Isotope Effects and Medium Effects on Sulfuryl Transfer Reactions

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Abstract: Kinetic isotope effects and medium effects have been measured for sulfuryl-transfer reactions of the sulfate ester *p*-nitrophenyl sulfate (pNPS). The results are compared to those from previous studies of phosphoryl transfer, a reaction with mechanistic similarities. The N-15 and the bridge O-18 isotope effects for the reaction of the pNPS anion are very similar to those of the *p*-nitrophenyl phosphate (pNPP) dianion. This indicates that in the transition states for both reactions the leaving group bears nearly a full negative charge resulting from a large degree of bond cleavage to the leaving group. The nonbridge O-18 isotope effects support the notion that the sulfuryl group resembles SO_3 in the transition state. The reaction of the neutral pNPS species in acid solution is mechanistically similar to the reaction of the pNPP monoanion. In both cases proton transfer from a nonbridge oxygen atom to the leaving group is largely complete in the transition state. Despite their mechanistic similarities, the phosphoryl- and sulfuryl-transfer reactions differ markedly in their response to medium effects. Increasing proportions of the aprotic solvent DMSO to aqueous solutions of pNPP cause dramatic rate accelerations of up to 6 orders of magnitude, but only a 50-fold rate increase is observed for pNPS. Similarly, phosphoryl transfer from the pNPP dianion to *tert*-amyl alcohol is 9000-fold faster than the aqueous reaction, while the sulfuryl transfer from the pNPS anion is some 40-fold *slower*. The enthalpic and entropic contributions to these differing medium effects have been measured and compared.

In recent years there has been a growing realization of the biochemical importance of sulfuryl transfer. Sulfation has a crucial biological role in detoxification. In addition, sulfate monoesters are found among all the classes of natural products, including nucleotides, peptides and proteins, polysaccharides, steroids, and lipids. Despite its biological importance, sulfate ester chemistry has been the subject of much less study than its phosphate ester chemistry counterpart. We report here kinetic isotope effect measurements and medium effects studies of sulfuryl-transfer reactions of *p*-nitrophenyl sulfate (pNPS). These studies parallel prior work on phosphoryl transfer and add to the body of knowledge of sulfuryl-transfer reactions and how they compare with phosphoryl transfer.

Some aspects of the sulfuryl-transfer mechanism are known from previous studies. Sulfate ester hydrolysis is accelerated under strongly acidic and strongly basic conditions, with a broad pH-independent range between pH 4 and 12.[1,2] Sulfate monoesters may exist in either of two protonation states, the anion or the neutral species (Figure 1). There is evidence that different mechanisms are followed by these species; the chemistry of the anionic compound (A in Figure 1) will be summarized first.

^{18}O tracer studies have shown that aryl sulfates undergo hydrolysis by S−O bond fission in the pH-independent region.[2] The reactions of alkyl sulfate esters in the pH-independent region have not been studied. In very basic solutions (pH ≥ 13), where the rate of hydrolysis increases with [HO^-], both aryl and alkyl sulfate esters undergo hydrolysis via C−O bond cleavage. [3,4]

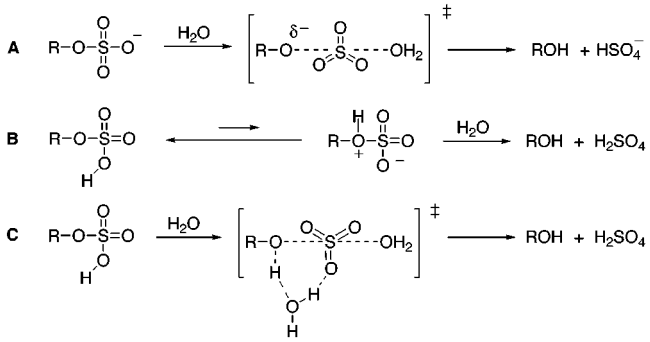

Figure 1. Mechanisms for the hydrolysis (sulfuryl transfer to water) of a sulfate monoester as the anion in the pH-independent region (A) and the neutral species (B). The transition state of the anion mechanism may in principle lie anywhere between the tight and loose extremes.

Most of the information about the mechanism in the pH-independent region comes from linear free energy relationships with aryl sulfates. These studies reveal a large sensitivity to the pK_a of the leaving group but a very small sensitivity to the nucleophile, suggesting a concerted pathway having a loose transition state.[1,2,5−7] These LFER results are similar to those for reactions of the analogous phosphate monoester dianions. Accordingly, it has been suggested that these reactions likely proceed by similar mechanisms and transition states. The

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possibility of a fully dissociative mechanism proceeding by way of a free SO_3 intermediate has been ruled out by stereochemical studies; incubation of phenyl[(*R*)-^{16}O,^{17}O,^{18}O]sulfate with an acceptor alcohol in carbon tetrachloride solution was found to form product of exclusively inverted configuration.[8] Similarly, metaphosphate is not typically an intermediate in hydrolysis reactions of phosphate esters,[9] although it does form in reactions where *tert*-butyl alcohol is the phosphoryl acceptor.[10] The small deuterium isotope effect of 1.26[2] indicates that no general acid/base catalysis takes place.

