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Solvent Isotope Effects in Intramolecular Catalysis: Acyl Transfer Reactions of 4-Nitrophenyl 5-Nitrosalicylate in Aqueous Tris Buffer

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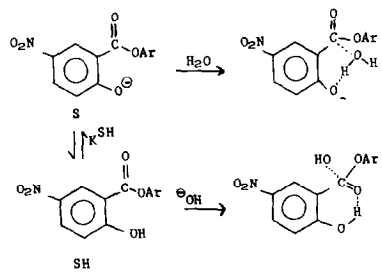
A kinetic study of the reactions of 4-nitrophenyl 5-nitrosalicylate in aqueous Tris buffer at 25°C has revealed three kinetically significant reactions: Tris aminolysis of the un-ionized substrate, Tris aminolysis of the ionized substrate, and spontaneous hydrolysis of the ionized substrate. Solvent isotope effects have been measured for all three reactions. Mechanisms are discussed, and the conclusion is reached that intramolecular catalysis is operating in only the spontaneous hydrolysis.

INTRODUCTION

In the past few years there has been a growing interest in chemical models of enzyme action. An especially useful class of reactions is one in which the model catalyst and substrate are bound to the same molecular framework. The basic premise is that reacting groups juxtaposed on the same molecule serve as a valid analogy for a reaction in an enzyme–substrate complex. A number of reviews have appeared on this topic (*1*). In this large body of research, two distinct types of models emerge. The first type is mimetic models; i.e., the reactions model specific enzymes. This type of chemical modeling is the subject of an excellent review by Fife (*2*). The second type is nonmimetic models; i.e., the reactions model a specific feature of the general process of enzyme catalysis. This type is more general and covers catalysis by functional groups.

A classic example of intramolecular catalysis of the nonmimetic type, the hydrolysis of 4-nitrophenyl 5-nitrosalicylate, was reported by Bender *et al.* (*3*). They suggested that the hydrolysis reaction from pH 7 to 10 occurred via intramolecular general base catalysis (Eq. [1]). Their conclusion was arrived at by excluding the kinetically equivalent mechanism, intramolecular general acid–specific base catalysis (Eq. [2]).



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Reactions of 4-nitrophenyl 5-nitrosalicylate with external nucleophiles were not exceptionally rapid when compared to the corresponding *O*-methyl compound, 4-nitrophenyl 2-methoxy-5-nitrobenzoate. Tis lack of rate enhancement was interpreted as meaning that if intramolecular general acid catalysis was not important for weaker nucleophiles it would not be important for hydroxide ion. The observed solvent isotope effect of 1.68 was acknowledged as being low for a general-base-catalyzed reaction. Bender *et al.* (*3*) pointed out that since both Eqs. [1] and [2] would give similar isotope effects, this mechanistic criterion was not useful for this case.

Since the time this paper was published, a number of solvent isotope effects on general-base-catalyzed reactions have been reported (*4–6*) which reinforce the idea that a solvent isotope effect for general-base catalysis should be in the range of 2–3. Since the original kinetics were determined in 34.4% dioxane/water and the solvent isotope effect was measured at only one pD value, it was decided to determine a pH-rate profile in water and a pD-rate profile in deuterium oxide in the pL range of 7–10. Tris buffer was employed for these pL ranges, and significant terms for buffer reactions were observed.

Solvent isotope effects have been determined for all the measured rate constants, and mechanistic deductions from these effects form the subject of this report.

EXPERIMENTAL

Materials. Tris (Calbiochem, Ultrol grade) and Tris–hydrochloride (Calbiochem, A grade) were used as received. Potassium chloride (MCB, reagent grade) was dried in an oven for 24 hr at 130°C and stored in a desiccator until needed. 4-Nitrophenyl 5-nitrosalicylate was prepared according to the previous procedure (*7*). Acetonitrile was distilled from phosphorous pentoxide. Water was double distilled and deuterium oxide (Aldrich, gold label) was distilled.

Solutions. Buffers were prepared gravimetrically in the order Tris, Tris–hydrochloride, and potassium chloride, and diluted to the mark, 50 ml of water or 25 ml of deuterium oxide. A 2.5×10^{-3} *M* solution of 4-nitrophenyl 5-nitrosalicylate in acetonitrile was prepared as a stock solution for the kinetic runs. The pH or pD of each cuvette at the termination of a run was determined with a Beckman Model 4500 pH meter.[4] For pD, a correction factor of 0.4 was added to the meter reading (*8*).

