JOC The Journal of Organic Chemistry

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Proton-in-Flight Mechanism for the Spontaneous Hydrolysis of *N*-Methyl *O*-Phenyl Sulfamate: Implications for the Design of Steroid Sulfatase Inhibitors

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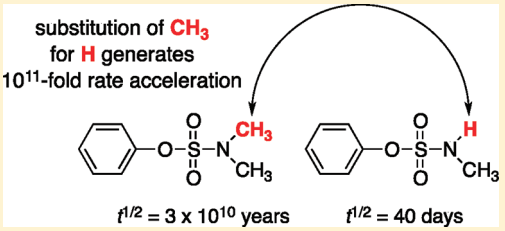
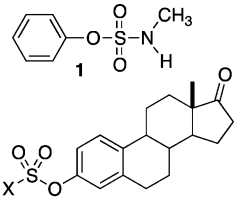
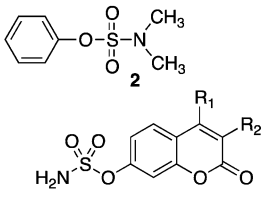
Supporting Information

ABSTRACT: The hydrolysis of *N*-methyl *O*-phenyl sulfamate (**1**) has been studied as a model for steroid sulfatase inhibitors such as Coumate, 667 Coumate, and EMATE. At neutral pH, simulating physiological conditions, hydrolysis of **1** involves an intramolecular proton transfer from nitrogen to the bridging oxygen atom of the leaving group. Remarkably, this proton transfer is estimated to accelerate the decomposition of **1** by a factor of 10^{11}. Examination of existing kinetic data reveals that the sulfatase *Pa*AstA catalyzes the hydrolysis of sulfamate esters with catalytic rate accelerations of ~10^4, whereas the catalytic rate acceleration generated by the enzyme for its cognate substrate is on the order of ~10^{15}. Rate constants for hydrolysis of a wide range of sulfuryl esters, $ArOSO_2X^-$, are shown to be correlated by a two-parameter equation based on pK_a^{ArOH} and $pK_a^{ArOSO2XH}$.



Sulfamate esters such as Coumate, 667 Coumate, and EMATE have been investigated as time-dependent and irreversible inhibitors of human steroid sulfatase for the treatment of hormone-dependent breast cancer and other ailments.[1] Sulfatase inhibition appears to involve decomposition of the parent sulfamate ester to generate a reactive electrophile [HN=S(=O)$_2$] that undergoes nucleophilic capture by groups positioned in and around the enzyme's active site.[2] Here, we describe a mechanistic study on the hydrolysis of **1**, which serves as a simulant for the sulfamate esters being developed as enzyme inhibitors. The *N*-methyl substituent of **1** precludes formation of the dianion $PhOSO_2N^{2-}$, simplifying the interpretation of kinetic effects at high pH.[3] Based on the accumulated kinetic data, we propose a novel mechanism for the spontaneous hydrolysis of sulfamate esters under physiological conditions. We also present a physical organic description of sulfuryl transfer that should prove useful in predicting the hydrolytic stability of the next generation of sulfatase inhibitors.





Pseudo-first-order rate constants for the hydrolysis of **1** were determined by UV−vis spectrophotometry monitoring phenol or phenoxide production at 60 °C from 4.8 < pH < 13.6 (I = 550 mM, NaCl). A nonlinear least-squares fit of the rate data to eq 1 indicates rate constants of $k^{1-} = (1.09 \pm 0.07) \times 10^{-5}$ and $k^{1} = (2.0 \pm 0.1) \times 10^{-5}$ s^{-1} corresponding to reaction along the high and low pH plateaus, respectively (Figure 1). The kinetic