The significant negative entropy of activation of −18.5 eu[2] for the pH-independent hydrolysis of *p*-nitrophenyl sulfate anion raises the question of whether this reaction might proceed with significantly more nucleophilic participation than the corresponding phosphoryl transfer, which exhibits an entropy of activation of +3.5 eu.[11] Negative values for $\Delta S^{\ddagger}$ of this magnitude are typically indicative of associative mechanisms with significant nucleophilic participation. The reason for the discrepancy between the entropies of activation of these two reactions remains unexplained.

In the acidic hydrolysis of sulfate esters, the reactive species is the neutral molecule (B in Figure 1) and this form is much more reactive than the anion. A reduced value for β_{lg}[1] and a solvent isotope effect of 2.43[12] led to the proposal that the hydrolysis of the neutral species proceeds via a two-step pathway involving equilibrium proton transfer to the incipient leaving-group oxygen atom followed by O−S bond cleavage. This is mechanism B in Figure 1. The intermediacy of free SO_3 in the acid hydrolysis is often assumed but has never been proven. Also consistent with previous data is mechanism C, in which a proton is transferred to the leaving group in the same step as sulfuryl transfer.

It has been noted that the addition of organic cosolvents, especially DMSO, to aqueous solutions of phosphate monoesters increases the rate constant for hydrolysis by the dianion by up to 10^6-fold.[13] An analogous effect resulting from binding has been postulated to be a possible contributor to enzymatic catalysis.[13] Whether sulfate ester anion hydrolysis is subject to similar medium effects has not been determined, although the acid-catalyzed hydrolysis of alkyl sulfate esters is accelerated in moist dioxane compared to the rate in aqueous solution.[14,15]

To further understand the mechanism and the nature of the transition state for sulfuryl transfer we have measured kinetic isotope effects for the acid-catalyzed and the pH-independent hydrolysis of pNPS. The substrate is shown in Figure 2, with the positions indicated where isotope effects were measured. The notation used to express isotope effects is that of Northrop[16] where a leading superscript of the heavier isotope is used to indicate the isotope effect on the following kinetic quantity; for example ^{15}k denotes k_{14}/k_{15}, the nitrogen-15 isotope effect

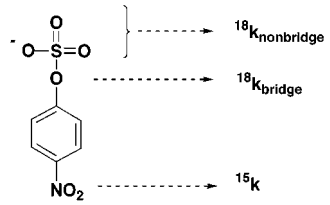

Figure 2. A diagram of *p*-nitrophenyl sulfate showing the positions at which isotope effects were measured and the nomenclature used.

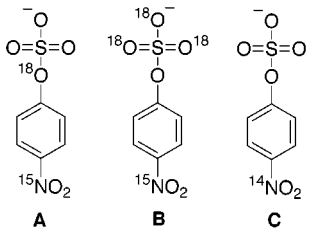

Figure 3. Isotopic isomers used for measurement of the bridge (A and C) and nonbridge (B and C) ^{18}O isotope effects.

on the rate constant *k*. Previous isotope effect studies on reactions of *p*-nitrophenyl phosphate (pNPP) allow for direct comparisons to be made between the two reactions. We have also examined medium effects on the sulfuryl-transfer reaction of pNPS in neat *tert*-amyl alcohol and in DMSO/water mixtures. Since medium effects on phosphate monoester hydrolysis have been well characterized under these conditions, the results allow a comparison of medium effects on reactions of the two types of esters.

Experimental Section

^{18}O-labeled sulfuric acid (95% H_2SO_4 in H_2O) 95% atom percent ^{18}O, was purchased from Isotec. Natural abundance *p*-nitrophenol was a commercial product, recrystallized from toluene.

The potassium salt of pNPS was obtained from a commercial source but was found to contain excessive free phenol. This commercial potassium salt after recrystallization from aqueous KOH was used for some work, but most kinetic work was done using locally synthesized salts. Two different literature methods[17,18] were used to synthesize pNPS, in an effort to optimize the process for the later synthesis of labeled compounds.

Unlabeled pNPS was converted to the tetra-*N*-butylammonium salt by ion exchange using DOWEX-50A cation-exchange resin. A 1:1 stoichiometry was confirmed by integration of the proton NMR.

The isotopic isomers of *p*-nitrophenyl sulfate needed to measure the isotope effects are shown in Figure 3. [^{14}N]-*p*-nitrophenol, [^{15}N]-*p*-nitrophenol and [^{15}N,^{18}O]-*p*-nitrophenol were synthesized as described previously.[19] [^{14}N]-*p*-nitrophenol, and [^{15}N,^{18}O]-*p*-nitrophenol were mixed to reconstitute the natural abundance of ^{15}N, and then the mixture was sulfurylated to produce *p*-nitrophenyl sulfate using the method referred to above. This mixture of isotopic isomers (A and C, Figure 3) was used for determination of $^{18}k_{bridge}$.