Kinetics. The release of 4-nitrophenoxide ion was monitored at 415 nm with a Cary 118c ultraviolet-visible spectrophotometer equipped with a constant temperature apparatus and an auto sample changer. The reaction was initiated by injecting 25 μl of the stock solution into 3.0 ml of buffer solution in a 1-cm quartz cuvette, thermally equilibrated at 25°C. Reactions were followed for at least three half-lives, and the first-order rate constants were calculated by a nonlinear least-squares computer program from given time and absorbance values. The time and absorbance values were determined by digitizing the traces on the chart paper with a CALMAgraphic II stand-alone

[4] It should be pointed out that pH and pD are not defined in acetonitrile/water mixtures. We have observed that addition of a small amount of acetonitrile has a negligible effect on the pH meter reading of the buffer solutions. We are assuming in this report then that the pH meter reading is a fair approximation of the actual pH.

TABLE 1

FIRST-ORDER RATE CONSTANTS ($10^7\ k_{obsd}$ FOR THE REACTION OF 2×10^{-5}-*M* 4-NITROPHENYL 5-NITROSALICYLATE IN TRIS BUFFERS IN WATER AT 25°C, IONIC STRENGTH = 0.2

[Tris]/[Tris–HCl]	pH	$10^7\ k_{obsd}$ (sec^{-1})					
		[0.00799][a]	[0.01210]	[0.01991]			
0.0997	7.21	1620 ± 40[b] (4)[c]	1838 ± 5 (5)	2360 ± 10 (5)			
		[0.01018]	[0.02030]	[0.03005]	[0.04001]		
0.2001	7.49	1481 ± 6 (5)	1793 ± 5 (4)	2049 ± 3 (5)	2547 ± 10 (5)		
		[0.02991]	[0.06050]	[0.08971]			
0.5168	7.83	1746 ± 11 (5)	2429 ± 7 (5)	3022 ± 7 (5)			
		[0.01024]	[0.03944]	[0.06748]	[0.07987]	[0.1012]	[0.1201]
0.9716	8.09	1317 ± 4 (5)	1752 ± 10 (9)	2212 ± 5 (5)	2544 ± 7 (4)	2829 ± 9 (5)	3060 ± 33 (3)
		[0.03995]	[0.08007]	[0.1201]			
9.997	8.92	1719 ± 2 (4)	2259 ± 6 (5)	2703 ± 5 (4)			

[a] [Tris].
[b] Standard error of the mean.
[c] Number of determinations.

digitizing system. Typically, 80 points were determined for each run. The standard error for an individual rate constant was consistently less than ±0.6%.

RESULTS

The kinetics of the reactions of 4-nitrophenyl 5-nitrosalicylate in aqueous Tris buffer are listed in Table 1. In Table 2 is a similar listing for reactions in Tris-buffered deuterium oxide.

TABLE 2

FIRST-ORDER RATE CONSTANTS ($10^7\ k_{obsd}$) FOR THE REACTION OF 2 × 10^{-5} *M* 4-NITROPHENYL-5-NITROSALICYLATE IN TRIS BUFFERS IN DEUTERIUM OXIDE AT 25°C, IONIC STRENGTH = 0.2

[Tris]/[Tris–HCl]	pD	$10^7\ k_{obsd}$ (sec^{-1})			
		[0.00227][a]	[0.01044]	[0.02000]	
0.1992	8.01	574.9 ± 4.2[b] (5)[c]	741.6 ± 3.3 (5)	981.6 ± 2.8 (5)	
		[0.00502]	[0.01057]	[0.01510]	[0.03012]
0.5012	8.41	575.2 ± 6.5 (5)	658.0 ± 2.6 (5)	717.9 ± 2.3 (5)	914.2 ± 5.5 (5)
		[0.01984]	[0.0999]	[0.15010]	
0.9736	8.59	769.1 ± 4.4 (4)	1587 ± 14 (5)	2093 ± 9 (5)	
		[0.04016]	[0.07995]	[0.11980]	
9.833	9.53	961.7 ± 3.9 (5)	1293 ± 10 (5)	1653 ± 5 (5)	

[a] [Tris].
[b] Standard error of the mean.
[c] Number of determinations.

These data support the rate law that has been suggested by Bender *et al.* (*3*) Eq. [3] where the observed rate constant, k_{obsd}, can be further partitioned into solvolytic, k_0, and buffer, k_A and k_{HA}, rate constants (Eq. [4]).

$$d[ArO^-]/dt = k_{obsd}([S] + [SH]) \quad [3]$$

$$k_{obsd} = k_0 + k_A[Tris] + k_{HA}[Tris–HCl] \quad [4]$$

In order to evaluate these three rate constants, Eq. [4] is rearranged to Eq. [5], where R = [Tris]/[Tris–HCl].

$$k_{obsd} = k_0 + (k_A + k_{HA}/R)[Tris] \quad [5]$$

Plots of mean values of k_{obsd} versus [Tris] for the same value of R are shown in Figs. 1 and 2, employing the data from Tables 1 and 2, respectively. The lines are calculated by linear least-squares analysis (*9*).

