on the basis of the results of Ogilvie *et al.*[4c,*] confirms that sensitivity to the basicity of the nucleophile in reactions of *p*-NPA is lower for sulphur bases than for oxygen and nitrogen bases. This, in accord with the view of Ogilvie *et al.*,[4c] is of considerable importance from a biological standpoint, since it signifies that a mercapto-group with a low pK_a would still be an efficient nucleophile in the physiological pH range.

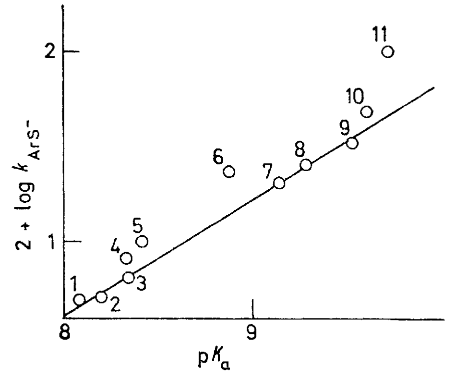

FIGURE 1 Plot of the rate constants ($2 + \log k_{\mathrm{ArS^-}}$) of the reaction of *p*-NPA with X-substituted arenethiols in ethanol at 22° *versus* the pK_a of the corresponding arenethiol. The slope calculated from *meta*-substituents (Cl, Br, F, OMe, Me, H) is 0·61 (*r* 0·999). Numbering of substituents corresponds to that in Table 2

From comparison of the β constants for sulphur, nitrogen, and oxygen nucleophiles, in the light of general concepts, it can be inferred that a greater degree of bond formation occurs in the reaction of esters with oxygen and nitrogen than with sulphur bases. However, recent criticism of some authors[9] on the use of β values as a guide to changes in transition state structures suggests caution in reaching a firm conclusion.

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[*] Caution must be exercised in these comparisons since different conditions were used in the experiments.

[7] R. P. Bell, 'The Proton in Chemistry,' Methuen, London, 1959, p. 155.

[8] (*a*) T. C. Bruice and R. Lapinski, *J. Amer. Chem. Soc.*, 1958, **80**, 2265; (*b*) G. Biggi and F. Pietra, *J. Chem. Soc.* (*B*), 1971, 44.

[9] F. G. Bordwell and W. J. Boyle, *J. Amer. Chem. Soc.*, 1972, **94**, 3907; C. D. Johnson and K. Schofield, *ibid.*, 1973, **95**, 270; M. R. Crampton and M. J. Willison, *J.C.S. Perkin II*, 1974, 238.

Figure 1 shows that the points corresponding to electron-donating substituents such as *p*-OMe, *p*-Me, and *p*-halogen deviate from linearity. This indicates that the arenethiolates with *para*-substituents having a large $+M$ effect have a nucleophilicity higher than expected on the basis of proton basicity. The magnitude of the positive deviation of the points for the substituents mentioned depends on their ability to release electrons mesomerically. Such deviations have been observed [10] in reactions involving *para*-substituted arenethiolates with other types of substrates and different interpretations have been proposed.

It is well known that there exists a limit to the application of the Brönsted law of catalysis to nucleophilic reactions because of the fact that pK_a refers to proton release while nucleophilicity refers to the properties of the carbon atom.[11] It is also known that deviations from the law generally increase as the polarizability of the nucleophile increases and the electronegativity decreases.[12] Thiolate anions have high polarizability and low electronegativity. To see whether a more satisfactory correlation could be obtained, the rate constants from Table 2 have been plotted logarithmically against the carbon basicities (log k_{TNB}) of the arenethiolates (Figure 2). The carbon basicities of the arenethiolates were determined by Crampton [10c] by measurement of the equilibrium constants for the formation of 1 : 1 covalently bound addition complexes of the arenethiolates with 1,3,5-trinitrobenzene in 95% ethanol at 22°. In this plot all the points fall on a straight line with slope 0·54 (r 0·998). The excellent correlation obtained indicates that, as found for reactions with other types of substrates,[9] with ester as substrate the nucleophilic reactivities of the substituted arenethiolates are better correlated with their carbon than with their proton basicities.