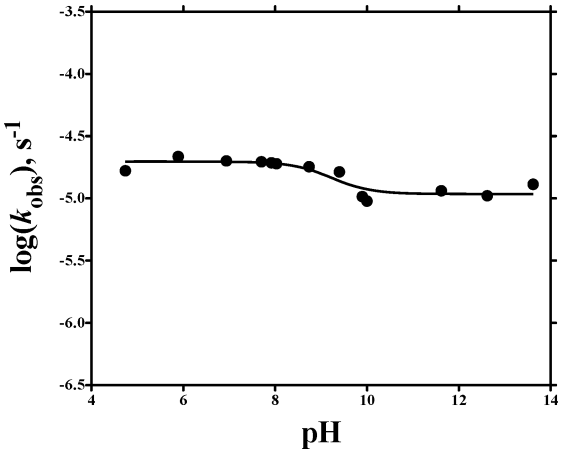


Figure 1. Plot of log(k_{obs}) versus pH for the hydrolysis of **1** at 60 °C, I = 0.55 M (NaCl). The fitted parameters $k^{1-} = (1.09 \pm 0.07) \times 10^{-5}$ s^{-1}, $k^{1} = (2.0 \pm 0.1) \times 10^{-5}$ s^{-1} and $pK_a = 9.1 \pm 0.3$ were obtained from a fit of the data to eq 1.

pK_a of 9.1 ± 0.3 is somewhat lower than that determined by spectrophotometric titration at 25 °C where $pK_a = 9.7 \pm 0.1$ (Figure S1, Supporitng Information). The activation parameters for the hydrolysis of **1** at pH 5.9 were measured from

Received: February 23, 2012
Published: April 9, 2012

   dx.doi.org/10.1021/jo300386u | *J. Org. Chem.* 2012, 77, 4450−4453

25 < T < 80 °C and determined to be $\Delta H^{\ddagger}$ = 18.7 ± 0.5 kcal/mol and $\Delta S^{\ddagger}$ = −24 ± 1 cal/mol·K (Table 1).

Table 1. Kinetic and Thermodynamic Data for the Hydrolysis of Sulfamate Esters 1 and 2

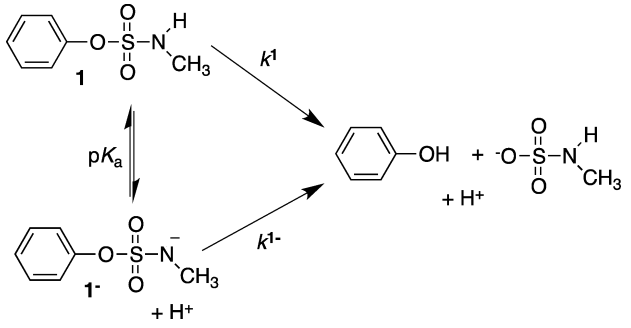
	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (cal/mol·K)	k_{25} (s^{-1})	k_{60} (s^{-1})
1	18.7 ± 0.5	−24 ± 1	2×10^{-7}	$(2.0 \pm 0.1) \times 10^{-5}$
$\mathbf{1}^-$				$(1.1 \pm 0.1) \times 10^{-5}$
2[a]	38 ± 3	−14 ± 6	7×10^{-19}	7×10^{-16}

[a]The rate constants k_{25} and k_{60} for hydrolysis of **2** at 25 and 60 °C were calculated from the activation parameters using the Eyring equation.

$$\log(k_{obs}) = \log\left[k^{\mathbf{1}}\frac{10^{-pH}}{10^{-pH} + 10^{-pKa}} + k^{\mathbf{1}-}\frac{10^{-pKa}}{10^{-pH} + 10^{-pKa}}\right] \quad (1)$$

The appearance of the pH−rate profile (Figure 1) is consistent with an equilibrium mixture of **1** and $\mathbf{1}^-$ undergoing product formation by the two pathways $k^{\mathbf{1}}$ and $k^{\mathbf{1}-}$ shown in Scheme 1. Williams previously studied a series of *N*-methyl

Scheme 1. Hydrolysis of 1 and $\mathbf{1}^-$



O-aryl sulfamates hydrolyzing at elevated pH via $k^{\mathbf{1}-}$ and found these to undergo spontaneous unimolecular decomposition to $^-$OAr and MeN═SO_2.[4] Subsequent fast nucleophilic capture of MeN═SO_2 by solvent (or amine buffer) completes the transformation. The focus of the current study is on the $k^{\mathbf{1}}$ process, which for **1** predominates below pH ∼9 and provides an appropriate model for hydrolysis under physiological conditions of the *O*-aryl sulfamates being investigated as steroid sulfatase inhibitors.