The intercept, k_0, for each line is reported in Table 3. As illustrated in Fig. 3, a log k_0–pH (pD) profile, there is almost no dependence of k_0 on pH (pD) in the range studied. This is because the substrate is predominantly in the form of S. The pK of SH has been

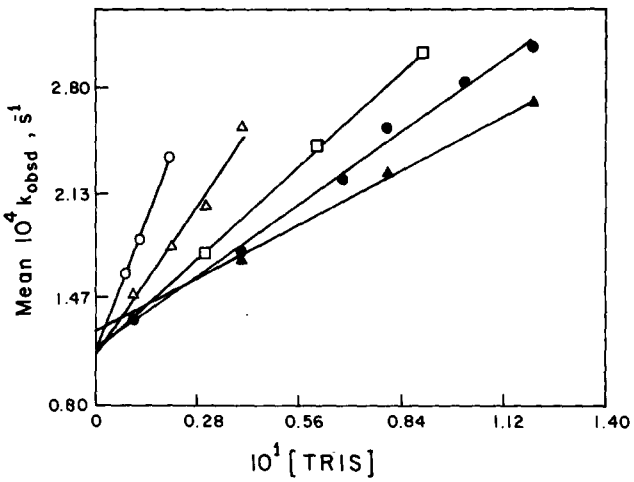


FIG. 1. Plots of the means of observed rate constants versus Tris concentration at constant buffer ratio in water. Buffer ratio = 0.0997 (○), 0.2001 (△), 0.5158 (□), 0.9716 (●), and 9.997 (▲).

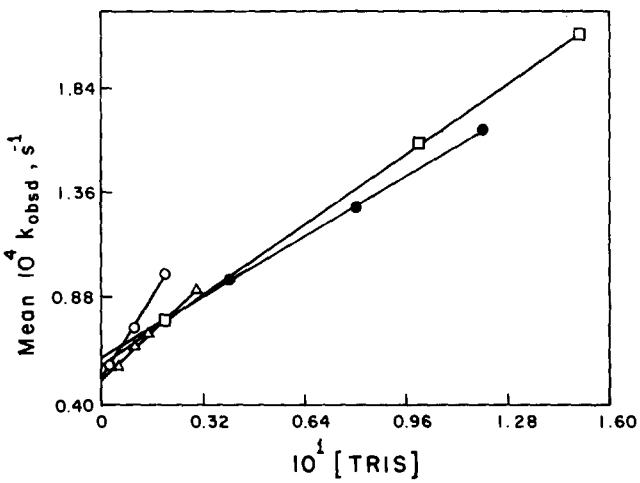


FIG. 2. Plots of the means of the observed rate constants versus Tris concentration at constant buffer ratio in deuterium oxide. Buffer ratio = 0.1992 (○), 0.5012 (△), 0.9736 (□), and 9.833 (●).

measured (*3*) in 34.4% dioxane/water as 6.023. The pK in water is estimated[5] at 5.6. Thus at pH 7.2, the substrate is 97.5% in the form of S.

From Eq. [5] it is seen that slopes from the lines in Figs. 1 and 2 equal $(k_A + k_{HA}/R)$. These slopes decrease as R increases, indicating a significant k_{HA} term. A plot, Fig. 4, of $(k_A + k_{HA}/R)$ versus $1/R$ solves for k_A (intercept) and k_{HA} (slope). The results of linear least-squares analysis for the values of k_A and k_{HA} are given in Table 4.

[5] Determinations of pK have been done (*3*) on 4-nitrophenol, 7.62, and 5-nitrosalicylic acid, 2.55 and 11.50, in 34.4% dioxane/water. If the pK's of 4-nitrophenol (*10*) and 5-nitrosalicylic acid (*11*) in water, 7.14, 2.31, and 10.22, respectively, are compared with those in the solvent mixture, a trend is seen in the pK differential as acid strength decreases, i.e., 2.55 − 2.31 = 0.24, 7.62 − 7.14 = 0.48, and 11.50 − 10.22 = 1.28. A correction factor of 0.4 at pK = 6 can be determined from a plot of the three pK differentials vs pK in the solvent mixture.