Measurement of the isotope effect in the nonbridge oxygen atoms requires the isomers B and C in Figure 3. ^{14}N-labeled pNPS (C) was synthesized from [^{14}N]-*p*-nitrophenol using the sulfur trioxide−pyridine complex method.[18] [$^{18}O_3$]-chlorosulfonic acid was prepared from [$^{18}O_4$]-sulfuric acid (obtained from Isotec) using the method of Williamson, from sulfuric acid and phosphorus pentachloride.[20] The [$^{18}O_3$]-chlorosulfonic acid thus obtained was reacted with ^{15}N-labeled *p*-nitrophenol to form isomer B (Figure 3), using the method of Burkhardt and Lapworth.[17] The percent of ^{18}O incorporation in the product was

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measured by matrix assisted laser desorption/ionization time-of-flight mass spectrometry, using a nitrogen laser at 337 nm and a 2.1 m linear TOF mass spectrometer. The results are shown in Figure 1S of the Supporting Information. Integration of the results showed that 64.6% of the product was triply labeled in the nonbridge position, with the remainder doubly labeled. This percentage was used in the mathematical correction of the observed isotope effect.

Isotope Effect Determinations. The protocols for carrying out these experiments were the same as those previously described for *p*-nitrophenyl phosphate.[19] In this method the reactions are allowed to proceed to partial completion and stopped, followed by separation of the product and residual substrate. These are then subjected to isotopic analysis by isotope ratio mass spectrometry to determine the nitrogen isotope ratios.

Isotope effects were measured for the hydrolysis of pNPS under three conditions: in 100 mM CHES buffer at pH 9.0 at 85 °C, in 1.0 N HCl at 65 °C and 21 °C, and in 10 N HCl at 15 °C. Reactions were begun with 100 μmol of pNPS. Experiments were performed in triplicate with individual experiments stopped at fractions of reaction ranging from 30 to 60%. Extents of reaction were measured by assaying for *p*-nitrophenol in 0.1 N NaOH at 400 nm. The reactions at pH 9 were stopped by chilling the solutions on ice; the acidic reactions were stopped by titration to pH 5. After the reaction solutions were titrated to pH 5 they were extracted three times with an equivalent volume of ethyl ether to quantitatively remove the *p*-nitrophenol product. The aqueous layer was evaporated briefly under vacuum to remove dissolved ether, sufficient 10 N HCl was added to bring the solution to at least 1.0 N in HCl, and the solutions were heated overnight to 80 °C to completely hydrolyze the remaining pNPS. They were then titrated back to pH 5 and extracted with ether, the *p*-nitrophenol in this ether fraction representing the residual substrate from the initial reaction. The ether fractions were dried over magnesium sulfate and filtered, and the solvent was removed by rotary evaporation. The *p*-nitrophenol was sublimed under vacuum at 90 °C, and 1.0 mg samples were prepared for isotopic analysis using an ANCA-NT combustion system in tandem with a Europa 20−20 isotope ratio mass spectrometer.

Reactions performed in 10 N HCl provided some challenges in terms of kinetic control. At this concentration of acid, the hydrolysis rate was very high; hence, time spent in mixing the reactant into the solution was critical. The pNPS was dissolved in a small measured volume of water. The volume of water was calculated to bring a measured volume of concentrated HCl to 10 N when the two were combined. To prevent a large temperature rise due to the production of heat upon mixing, the solutions were cooled to a temperature below the desired reaction temperature such that when the reactants were combined the heat evolved would not raise the temperature of the mixture above the desired temperature. To stop these reactions, the 10 N acid solution was rapidly combined with 10 times its volume of ice-cold 1.0 N NaOH. A small (<5%) excess of base was used to ensure the resulting solution would be basic, where the reaction is orders of magnitude slower. This cold solution was assayed for *p*-nitrophenol and then titrated to pH 5 and handled as described above to isolate product from unreacted pNPS.

Isotope effects were calculated from the isotopic ratios at partial reaction in the *p*-nitrophenol product (R_p), in the residual substrate (R_s), and in the starting material (R_o). Equations 1 and 2 were used to calculate the observed isotope effect either from R_p and R_o or from R_s and R_o respectively at fraction of reaction f.[21] Thus each experiment yields two independent determinations of the isotope effect.

$$\text{isotope effect} = \log(1 - f)/\log(1 - f\,(R_p/R_o)) \qquad (1)$$

$$\text{isotope effect} = \log(1 - f)/\log\,[(1 - f)\,(R_s/R_o)] \qquad (2)$$

R_O was determined by two methods: from unreacted pNPS by isotope ratio mass spectrometry, and, as a control, from *p*-nitrophenol isolated after complete hydrolysis of a sample of pNPS using the same isolation and purification procedures used in the isotope effect experiments. The agreement of these two numbers demonstrated that, within experimental error, no isotopic fractionation occurs as a result of the procedures used to isolate and purify the *p*-nitrophenol.