At 60 °C, the ratio of $k^{\mathbf{1}}/k^{\mathbf{1}-}$ at ∼2 is small, and yet there is a strong indication that the N-*H* group has a profound effect on the reactivity of **1**. Consider, for example, the hydrolysis of *N*,*N*-dimethyl *O*-phenyl sulfamate (**2**), which does not possess N−H groups. Spontaneous hydrolysis of **2** is too slow to monitor at 60 °C ($t^{1/2}$ = 3 × 10^7 years, vide infra), and kinetic experiments were carried out at pH 5.9 from 240 < T < 279 °C. Observed first-order rate constants for the hydrolysis of **2** were determined by ^{1}H NMR comparing the normalized signal intensities for unreacted starting material and phenol.[5] Fitting the rate data for hydrolysis of **2** to the Eyring equation yielded the activation parameters $\Delta H^{\ddagger}$ = 38 ± 3 kcal/mol and $\Delta S^{\ddagger}$ = −14 ± 6 cal/mol·K (Table 1). The thermodynamic data allow us to estimate a rate constant of $k^{\mathbf{2}}$ = 7 × 10^{-19} s^{-1} for the spontaneous hydrolysis of **2** at 25 °C (Table 1). Remarkably, comparing this value to $k^{\mathbf{1}}$ indicates that substitution of one of the *N*-methyl substituents in **2** with a hydrogen atom results in a greater than 10^{11}-fold rate acceleration at 25 °C. A rate constant of 7 × 10^{-7} s^{-1} for the hydrolysis of $PhOSO_2NH_2$ in 50% aqueous acetonitrile at 25 °C can be calculated from the reported activation parameters.[6] This value is very similar to that for **1**, indicating that a single N-*H* group is sufficient to achieve the full catalytic effect.

A solvent deuterium kinetic isotope effect of k^{H}/k^{D} = 1.8 ± 0.1 was determined on $k^{\mathbf{1}}$ for hydrolysis at 60 °C (Figure S4, Supporting Information). Solvent isotope effects of 2.6 and 3.9 have previously been reported for the hydrolysis of *O*-4-nitrophenyl sulfamate [4-$NO_2C_6H_4SO_2NL_2$] and EMATE for reaction along the low pH plateau at 60 °C.[7,8] These solvent isotope effects were used to support the proposal of an associative and bimolecular S_N2 pathway. Notably, any contribution of the N-*L* hydron to the observed kinetic isotope effects was specifically ruled out as it was deemed not to be involved in the slow step of sulfamate ester hydrolysis.[8] However, the greater than 10^{11}-fold difference in reactivity between **1** and **2** seems to contradict this conclusion and suggests that the sulfamate N-*H* group is actually engaged in bonding interactions at the transition state that substantially reduce the free energy for hydrolysis ($\Delta\Delta G^{\ddagger}$ = 15.6 kcal/mol).

We propose an alternative mechanism for the hydrolysis of **1** where the N-*H* proton is transferred, either directly or through a network of intervening water molecules,[9] to the phenoxy leaving-group as in transition structures **3** and **4** (Figure 2).

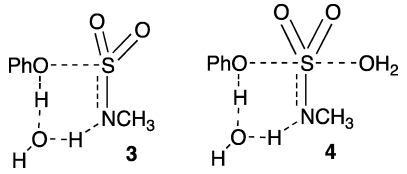


Figure 2. Possible transition structures for the hydrolysis of **1**.