TABLE 3

SUMMARY OF SOLVOLYTIC RATE CONSTANTS (10^7 $k_0^{L_2O}$) AND THEIR SOLVENT ISOTOPE EFFECTS[a]

pH	$k_0^{H_2O}$ (sec^{-1})	pD	$k_0^{D_2O}$(sec^{-1})	$k_0^{H_2O}/k_0^{D_2O}$
7.21	1103 ± 50[b]	—	—	—
7.49	1092 ± 100[b]	8.01	515.3 ± 17.6	2.12 ± 0.21[c]
7.83	1117 ± 38	8.41	512.6 ± 4.7	2.18 ± 0.08
8.09	1142 ± 53	8.59	568.2 ± 3.4	2.01 ± 0.09
8.92	1244 ± 59	9.53	608.2 ± 17.7	2.04 ± 0.11

[a] Listed in order of similar buffer ratios in each.
[b] Standard error.
[c] Propagated error.

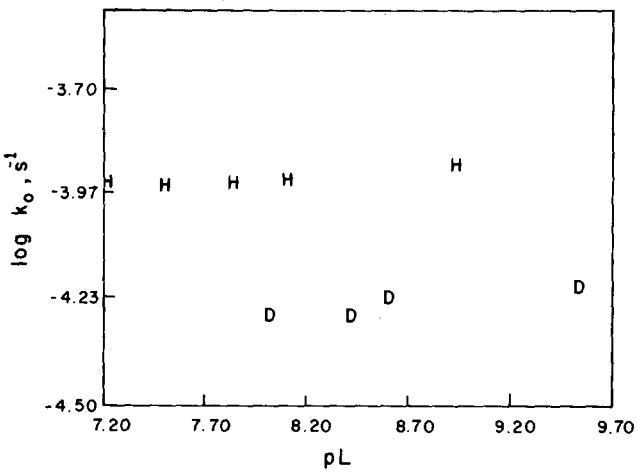


FIG. 3. Log of solvolysis rate constants versus pH (H) and pD (D).

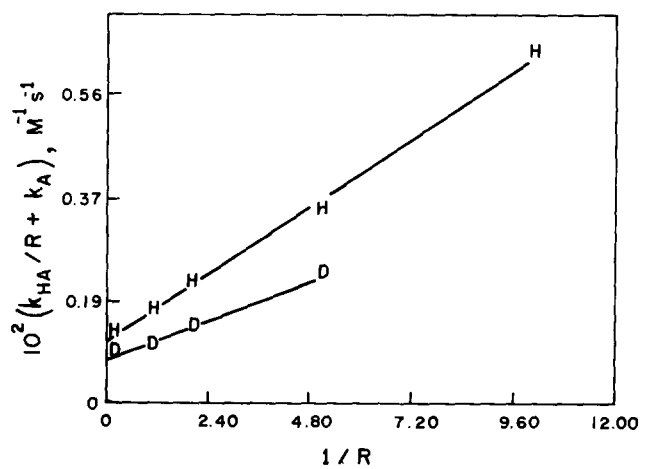


FIG 4. Plots of the slopes of the lines from Fig. 1 (H) and Fig. 2 (D) versus the reciprocal of the buffer ratio. See Results.

TABLE 4

SUMMARY OF BUFFER RATE CONSTANTS ($10^7\ k_{HA}$, $10^7\ k_A$) AND THEIR SOLVENT ISOTOPE EFFECTS[a]

$k_{HA}^{H_2O} = 5{,}059 \pm 140$[b]	$k_A^{H_2O} = 11{,}200 \pm 720$
$k_{HA}^{D_2O} = 3{,}002 \pm 189$	$k_A^{D_2O} = 7{,}701 \pm 519$
$k_{HA}^{H_2O}/k_{HA}^{D_2O} = 1.69 \pm 0.12$[c]	$k_A^{H_2O}/k_A^{D_2O} = 1.45 \pm 0.14$

[a] Constants expressed as $M^{-1}\ sec^{-1}$.
[b] Standard error of estimate.
[c] Propagated error.

DISCUSSION

Buffer Reactions

The buffer terms in the rate expression could describe a number of mechanistic possibilities. It is readily seen from Eqs. [3] and [4] that there are two possible reactions corresponding to each buffer term:

$$k_{buffer} = k_A[\text{Tris}]([S] + [SH]) + k_{HA}[\text{Tris–HCl}]([S] + [SH]), \qquad [6]$$

where

$$k_{buffer} = k_{obs}([S] + [SH]) - k_0[S],$$

viz. reaction of each form of the buffer with either the ionized or un-ionized form of the substrate. The k_{HA} [Tris–HCl] term describes two mechanistic possibilities: (a) general acid catalysis, and (b) specific acid–general base catalysis. The k_A[Tris] term describes three: (a) general base (protolytic) catalysis, (b) nucleophilic reaction (aminolysis), and (c) specific base-general acid catalysis. The kinetic situation is complicated further by the ionization of the substrate, hence $k_A^{SH}[\text{Tris}][SH] \equiv k_{HA}[\text{Tris–HCl}][S]$. Fortunately, some of the above mechanisms are less likely than others.