The ^{15}N isotope effects were measured using natural abundance pNPS. The ^{18}O isotope effects were measured by the remote-label method.[22] In these experiments the nitrogen atom in the reactant is used as a reporter for the bridging oxygen atom or the nonbridging oxygen atoms. These experiments yield an observed isotope effect that is the product of the effect due to ^{15}N and that due to ^{18}O substitutions. The observed isotope effects from these experiments were then corrected for the ^{15}N effect and for incomplete levels of isotopic incorporation in the starting material as previously described.[23]

Kinetics Experiments. The potassium salt of pNPS was used in all aqueous kinetic experiments. Concentrations of pNPS used for kinetic runs were 5−13 mM. Thermostatically controlled water baths or dry blocks were used to maintain reaction temperatures over the course of the experiments.

The pH-independent aqueous reaction was examined in 100 mM TRIS buffer at pH 9.0 at temperatures from 35 to 95 °C. Over time, aliquots were removed and added to measured portions of 0.1 N NaOH, and reaction progress was monitored spectrophotometrically by tracking the evolution of the *p*-nitrophenol hydrolysis product at 400 nm (ϵ = 18 320).[24] The plot of absorbance versus time over the first 1−5% of reaction was analyzed by assuming first-order kinetics using the initial rates method. To determine the initial substrate concentration, an aliquot of the reaction mixture was subjected to complete hydrolysis in a measured amount of 1.0 N HCl, which was heated at 80 °C for 4 h and then assayed for *p*-nitrophenol.

Reactions in *tert*-amyl alcohol were performed using the tetrabutylammonium salt of pNPS. In cases where the reactions were very slow, concentrations as high as 80 mM were used to obtain measurable changes in the composition of the reaction mixture. Evolution of free phenol was quantified by adding aliquots of the reaction solution to measured portions of 50% aqueous ethanol, which was 0.1 N in NaOH. The solutions were then analyzed spectrophotometrically for phenolate ion at 405 nm (the λ_{max} is slightly shifted in the ethanolic solution). Rate constants were measured using the initial rates method.

Reactions in DMSO/water mixtures were carried out using the potassium salt of pNPS. Reactions were 5 mM in pNPS, 0.1 M in CHES buffer, pH 9, with ionic strength of 1.0 maintained using tetraethylammonium chloride. Fractions of DMSO ranged up to 95%, and rates were measured at temperatures from 60 to 90 °C. The pK_a of the buffer and of the pNPS, and the effective pH of the solution, will vary somewhat with the DMSO content of the solution. However the very broad pH-independent range for hydrolysis and the very low pK_a of pNPS give confidence that the anion is the reactive species present. Rate constants were measured using the initial rates method, assaying for released *p*-nitrophenol by adding aliquots of the reaction mixtures to 0.1 N NaOH.

Results

Values of the first-order rate constants for the reactions of pNPS at pH 9.0 in aqueous solution, in 95% DMSO/water mixtures, and in neat *tert*-amyl alcohol were determined by following the liberation of *p*-nitrophenolate anion spectrophotometrically. The rate constants for these reactions gave linear Eyring plots (Figures 2S and 3S) which were used to calculate the enthalpies and entropies of activation. The enthalpy of activation for each reaction was calculated from the slope. The free energy of activation for each reaction was calculated at 35 °C using the equation $\Delta G^{\ddagger} = -RT\ln(k_T h/kT)$, and the entropy of activation was then calculated using the relation $\Delta S^{\ddagger} = (\Delta H^{\ddagger} - \Delta G^{\ddagger})/T$. Table 1 shows the rate and activation parameter data for these reactions. The pH-independent hydrolysis rate of pNPS at 35 °C, and the activation parameters are in reasonable agreement with previously reported data.[2]

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Table 1. Rates and Activation Parameters for Reactions of *p*-Nitrophenyl Sulfate Anion

	aqueous		*tert*-amyl alcohol	95% DMSO
k at 35 °C, s^{-1}	1.87×10^{-9}	2.47×10^{-9}	5.07×10^{-11}	9.26×10^{-8}
$k_{solv}/k_{aqueous}$ at 35 °C	1		0.027	50
$\Delta H^{\ddagger}$, kcal/mol	24.4 ± 0.2	24.6	25.0 ± 0.7	23.2 ± 0.5
$\Delta S^{\ddagger}$, eu	-19.5 ± 0.4	−18.5	-25 ± 1	-16 ± 1
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Table 2. Kinetic Isotope Effects for the Aqueous Hydrolysis of pNPS and pNPP[a]