This mechanism accounts for the small β^{LG} of −0.41 reported for the spontaneous hydrolysis of *O*-aryl sulfamates [$ArOSO_2NH_2$].[6] The magnitude of β^{LG} for these reactions indicates a modest degree of negative charge is accumulating on the aryloxy leaving-group as the sulfamate progresses from ground state to transition state.[10] The transfer of the N-*H* proton to the bridging oxygen atom of the leaving group partially neutralizes the accumulating negative charge resulting from S−O bond fission and provides for the appearance of a shallow Brønsted coefficient or β^{LG}. Similar conclusions have been drawn regarding the hydrolysis of aryl phosphate monoester monoanions (β^{LG} = −0.27) and the acid-catalyzed hydrolysis of sulfate monoesters (β^{LG} = −0.33).[11,12]

Impressive second-order rate constants of $k_i/K_i \approx 10^6$ M^{-1} s^{-1} for irreversible inhibition of an aryl sulfatase from *P. aeruginosa* (*Pa*AstA) have been reported for the inhibitors Coumate, 667 Coumate, and EMATE.[2] The strong binding affinities of these compounds (K_i < 1 μM) might be expected to translate into high target selectivity; however, this does not appear to be the case. Active site titrations reveal that up to six equivalents of inhibitor are required for complete inactivation of *Pa*AstA.[2] This suggests that off-target sulfamolyations are occurring with solvent and/or nucleophilic groups on the enzyme's surface acting as acceptors for the electrophile HN═S(═O)$_2$.

dx.doi.org/10.1021/jo300386u | J. Org. Chem. 2012, 77, 4450−4453

The high reactivity of the sulfamate esters likely contributes to their poor target selectivity; for example, Coumate has a half-time in dilute aqueous solution of only ~2 h at pH 7 and 37 °C.

Shown in Figure 3 (■, dashed line) is a plot of $\log(k_i)$ versus pK_a^{LG}, where k_i is the first-order rate constant for irreversible

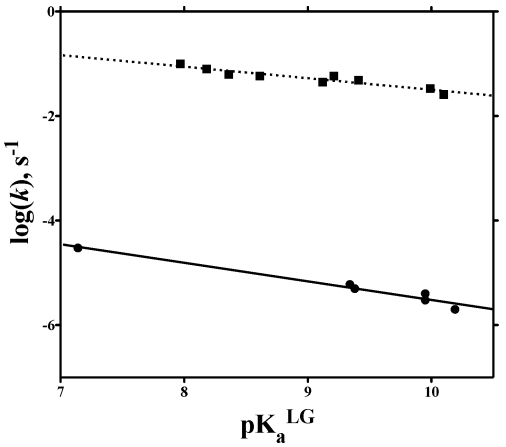


Figure 3. (a) Plot of $\log(k_i)$ versus pK_a^{LG} (■, dashed line) for the irreversible inhibition of *Pa*AstA by $ArOSO_2NH_2$ (pH 7, 37 °C). The data were obtained from ref 2. (b) Plot of $\log(k_{hydrolysis})$ versus pK_a^{LG} (●, solid line) for the uncatalyzed hydrolysis of $ArOSO_2NH_2$ at 37 °C. The data for this series were obtained from ref 7. See Table S1 of the Supporting Information.

inhibition of *Pa*AstA at pH 7, 37 °C under saturating concentrations of $ArOSO_2NH_2$.[2] Also included in Figure 3 is a plot of $\log(k_{hydrolysis})$ versus pK_a^{LG} (●, solid line) for the uncatalyzed hydrolysis of $ArOSO_2NH_2$ at 37 °C.[13] The vertical separation between the best-fit lines indicates that *Pa*AstA provides a ~10^4–fold rate acceleration for the cleavage of the S-OAr bond of simple sulfamate esters. This rate acceleration is small when compared with the level of catalysis achieved with sulfate monoesters, which are the enzyme's natural substrates. For example, a catalytic rate acceleration of k_{cat}/k_{uncat} = 16.7/(2×10^{-14}) = 8×10^{14} can be calculated for the hydrolysis of 4-methoxyphenyl sulfate.[2,12b] Steroid sulfatase inhibitors showing higher ratios of $k_i/k_{hydrolysis}$ are likely to result in fewer off-target sulfamoylations but it remains to be seen if this strategy will also result in a reduction in potency.