Specific acid–general base catalysis does not appear likely. A mechanism is described in Eq. [7].

$$\text{ArO–}\overset{\overset{O}{\|}}{C}\text{–Ar} + H_3O^+ \underset{}{\overset{K}{\rightleftharpoons}} \text{ArO–}\overset{\overset{+OH}{\|}}{C}\text{–Ar} + H_2O + \text{Tris} \xrightarrow{k'} \text{products} \qquad [7]$$

The rate constant, k', for the rate-limiting step can be estimated in the following manner. Since

$$k_{HA}[\text{Tris–HCl}] = (k_{HA}/K^{TH})[H_3O^+][\text{Tris}],$$

then

$$Kk' = k_{HA}/K^{TH}$$

or

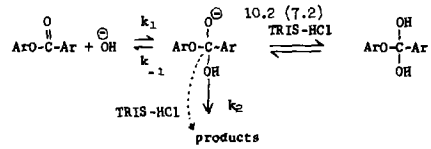
$$k' = k_{HA}/KK^{TH},$$

where K^{TH} = acid dissociation constant of Tris = $10^{-8.2}$. The value of K is the reciporcal of K_a for (Ar–COH–OR)$^+$ (*12*), ca. $10^{7.4}$. Using the value for $k_{HA}^{H_2O}$ from Table 4, then

$$k' = 10^{-3.3}/10^{-7.4}\ 10^{-8.2} = 10^{12.3},$$

which is greater than the diffusion limit. Therefore, the mechanism described by Eq. [7] is probably not operational in this system.

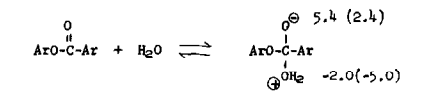
Specific base–general acid catalysis can also be considered as an unlikely mechanism. Jencks (*13*) has elaborated criteria for reactions that are subject to enforced general acid–base catalysis. Examination of hydrolysis mechanisms of S, involving specific base–general acid catalysis, reveals that these mechanisms would not meet the Jencks' criteria. The primary objection lies in the stability of the addition intermediate formed when $^-$OH adds to S:



[8]

This intermediate is more likely to breakdown to products than regenerate reactants ($k_2 > k_{-1}$), since ArO^- is a better leaving group than OH^-, thus obviating the need for any acid catalysis. Acid catalysis of the breakdown step (dashed line) is not likely, since the pK of the leaving group is less than the pK of catalyst. Although the pK of the intermediate[6] (see Appendix) satisfies Jencks' Rule (*14*) for acid catalysis, the intermediate formed is not on a likely pathway to products.

General acid catalysis is probably not a likely mechanism either. Examination of an intermediate formed by the addition of a water molecule, Eq. [9], reveals that the oxyanion generated would not be basic enough for a thermodynamically favorable proton transfer.



[9]

As the analysis reveals, loss of a proton from the oxonium ion (base catalysis) is a necessity for hydrolysis to occur. Thus, if general catalysis is taking place, it must be general base and not general acid.

Now that all of the acid-catalyzed mechanisms are ruled out, there are only two remaining choices—general base catalysis or a nucleophilic reaction (aminolysis). At this point, it can be suggested that the two buffer reaction terms represent different ionization states of the substrates; i.e., the general acid term represents k_A^{SH}[Tris][SH]. All that remains is to decide between a general base mechanism and an aminolysis.

The question of determining whether a compound acts as a proton transfer agent or a nucleophile is a common one. Bender has provided (*15*) a superb outline of the methods used to achieve this differentiation. There are two pieces of evidence that support the choice of aminolysis. It has been observed (*16*, *17*) that reaction of Tris with phenyl esters is aminolysis. Our observed solvent isotope effects, 1.45 and 1.69, are more in line

[6] pK for S is given and pK for SH is in parentheses.

with aminolysis than general base catalysis. For example, it is proposed that if the solvent isotope effect is less than 1.5, the mechanism is nucleophilic. If the solvent isotope effect is greater than 2.0, a general base mechanism is preferred (*4–6*). Thus, it is suggested here that the reaction of Tris with S and SH is aminolysis.