pNPS KIEs	pH 9.0, 85 °C	1 N HCl, 65 °C	1 N HCl, 21 °C	10 N HCl, 15 °C
$^{18}k_{nonbridge}$	0.9951 (3)	1.0067 (1)	1.0083 (2)	1.0098 (3)
$^{18}k_{bridge}$	1.0210 (10)	1.0069 (2)	1.0097 (2)	1.0101 (2)
^{15}k	1.0026 (1)	1.0002 (1)	1.0002 (1)	1.0004 (1)

pNPP KIEs	dianion, 95 °C	monoanion, 95 °C	monoanion, 30 °C
$^{18}k_{nonbridge}$	0.9994 (5)	1.0184 (5)	1.0199 (3)
$^{18}k_{bridge}$	1.0189 (5)	1.0087 (3)	1.0094 (3)
^{15}k	1.0028 (2)	1.0004 (2)	1.0005 (1)

[a] Results for the sulfate monoester are from this work, phosphate results were previously reported elsewhere.[19,36] Values for $^{18}k_{nonbridge}$ are the KIEs for all three nonbridge atoms labeled. Standard errors in the last decimal place(s) are shown in parentheses.

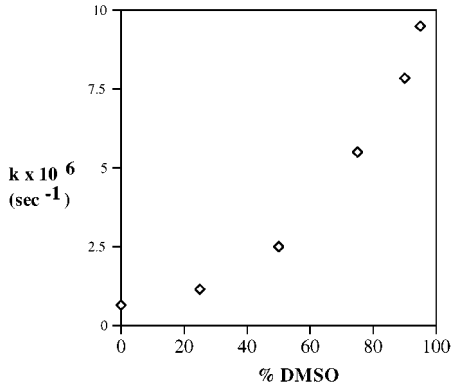

Figure 4. Dependence of the pseudo-first-order rate constant at 80 °C for hydrolysis of the anion of pNPS as a function of DMSO content in DMSO/water mixtures.

The monoanion of pNPS was found to undergo sulfuryl transfer to the solvent approximately 40 times slower in *tert*-amyl alcohol than in water at 35 °C. This is in contrast to the solvolysis of *p*-nitrophenyl phosphate, which is approximately 9000-fold faster in *tert*-amyl alcohol at 39 °C.[25] The activation parameters for the reactions of pNPS in the two solvents are similar, in contrast to the pNPP phosphoryl-transfer reaction, in which the entropy of activation changes from +3.5 in water to +23 eu in *tert*-amyl alcohol.[25]

The effect of DMSO on the rate of hydrolysis of pNPS is shown in Figure 4. The rate constant increases with the fraction of DMSO, as has been observed with the hydrolysis of pNPP,[13] but to a much smaller extent. At 80 °C only a 15-fold rate increase is observed in 95% DMSO relative to the aqueous reaction. Comparison of the extrapolated rate constants at 35 °C for the pNPS reaction in 95% DMSO with the aqueous rate at the same temperature yields a 50-fold rate acceleration. By comparison, a 10^6-fold increase is observed in the phosphoryl transfer of pNPP at 39 °C.[13]

The isotope effects for the solution reactions of pNPS and their standard errors are given in Table 2. The isotope effects obtained from isotopic ratios of product, and those obtained from isotopic ratios of residual substrate, agreed within experimental error in all cases and were averaged together to give the results shown. Six or more determinations of each isotope effect were made. Isotope effects were measured for the pNPS monoanion at pH 9.0, under conditions where the substrate was partially protonated in 1.0 N HCl, and in 10 N HCl where a greater fraction of the reactant is protonated. The values for the ^{18}O isotope effects have been corrected for the ^{15}N effect and for levels of isotopic incorporation.

It was not feasible to measure the KIEs for the hydrolysis of the pNPS anion and of the conjugate acid at the same temperature, due to the large difference in the reactivities of the two species. The temperature difference was minimized to make the temperature-induced differences in isotope effects as small as possible. The KIEs for the hydrolysis of the anion were measured at 85 °C, and those for the acid-catalyzed reaction in 1 N HCl were measured at 65 °C. To provide results in 1.0 N HCl to compare both the very slow reaction of the anion and the very fast reaction in 10 N HCl, the isotope effects in 1.0 N HCl were measured at 65 and at 21 °C.

Discussion

Mechanistic Similarities between Phosphoryl and Sulfuryl Transfer. LFER results suggest that the reactions of phosphate monoester dianions and of sulfate ester anions are similar. In both of these reactions the phosphoryl and sulfuryl groups are fully deprotonated, and the major impetus for leaving-group departure is internal electron donation from charge borne on the nonbridge oxygen atoms. Another mechanistic similarity exists between reactions of the phosphate monoanion and the neutral sulfate ester. In both of these species, one of the nonbridge oxygen atoms is protonated. In hydrolysis reactions of both, it is proposed that the proton is transferred to the leaving group during the reaction. The kinetic isotope effect data and results from medium effects give further insights into the similarities and differences of these sets of reactions.