It would be desirable to predict the hydrolytic stability of sulfuryl esters before committing valuable resources toward synthesis of steroid sulfatase inhibitors. A plot of $\log(k)$ versus $pK_a^{ROSO2XH}$ for the hydrolysis of a series of *anionic* sulfuryl esters with a common 4-nitrophenoxide leaving group at 25 °C adheres to the equation $\log(k) = (-0.70 \pm 0.05)pK_a^{ROSO2XH} - (8.0 \pm 0.5)$ (Figure 4).[14] The 13 sulfuryl esters used to construct the plot are $4\text{-}NO_2C_6H_4OSO_3^-$, $4\text{-}NO_2C_6H_4OSO_2N^-CH_2Ar$, $4\text{-}NO_2C_6H_4OSO_2N^-Me$, $4\text{-}NO_2C_6H_4OSO_2C^-(H)SO_2CH_3$, $4\text{-}NO_2C_6H_4OSO_2C^-(CH_3)SO_2CH_3$, and $4\text{-}NO_2C_6H_4OSO_2\text{-}C^-C_6H_5$ (details are provided in the Supporting Information).[3,4,6,12b,15,16] The inset in Figure 4 shows a series of parallel lines corresponding to the hydrolysis of sulfuryl esters $ArOSO_2X^-$ with different aryloxy leaving groups (where ArO = PhO, 4-Cl-PhO, 3-NO_2-PhO, and 4-NO_2-PhO). The best-fit lines indicate an average gradient of 0.71 ± 0.01 that is invariant with respect to leaving group and *y*-intercepts that increase with decreasing pK_a^{ArOH}.

Equation 2 can be derived from these data to estimate rate constants for the decomposition of *anionic* sulfuryl esters based

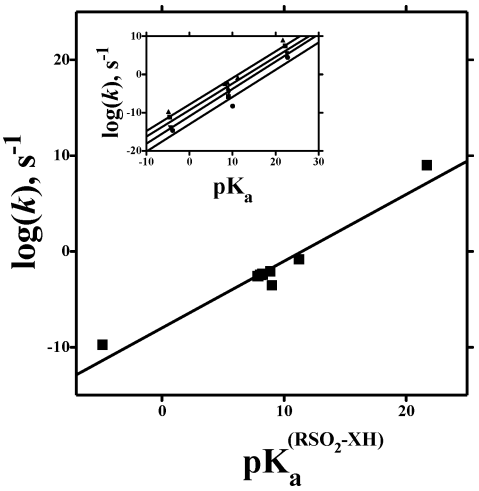


Figure 4. (a) Plot of $\log(k, s^{-1})$ versus $pK_a^{ROSO2XH}$ for the hydrolysis of $4\text{-}NO_2\text{-}C_6H_4O\text{-}SO_2X^-$ at 25 °C. The data are fit to $\log(k) = (-0.70 \pm 0.05)pK_a^{ROSO2XH} - (8.0 \pm 0.5)$ with $r^2 = 0.95$ (13 data). Full details are provided in the Supporting Information.

on pK_a^{ArOH} and $pK_a^{ArOSO2XH}$. The sulfuryl esters $F_5C_6OSO_3^-$ and $4\text{-}CH_3C(=O)C_6H_4OSO_2C^-HC_6H_5$ are estimated to decompose at 25 °C with rate constants of 5×10^{-9} and 4×10^6 s^{-1}, respectively, which do not differ greatly from the experimental values of 10^{-7} and 4×10^7 s^{-1}.[12b,16] The accuracy of the predictions, estimated to be within 2 orders of magnitude, is remarkable considering that the reactivities of the sulfuryl esters span a range of almost 20 orders of magnitude. At present, there is insufficient kinetic data on the hydrolysis of *neutral* sulfuryl esters to construct an analogous correlation to that in Figure 4.

$$\log(k) = (0.71 \pm 0.01)pK_a^{ROSO2XH} - (1.7 \pm 0.3)pK_a^{ArOH} + (4.7 \pm 2.5) \quad (2)$$