The question arises as to why the reaction of Tris with SH is kinetically significant since only a small fraction of SH is present at these pH's. This means that the observed value for k_{HA} is not a true reflection of aminolysis rate constant for Tris and SH. A value for the overall rate constant for aminolysis of SH, k_A^{SH}, can be derived from the measured value of k_{HA} and the kinetic equivalence,

$$k_A^{SH}[SH][Tris] = k_{HA}[S][Tris\text{–}HCl].$$

Rearranging this expression gives

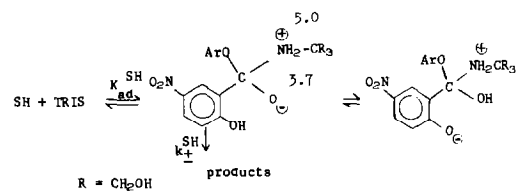
$$k_A^{SH} = k_{HA}\frac{[S][Tris\text{–}HCl]}{[SH][Tris]} = k_{HA}\frac{K^{SH}}{K^{TH}}, \qquad [10]$$

where K^{SH} and K^{TH} are the acid dissociation constants for SH and Tris–HCl, respectively. Then

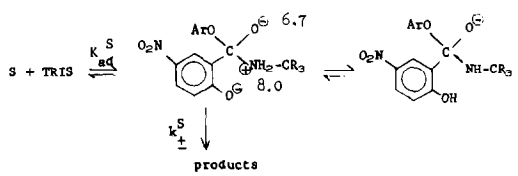
$$k_A^{SH} = (5.06 \times 10^{-4})(10^{-5.6}/10^{-8.2}) = 2.01 \times 10^{-1}\,M^{-1}\,\text{sec}^{-1}.$$

From this calculation it is seen that Tris aminolysis of SH is 180-fold faster than Tris aminolysis of S (from Table 4, $k_A^{H_2O} = 1.12 \times 10^{-3}\,M^{-1}\,\text{sec}^{-1}$).

Examination of the adducts formed in the two reactions (Eqs. [11] and [12]) leads to some interesting conclusions about the rate difference.



[11]



[12]

If the pK's of the phenolic groups in the adducts[7] lie somewhere between 5.6 and 7.2, then intramolecular trapping of the initial adducts does not appear likely in either reaction. The mechanism of both reactions would appear, according to the classification of Satterthwait and Jencks (*18*), to be "Class II" reactions, i.e., rapid formation of the adduct followed by a rate-limiting expulsion of ArO^-. The difference in rate between the

[7] It is assumed that the $-C(OAr)(O^-)NH_2CR_3^+$ group is not more electron withdrawing than a –COOAr group. This would suggest a lower limit of 5.6 (see footnote 5). It is also assumed the $-C(OAr)(O^-)NH_2CR_3^+$ group is not electron donating and thus the upper limit of 7.2, the approximate pK of 4-nitrophenol (*10*).

two reactions can be explained as a difference in the rate of breakdown ($k_{\pm}^{SH} > k_{\pm}^{S}$), a difference in the formation of adduct ($K_{ad}^{SH} > K_{ad}^{S}$), or a combination of both factors. The latter two explanations seem more probable to us, although mechanisms involving proton donation to the leaving group have been proposed for a related reaction in an aprotic solvent (*19*).

Differences in rates of reactions of other nucleophiles with S and SH have been observed (*3*) previously. Azide ion reacts 94-fold faster with SH than with S, while for imidazole the rate difference is only 6-fold. When the rates of the reactions of azide and imidazole with 4-nitrophenyl 2-methoxy-5-nitrobenzoate are compared (*3*) with the similar reactions with SH, there is observed only a small rate increase when a hydroxyl is present. Bender *et al.* (*3*) have concluded from this observation that the hydroxyl group is not acting as an acid catalyst.

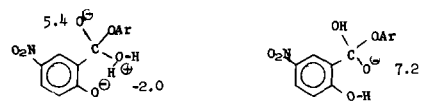
In summary, the aminolyses of S and SH by Tris follow a pattern similar in rate difference to that for other nucleophilic reactions with these substrates. Participation of the hydroxyl group in SH does not appear to be a likely explanation for the increased rate with SH. The difference in the solvent isotope effects for the two reactions, 1.45 for S and 1.69 for SH, likely reflects the difference in the number of exchangeable hydrogens in the two transition states.[8]

Solvolysis

Table 3 lists the solvent isotope effects for comparable positions on the pH- and pD-rate profiles (Fig. 3). These solvent isotope effects are for all intents and purposes the same, ca. 2.1. It is hard to provide a definitive explanation for the difference in the value of the solvent isotope effect, 1.68, measured in dioxane/water (*3*), and our value. Although a pD-rate profile was not determined previously, the point chosen for comparison, pD 8.63, would certainly be expected to be in the plateau region of the profile. The only conclusion that can be reached is that the solvent isotope effect varies in the presence of a co-solvent.