Isotope Effects and the Sulfate Monoester Hydrolysis Transition State. Reaction of the pNPS Anion. The kinetic isotope effects at pH 9.0 represent the reaction in the pH-independent range where pNPS is present as the anion. The secondary ^{15}k isotope effect of 1.0026 is comparable to the equilibrium ^{15}K effect for the deprotonation of *p*-nitrophenol of 1.0023.[26] This isotope effect is sensitive to the amount of

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negative charge delocalized into the nitrophenolate group,[19,26] and the observed value indicates that the leaving group bears essentially a full negative charge in the transition state. In phosphoryl-transfer reactions from the dianion of pNPP this isotope effect can also be slightly larger than the equilibrium effect for deprotonation.[27] This is likely due to the fact that in the transition state the leaving group, which resembles *p*-nitrophenolate anion, remains in close proximity to the phosphoryl or sulfuryl group and the phenolic oxygen is not well-solvated. This would result in greater charge delocalization (a larger contribution from the quinonoid resonance form). Relatively less charge will be delocalized in the phenolate anion in aqueous solution as measured in the equilibrium isotope effect, where charge on the phenolate oxygen atom is stabilized by hydrogen bonding with solvent.

The $^{18}k_{bridge}$ is a primary isotope effect as the S−O bond is broken in the transition state. The value of 1.0210 for pNPS hydrolysis is similar to the $^{18}k_{bridge}$ value of 1.0189 observed for reaction of the pNPP dianion at the slightly higher temperature of 95 °C. This indicates that the bond to the leaving group is largely broken in the transition state, as is the case for phosphoryl transfer. Together the two isotope effects, $^{18}k_{bridge}$ and ^{15}k indicate that sulfuryl and phosphoryl transfer from the *p*-nitrophenyl esters have very similar transition states both with respect to the extent of leaving-group bond cleavage and the amount of charge borne on the leaving group.

An interpretation of the secondary isotope effect, $^{18}k_{nonbridge}$, may be considered in part by analogy with a fairly considerable body of data for phosphoryl transfer. In phosphoryl-transfer reactions, $^{18}k_{nonbridge}$ is small and inverse for loose transition states in which the phosphoryl group resembles metaphosphate and is normal for reactions in which the transition state resembles a pentacoordinate phosphorane.[27] The change in hybridization and in bonding to the nonbridge oxygen atoms is directly analogous in sulfuryl and phosphoryl-transfer reactions. This suggests that a similar trend might be expected for the analogous sulfuryl-transfer reaction. Calculations at the 6-31++G** level of the expected equilibrium isotope effect between pNPS and sulfur trioxide yield a value of 0.9910 (P. Czyryca and A. Hengge, unpublished results). However we have not been able to successfully model $^{18}k_{nonbridge}$ for an associative mechanism for comparison. Thus, firm conclusions cannot be drawn from the value of $^{18}k_{nonbridge}$. However, the experimental value of 0.9951 is consistent with expectations from the calculation and from KIEs for analogous phosphoryl-transfer reactions, for a transition state in which the sulfuryl group resembles SO_3. The value of $^{18}k_{nonbridge}$ is more inverse than in the reaction of the pNPP dianion. This suggests that nonbridge S−O bond order increases more than nonbridge P−O bond order in the corresponding phosphate ester reaction. The metaphosphate-like PO_3^- unit has considerable contribution from a resonance form with a positively charged phosphorus atom, two negative charges on oxygen atoms, and diminished nonbridge P−O bond order.[28,29]

Together, the isotope effects suggest a transition state like the one shown diagrammatically in Figure 1 A. The bond to the leaving group is largely broken, and the leaving group bears essentially a full negative charge. The sulfuryl group resembles sulfur trioxide.

Isotope Effects and the Sulfate Monoester Hydrolysis Transition State. The Reaction of Neutral pNPS. Isotope effects were measured for the solution reaction of pNPS in acid conditions for comparison with the reaction of the anion. At 35 °C the reaction is 10^5 fold faster in 1.0 N HCl than in the pH-independent region at pH 9.0. The mechanism of reaction of the neutral species has been postulated to proceed via the zwitterionic species shown in part B of Figure 1.

Kinetic isotope effects are sensitive to the reaction temperature, but due to the extreme difference in reactivity, these two reactions could not be investigated at the same temperature. A compromise was reached, with the pH 9 reaction studied at 85 °C and the 1.0 N HCl reaction at 65 °C.

In the reaction under acidic conditions the ^{15}k isotope effect is essentially unity, indicating that no significant negative charge is developed on the nitrophenyl group in the transition state. The $^{18}k_{bridge}$ isotope effect is reduced from its value in the reaction of the anion by an amount close to the equilibrium ^{18}K isotope effect for deprotonation of *p*-nitrophenol, which is 1.0153[30] (protonation will result in an inverse contribution to $^{18}k_{bridge}$ of the same magnitude). Thus, the observed kinetic isotope effect is consistent with a transition state in which the O−S bond is largely broken, and protonation of the leaving group is essentially complete. Both ^{15}k and $^{18}k_{bridge}$ are very similar to the values from the reaction of the pNPP monoanion (Table 2).