In summary, the spontaneous hydrolysis of neutral sulfamates, containing one or more N-*H* groups, is shown to proceed through a novel proton-in-flight mechanism (Figure 2). The hydrolysis of **1** is accelerated by an impressive factor of 10^{11} relative to the hydrolysis of **2**, and this effect is attributed to the simultaneous neutralization of charge on the bridging oxygen and nonbridging nitrogen atoms as a proton is transferred between these two atoms at the transition state. This mechanism suggests a rationale for the lack of irreversible inhibition observed with *N,N*-dialkyl *O*-aryl sulfamates such as **2**, which cannot react in a like manner.[17] Moreover, the observed rates of sulfuryl transfer, at least for anionic esters with common leaving groups, can be satisfactorily correlated with their pK_a values. The data in Figure 4 suggests that a compound with a leaving group as activated as Coumate's (pK_a^{ArOH} = 7.8)[18] would require a pK_a value of ~5 in order to attain a half-time in aqueous solution of 24 h at 25 °C.

■ EXPERIMENTAL SECTION

N-Methyl *O*-phenyl sulfamate (**1**) was prepared as described and determined to be 95% pure by comparison of the ^1H NMR integrated signal intensities of **1** to a known amount of pyrazine.[4] The hydrolysis of **1** (2.9×10^{-4} M) was carried out in 1.5 mL of polypropylene centerfuge vials immersed in a temperature-controlled water bath. Reaction pH was maintained with 0.15 M acetate, phosphate, Tris, or CAPS buffer at I = 0.55 M (NaCl). Variations in buffer concentration at constant pH did not show any observable effect on the rate constants. Periodically, the reaction mixtures were transferred to 1 cm path length quartz cuvettes, and the UV–vis spectra were obtained.

dx.doi.org/10.1021/jo300386u | J. Org. Chem. 2012, 77, 4450–4453

Reaction progress was determined for reactions run below pH 9 by monitoring phenol production at 280 nm (ε^{280} = 1418 Abs/M/cm). Phenoxide production was monitored at 290 nm for higher pH reactions, and an effective ε^{290} was determined under the exact experimental conditions in these cases. Observed first-order rate constants were calculated by a nonlinear least-squares fitting of the absorbance versus time data to a standard first-order exponential equation. Good first-order behavior was generally observed for greater than three half-lives, and a comparison of the UV spectra before and after complete hydrolysis demonstrated a 1:1 stoichiometry in all cases. A rate constant for the hydrolysis of **1** at pH 5.9 and 25 °C was determined by the method of initial rates. *N*,*N*-Dimethyl *O*-phenyl sulfamate (**2**) was prepared as described and characterized by ^{1}H NMR.[19] Hydrolysis of **2** (17 mM) in H_2O was carried out in vacuum-sealed quartz tubes containing 0.2 M potassium phosphate buffer at pH 5.9. The sealed quartz tubes were inserted into stainless steel pipe bombs and placed in thermally equilibrated ovens as described.[20] Reaction progress was measured by diluting the reaction samples 5-fold with D_2O and then obtaining a ^{1}H NMR spectrum on the reaction mixture and integrating the signals corresponding to $PhOSO_2NMe_2$ to PhOH. Control experiments reveal that hydrolysis of **2** at pH 5.9 is independent of hydronium ion concentration and that the spontaneous reaction extends up to at least pH 8.

■ ASSOCIATED CONTENT

Supporting Information

Plot of absorbance versus pH for the UV−vis titration of **1**. Eyring plots for the hydrolysis of **1** and **2**. Plot of observed rate constant versus percent deuterium content used to calculate the solvent kinetic isotope effect on **1** (Figure S4). Table of kinetic constants for the hydrolysis of $ArOSO_2X^-$ (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant No. GM-18325.

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(5) We do not specify a mechanism for the spontaneous hydrolysis of **2** and consider that it could occur by S−O bond cleavage with direct displacement of phenol. An alternative process involving hydrolysis of **2** via S−N bond fission to initially afford phenyl sulfate and dimethylamine (or its ammonium ion) cannot be ruled out based on the kinetic data because of the subsequent hydrolysis of phenyl sulfate ($\Delta H^{\ddagger}$ = 32.9 kcal/mol $\Delta S^{\ddagger}$ −15 cal/mol/K, data from ref 12b) to form phenol and inorganic sulfate is predicted to occur faster than its formation.

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(14) A similar but less extensive plot appears in ref 16.

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