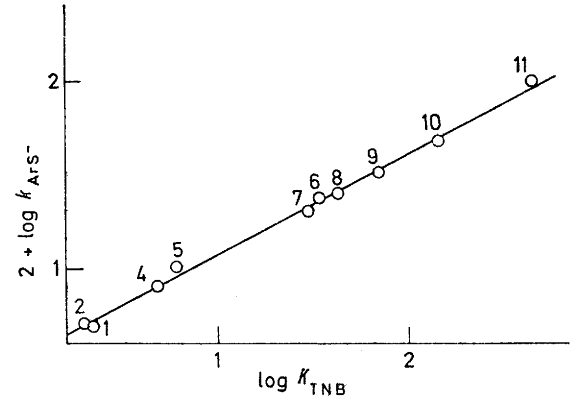

FIGURE 2 Plot of the rate constants (2 + log k_{ArS^-}) for the reaction of *p*-NPA with X-substituted arenethiols in ethanol *versus* log K_{TNB} (equilibrium constants for formation of 1 : 1 covalently bound addition complexes of the arenethiolates with 1,3,5-trinitrobenzene). The slope calculated from all the substituents is 0·54 (r 0·998). Numbering of substituents corresponds to that in Table 2

TABLE 3

Rate constants at different temperatures and activation parameters for the reaction of *p*-NPA with benzenethiol in ethanol (pH 9·22) and methanol (pH 8·55) at ionic strength 0·01M

[*p*-NPA] = 4×10^{-5}M; $[ArSH]_{total} = 2 \times 10^{-2}$M; $[ArSH]_{free} = 10^{-2}$M; $[ArS^-] = 10^{-2}$M

	EtOH			MeOH		
T/°C	$10^2 k_{ArS^-}$/ l mol^{-1} s^{-1}	$\Delta H^{\ddagger}_{22°}$/ kcal mol^{-1} [a]	$\Delta S^{\ddagger}_{22°}$/ cal mol^{-1} K^{-1} [b]	$10^2 k_{ArS^-}$/ l mol^{-1} s^{-1}	$\Delta H^{\ddagger}_{22°}$/ kcal mol^{-1} [a]	$\Delta S^{\ddagger}_{22°}$/ cal mol^{-1} K^{-1} [b]
22·0	25·4	11·22	−23·15	8·92	12·98	−19·33
28·0	41·3			14·45		
36·1	66·1			26·55		

[a] ±0·3 kcal mol^{-1}. [b] ±1 cal mol^{-1} K^{-1}.

A study on the effect of change of polarity of the medium from ethanol to methanol was also performed. Table 3 reports the rate constants at three different temperatures for the reaction of *p*-NPA with sodium benzenethiolate in methanol and ethanol. A comparison of the data at 22° shows that the process is about three-fold faster in ethanol than it is in methanol. The increase of reaction rate with decreasing polarity of the medium is apparently favoured by an enthalpy factor which compensates for the unfavourable entropy loss. This effect is expected for a reaction in which charge is more dispersed in the transition state than in the ground state.[13] However, in the light of the anomalous solvent effects observed in other reactions involving esters,[14] no conclusion on the mechanism of the reaction studied here can be drawn from this result.

EXPERIMENTAL

Solvents.—Ethanol (95%) was purified by distillation of the azeotrope; methanol was distilled on Mg activated by iodine.[15] Dioxan was purified following the method described in literature.[16] Solvents were degassed by bubbling in nitrogen and were stored under nitrogen.

Materials.—*p*-NPA was prepared as described [17] and recrystallized from n-hexane, m.p. 78—79°. The following

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[10] (*a*) R. F. Hudson and G. Klopman, *J. Chem. Soc.*, 1962, 1062; (*b*) G. Guanti, C. Dell'Erba, and P. Macera, *J. Heterocyclic Chem.*, 1973, **10**, 1007; (*c*) M. R. Crampton, *J. Chem. Soc.* (*B*), 1971, 2112; (*d*) P. De Maria and A. Fini, *ibid.*, p. 2335; (*e*) G. Bartoli, L. Di Nunno, L. Forlani, and P. E. Todesco, *Internat. J. Sulfur Chem. C.*, 1971, 77.

[11] A. J. Parker, *Proc. Chem. Soc.*, 1961, 371.

[12] C. Duboc, *Bull. Soc. chim. France*, 1970, 1768.

[13] E. D. Hughes and C. K. Ingold, *Trans. Faraday Soc.*, 1941, **37**, 6.

[14] E. Tommila and M. L. Savolainen, *Suomen Kem.*, 1967, **40B**, 212; E. Tommila, *ibid.*, 1964, **37B**, 117; G. J. Nolan and E. S. Amis, *J. Phys. Chem.*, 1961, **65**, 1556.