The $^{18}k_{nonbridge}$ isotope effect for this reaction will be influenced by the protonation state of the sulfuryl group. In the mechanistically analogous reaction of the pNPP monoanion, the isotope effects were measured at a pH (3.5) at which the phosphoryl group (pK_a 5.1) was essentially completely in the monoprotonated state. In this case there is no isotopic fractionation in the species that are present in the correct protonation state for reaction. However the pK_a of pNPS is too low to obtain a large proportion of the neutral species. The pK_a of unsubstituted sulfuric acid is on the order of −3,[31,32] and the *p*-nitrophenyl substituent will make this value even more negative. Thus, even in 1 N HCl much less than 1% of pNPS will be in the neutral form, and therefore the observed kinetic isotope effect should be corrected by the full equilibrium isotope effect for protonation. The ^{18}K isotope effect for protonation of sulfate, or of a sulfate ester, is unknown. However, reported values for the $^{18}k_{nonbridge}$ equilibrium isotope effect (EIE) for protonation of phosphate, of phosphate esters, for carboxylic acids and for formic acid, are all in the range of 1.015−1.02.[33] Assuming that the fractionation factor for the sulfate ester is similar to that for a phosphate ester (1.016)[34] yields a corrected isotope effect of 1.023 for the acidic hydrolysis of pNPS in 1 N HCl at 21 °C. This is reasonably close to the value of 1.0199 measured for the hydrolysis of the monoanion of pNPP at a similar temperature (Table 2) and thus supports the hypothesis of a similar mechanism for the two reactions, in which proton transfer from the sulfuryl group is essentially complete in the transition state.

The data for the reaction in 10 N HCl (Table 2) show that while negligible changes result in ^{15}k and in $^{18}k_{bridge}$, the

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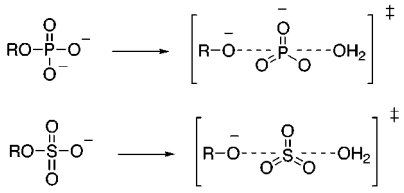

Figure 5. Charge dispersion in the transition state of reaction of phosphate monoester dianion, top, versus charge transfer in a sulfate ester anion reaction, bottom. Data suggest that in each transition state essentially a full negative charge resides on the leaving group, and bond formation to the nucleophile is minimal.

$^{18}k_{\text{nonbridge}}$ isotope effect increases by considerably more than the experimental uncertainty. This change is in the direction expected if a greater (although still very small) fraction of the pNPS present is in the correct protonation state for reaction, and less of the inverse EIE for protonation is expressed in the observed KIE. This indicates that the sulfuryl-protonated species is the reactive form that then transfers the proton (probably via the intermediacy of one or more water molecules) to the leaving group.

One could hypothesize that given the very low pK_a of the sulfuryl group of pNPS, the pK_a of the bridge oxygen atom might not be too much lower, and thus protonation might not occur on the sulfuryl group at all in the hydrolysis reaction. In such a scenario the bridge oxygen atom is protonated directly by hydronium ion to give the zwitterion. If this were the case, then the $^{18}k_{\text{nonbridge}}$ would be inverse, not normal.

If mechanism B in Figure 1 is followed, the EIE for protonation and that for deprotonation would effectively cancel, and $^{18}k_{\text{nonbridge}}$ would be inverse, as for the anion reaction. Only the reaction of the neutral form in which proton transfer occurs in the transition state, with the imaginary frequency factor arising from O−H bond cleavage giving a large normal contribution to the isotope effect,[35] will give the observed results. This is shown in mechanism C.

Kinetic Data and Medium Effects. Aqueous Reaction of the pNPS Anion. The rate of aqueous hydrolysis and the activation parameters calculated here agree well with those reported earlier.[1,2] The significant negative entropy of activation for the aqueous hydrolysis of the anion has always been an anomaly when all of the evidence is combined into a coherent model of the mechanism of this reaction. The individual activation entropies reported in Table 1 are calculated at 35 °C; however, the calculated values were very consistent across the entire temperature range evaluated (data not shown). The KIE data, in agreement with LFER studies, are fully consistent with a transition-state geometry similar to that of the analogous reaction of the pNPP dianion. If the different value for $\Delta S^{\ddagger}$ does not have a mechanistic origin, then it likely results from differing solvation effects. One possible origin for different solvation effects is the difference in charge distributions that occurs during the two reactions (Figure 5). In the loose transition state of the pNPP dianion reaction, the −2 charge of the reactant becomes dispersed between the phosphoryl group and the leaving group, in which each bears a charge of about −1. In the transition state of the sulfuryl-transfer reaction implied by the kinetic isotope effects and prior linear free energy relationships, charge is transferred rather than dispersed. The reactant's charge of −1 has been largely transferred to the leaving group, with the sulfuryl group resembling neutral sulfur trioxide. If these two processes have different solvent reorganization requirements, then they may be the source of the variance in the entropies of activation.