Bender *et al.* have reported (*3*) that the hydrolysis rate increases as the concentration of water increases. This rate increase may be due to an increase in solvent polarity or it may mean that more than one water molecule is involved, in other than a solvating capacity, in the transition state. It is of interest to note that in another case of intramolecular general base catalysis, aspirin anion hydrolysis (*20*), where there is strong support for a one-water bridge (*6*), the spontaneous hydrolysis rate is independent of water concentrations in dioxane/water mixtures (*21*).

Shown below are the two possible adducts, water plus S and hydroxide ion plus SH.



It can readily be seen, according to Jencks' criteria (*13*, *14*), that intramolecular base catalysis is more likely than intramolecular acid catalysis.

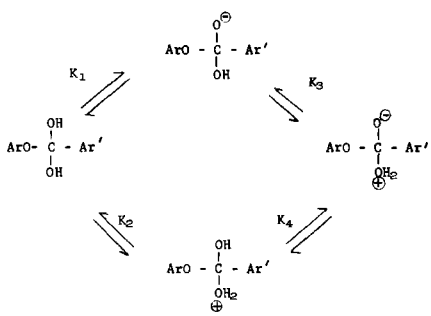
[8] It cannot be discerned at this time whether the solvent isotope effect results entirely from changes in fractionation factors at the exchangeable positions on the adducts or whether hydrogen-bonding solvent molecules need to be included.

Our solvent isotope effect of 2.1 is supportive of an intramolecular general base mechanism, as suggested previously (*3*). This new value would be harder to explain by an intramolecular general acid–specific base mechanism than would be the previous value. Attack of ^{-}OH would give an inverse contribution (*5*) to the net solvent isotope effect, which would have to be cancelled by the isotope effect on the bridging proton. Using the arguments presented above against the intermolecular general acid–specific base mechanism, we would conclude that the position of the bridging proton would not be far removed from the phenolic oxygen. Hence, a small normal isotope effect would be predicted. Consequently a net solvent isotope effect for this mechanism would be small or perhaps even inverse.

Whether or not the molecular details of the mechanism are those described previously (Eq. [1]) remains to be demonstrated. Certainly, the transition-state structure pictured in Eq. [1] is the simplest explanation. However, there are questions concerning the structural aspects of this transition-state structure (*22*, *23*). Unfortunately, the answers to these questions await the development of a more precise method for measuring the solvent isotope effect and the related "proton inventory" (*23*) for this reaction.

APPENDIX

Employing the reliable method of Fox and Jencks (*24*) we are able to estimate the pK values of our proposed tetrahedral intermediates. Their method involves the utilization of $\rho_I = -8.4$ along with the sum of σ_I values (*25*) (or estimates thereof) to approximate ionization constants in an equilibrium scheme, an example of which is Eq. [13].



[13]

Ar = 4-nitrophenyl
for S, Ar' = 5-nitro-2-oxyphenyl
for SH, Ar' = 2-hydroxy-5-nitrophenyl

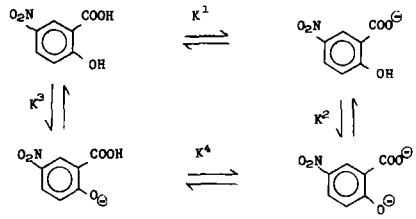
Approximation of three of the constants would then result in a definition of the fourth.

Estimates of σ_I. A value of σ_I for the 4-nitrophenoxy group can be determined from the ionization constant (*26*) of 4-NO_2-C_6H_4-OCH_2COOH, 1.28×10^{-3}. Substituting this value as a pK into Charton's (*25*) equation, $\sigma_I = 0.2512\ (2.89) + 1.186$, gives 0.46 as the value for σ_I.

A value of σ_I for the 2-hydroxy-5-nitrophenyl group can be estimated by first determining σ^* and then using the conversion factor (*25*), $\sigma_I = \sigma^*/6.23$. Utilizing the

equation of Takahashi *et al.* (*27*) and the pK_1 of 2-hydroxy-5-nitrobenzoic acid (*11*), 2.31, $\sigma^* = (2.31 - 5.275)/-1.795 = 1.65$. Thus, the value for σ_I is 0.26.