[15] L. F. Fieser 'Experiments in Organic Chemistry,' Heath, Boston, 1941, 2nd. edn., p. 360.

[16] A. I. Vogel 'Practical Organic Chemistry,' Longmans, London, 3rd edn., 1961.

[17] A. Spasov, *Ann. Univ. Sofia* II, *Fac. phys.-mat.*, 1938—1939, **35**, 289 (*Chem. Abs.*, 1940, **34**, 2343).

arenethiols were purified commercial specimens: benzenethiol, *m*- and *p*-toluenethiol, *p*-chlorobenzenethiol, *p*-bromobenzenethiol, *p*-fluorobenzenethiol, *m*- and *p*-methoxybenzenethiol. *m*-Bromo- and *m*-chlorobenzenethiol were synthesized from the corresponding anilines *via* the xanthate esters;[18] *m*-fluorobenzenethiol, b.p. 62—63° at 13 mmHg, was obtained in good yield (43·5%) by the same method. All the arenethiols were carefully distilled or recrystallized just prior to use.

Apparatus.—The spectrophotometer employed was a Zeiss PMQ II fitted with a thermostatted brass block for holding the cuvettes. The cell compartment was thermostatted at the required temperature by circulating water from a Haake FE temperature-controlled bath. pH Values were taken, on a Radiometer PHM4c pH meter employing a glass or a combination glass–KCl electrode, at the temperature at which the kinetics were run. In selected cases, the pH was measured before and after a kinetic run; in no case was a pH change $>0{\cdot}02$ unit detected.

Kinetics.—All vessels were flushed with nitrogen to minimize air oxidation of thiols. The release of *p*-nitrophenol was followed spectrophotometrically at 400 nm. Temperature control was better than ±0·1°. The buffer solution (thiol–thiolate) was prepared by mixing two thermostatted solutions of the appropriate thiol and of sodium ethoxide in ethanol. The concentration of the freshly prepared solutions of thiols was determined by iodometric titration; the concentration of thiolate anion was taken as equal to that of the sodium ethoxide titrated with 0·1N-hydrochloric acid (Methyl Red–Bromocresol Green). A stock solution of *p*-NPA was prepared in dioxan (*ca.* 4×10^{-3}M); the ester concentration was chosen so that the value of the optical density at infinite reaction was *ca.* 0·5—0·6. The reaction was initiated by the addition of *p*-NPA solution (20 μl; withdrawn with a microsyringe equipped with a reproducibility Chaney-type adapter) to buffer (2 ml) at the required temperature; thus the solvent used in the reaction was 1% dioxan–ethanol (95%) (v/v). Teflon-stoppered quartz cuvettes (1·0 cm path length; prefilled with nitrogen) were used to minimize oxygen access and consequent disulphide formation.

In order to maintain a constant pH the thiols were used as buffer as well as nucleophile: kinetic experiments were carried out with the thiol and the thiolate in great excess over *p*-NPA so that pseudo-first-order kinetics were followed. The pseudo-first-order rate constants were determined at a number of different concentrations of the reagents (see Table 1) from the slope of plots of log ($\text{O.D.}_{\infty} - \text{O.D.}_t$) against time. Linear rate plots were found to at least 90—95% reaction. Infinite time optical density readings were taken at approximately 10 half-lives and agreed, within experimental error, with those expected from the extinction coefficient of *p*-nitrophenol determined under identical conditions in the buffer used for the kinetic run. Second-order rate constants were obtained by dividing the observed pseudo-first-order constants by the thiolate concentration. Each k value is the average of at least four independent values, the mean deviation being ±2·5%.

Product Analyses.—Absence of solvolytic side reactions of *p*-NPA was established by control runs. Control experiments were performed to establish the reliability of the kinetic assays and to confirm the exclusive formation of *p*-nitrophenolate anion and thioester. T.l.c. analyses on silica gel [chloroform–light petroleum (b.p. 30—60°) (1 : 2)] gave two spots corresponding to the expected products when equimolar amounts of reactants were employed at suitable concentrations. A preparative reaction of *p*-NPA with benzenethiol was carried out using the conditions of the kinetic runs. Sodium *p*-nitrophenolate and thioester were isolated in quantitative yield.

[4/1236 *Received, 24th June,* 1974]

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[18] F. G. Bordwell and H. M. Andersen, *J. Amer. Chem. Soc.*, 1953, **73**, 6019.