Sulfuryl Transfer from the pNPS Anion to *tert*-Amyl Alcohol. In a study of the phosphoryl-transfer reaction of the pNPP dianion in *tert*-amyl alcohol, it was found that the switch from aqueous solution to *tert*-amyl alcohol was accompanied by a 9000-fold increase in reaction rate at 39 °C.[25] The rate increase is due to entropic effects; while the activation enthalpies in the two solvents are the same within experimental error, in *tert*-amyl alcohol $\Delta S^{\ddagger}$ is +23 eu, compared to +3 in the aqueous reaction.[25] Previous stereochemical studies imply that in the similar solvent and phosphoryl acceptor *tert*-butyl alcohol, the phosphoryl reaction becomes completely dissociative, with a metaphosphate intermediate.[10] Such a mechanism is consistent with a larger positive value for $\Delta S^{\ddagger}$. By contrast, in the sulfuryl transfer reaction of the pNPS anion the same solvent change from water to *tert*-amyl alcohol is accompanied by a 40-fold decrease in rate, and $\Delta S^{\ddagger}$ is somewhat more negative than the value for the reaction in water. The slower rate is due to a combination of enthalpic and entropic effects, but primarily the latter ($\Delta\Delta H^{\ddagger} = 0.6$ kcal/mol, $T\Delta\Delta S^{\ddagger} = 1.8$ kcal/mol).

Because solvent can heavily influence entropies of activation, it is difficult to assign a mechanistic cause to the more negative value in *tert*-amyl alcohol, particularly since the difference is modest. Clearly the data do not support a shift to a more dissociative mechanism in the pNPS reaction, as in the phosphoryl transfer from pNPP in *tert*-amyl alcohol. The more negative entropy of activation for the pNPS reaction in *tert*-amyl alcohol than for that in water might be taken to imply a transition state with more nucleophilic participation, but this is counterintuitive with such a weak and sterically hindered nucleophile.

Sulfuryl Transfer from the pNPS Anion in DMSO/Water Mixtures. Figure 4 shows the effects of increasing fractions of DMSO on the rate constant for hydrolysis of the pNPS anion at 80 °C. While the general shape of the profile is similar to that reported for the reaction of pNPP dianion, the rate acceleration is very modest. The rate constants in water and in 95% DMSO/water were calculated at 35 °C from the Eyring plot, for better comparison with previously reported data for the pNPP reaction at 39 °C. This gives a very modest rate acceleration of 50-fold at 35 °C, in contrast to the up to 10^6-fold rate increase with 95% DMSO in the corresponding phosphoryl-transfer reaction of pNPP. The internal energy of bond fission $\Delta H^{\ddagger}$ for pNPP hydrolysis is significantly lowered in 95% DMSO to 21 kcal/mol, from 31 kcal/mol in water. For pNPS hydrolysis this parameter is negligibly affected (Table 1). These results suggest that disruption of solvation of the monoanionic sulfuryl group of the sulfate ester gives rise to much less reactant destabilization than the analogous effect upon the dianionic phosphoryl group. This is a logical consequence of the difference in charge between the two species. This suggests that, while desolvation effects are important in phosphoryl transfer and may be a potential source for enzymatic catalytic power for phosphatases,[13] such effects are less important for sulfuryl transfer and are not a significant potential source for catalytic power.

Conclusions

The kinetic isotope effects support the notion that sulfuryl-transfer reactions from aryl sulfate esters have similar mecha-

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nisms and transition states very similar to those of their aryl phosphate ester counterparts. The reaction of the anion of pNPS and that of the dianion of pNPP both proceed via concerted reactions characterized by loose transition states in which the leaving group bears nearly a full negative charge. However, the two reactions differ markedly in their response to medium effects, which cause only very modest changes in the rate of sulfuryl transfer. This further suggests that the substantial difference between the activation entropies in the phosphoryl- and sulfuryl-transfer reaction is due to solvation effects, and not to a difference in mechanism. Neutral pNPS undergoes hydrolysis by a mechanism analogous to that of the pNPP monoanion, with a proton transferred from the sulfuryl group to the leaving group simultaneous with cleavage of the S−O bond.

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Supporting Information Available: MALDI/TOF mass spectrum for $^{18}O,^{15}N$-labeled pNPS; Eyring plots for the reaction of the pNPS anion in aqueous solution and in *tert*-amyl alcohol; Eyring plot for hydrolysis of the pNPS anion in 95% DMSO/water solution; rate data for hydrolysis of the anion of pNPS in DMSO/water mixtures at 80 °C, used to prepare Figure 4 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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