Determining a value for the 5-nitro-2-oxyphenyl group proceeds in an identical manner, once an estimate of the pK of 5-nitro-2-oxybenzoic acid is obtained. To estimate this pK we set up the equilibrium scheme as shown in Eq. [14].



[14]

Values for pK^1 and pK^2 have been measured (*11*) at 2.31 and 10.22, respectively. An estimate for pK^3 can be arrived at by the method of Williams and Norrington (*28*). Their method predicts a pK value of 5.97. Now, pK^4 = pK^1 + pK^2 − pK^3 = 6.36. Thus, $\sigma^* = (6.36 - 5.275)/-1.795 = -0.60$ and $\sigma_I = -0.10$.

Estimation of pK_1. The method of Fox and Jencks (*24*) requires the selection of a reference compound. If CH_3OH (pK value (*27*) of 15.54) is used, then for SH, pK = 15.54 − 8.4 (0.26 + 0.46 + 0.25) = 7.4 (where 0.25 is the σ_I value (*25*) for −OH) and for S, pK = 10.4. If $CH_2(OH)_2$ (pK value (*29*) of 13.27) is employed, then for SH, pK = 13.27 − 8.4 (0.26 + 0.46) = 7.2, and for S, pK = 10.2.

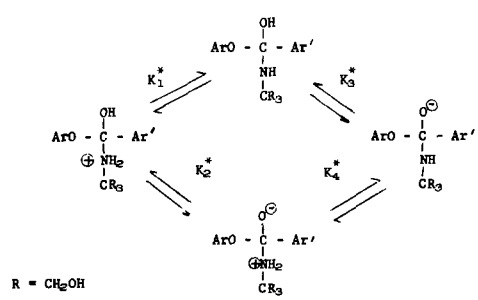
Since $CH_2(OH)_2$ is closer in structure to the adduct of interest, pK values of 7.2 for SH and 10.2 for S are chosen as better estimates.

Estimation of pK_2. The pK of $CH_3OH_2^+$ has been reported (*30*) as −2.5. Then for SH, pK_2 = −2.5 − 8.4 (0.97) = −10.6, and for S, pK_2 = −7.6.

Estimation of pK_3. Fox and Jencks have estimated that an α-oxy group in an ammonium ion increases the pK by 4.8 units. Employing this correction factor to pK_2 results in a value of pK_3 = −5.8 for SH, and pK_3 = −2.8 for S.

pK_4. The values for pK_4 are then derived from pK_1 + pK_2 − pK_3 = 2.4 for SH and 5.4 for S.

Estimation of the pK's for the addition compounds formed from Tris plus SH and Tris plus S proceeds in a similar manner. The associated equilibria are given in Eq. [15].



[15]

Estimation of pK_1.* The reference compound $CH_3NH_2CH_3^+$ has a pK value (*10*) of 10.64. The σ_I value (*25*) of CH_2OH is 0.09. Thus, pK_1* = 10.64 − 8.4 (0.97 + 3(0.09)) = 0.2 for SH and 3.2 for S.

*Estimation of pK_2**. The pK of the hydroxyl group of $CH_3NH_2CH_2OH^+$ has been estimated (*31*) to be 9.98. Substitution of $(CH_2OH)_3CNH_2$ for CH_3NH_2 should bring about only a small correction, if any, in the negative direction. An estimate of this correction can be made. The values of σ_I are 0.1 for an aliphatic amine and 0.25 for an amide. Assuming that σ_I values are linearly related to pK and approximating the pK of aliphatic amines at 10.5 and of amides at −0.5 suggest that $\Delta\sigma_I/\Delta pK = 0.15/11.0 = 0.014$. Since the p$K$ of Tris is 8.1, then σ_I for $(CH_2OH)_3CNH$ is estimated to be 0.13. The correction factor is approximated at $-8.4\ (0.13 - 0.1) = -0.25$ pK unit. This gives an estimate of a pK value of 9.73 for $(CH_2OH)_3CNH_2CH_2OH^+$. Now, pK_2* can be approximated as before. Thus, $pK_2^* = 9.73 - 8.4\ (0.46 + 0.26) = 3.7$ for SH and 6.7 for S.

*Estimation of pK_3**. Fox and Jencks (*24*) estimate the correction factor for the development of a positive charge on the ionization of a hydroxyl group to be 4.8. Applying this correction to pK_2*, estimates of $pK_3^* = 8.5$ for SH and 11.5 for S are obtained.

*pK_4**. The values of pK_4^* are then estimated from $pK_1^* + pK_3^* - pK_2^* = 5.0$ for SH and 8.0 for S.

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