However, this absorption does not show the usual shift to a longer wavelength in chloroform,[19] a characteristic of sulfoxides.

The thiol ester was converted to methyl phenylglyoxylate, **8a** (R′ = CH_3), by refluxing for 24 hr in methanol with a trace of sulfuric acid.

Mandelic Acid (11a). A solution of 9.1 g (0.050 mole) of **3a** in ml of warm ethanol was vigorously stirred for 1 hr with 7.59 g (0.037 mole) of powdered cupric acetate monohydrate. The precipitate was removed by filtration, and the filtrate was mixed with a solution of 8 g (0.2 mole) of sodium hydroxide in 20 ml of water and heated at 65–70° for 7 hr, shaking occasionally to break up the solid mass that formed. The mixture was poured into 200 ml of water, acidified to pH 1 with concentrated hydrochloric acid, filtered, and thoroughly extracted with 450 ml of chloroform in 75-ml portions. Evaporation of the solvent yielded 5.3 g (70%) of **11a**, mp 110–112°. Evaporation of the aqueous solution and extraction of the salts with acetone gave an additional 1.4 g (18%) of the acid. Recrystallization from carbon tetrachloride gave colorless crystals, mp 116–118°, lit.[10] mp 117–118.5°.

1-Phenylpropane-1,2-dione. A mixture of 6.47 g (0.033 mole) of **13** (R = CH_3) in 15 ml of water and 3 ml of 85% phosphoric acid was heated at reflux temperature for 94 hr with rapid stirring. The mixture was cooled, and 25 ml of chloroform was added. The chloroform layer was separated from the aqueous layer and the latter extracted with two 20-ml portions of chloroform. The combined chloroform extracts were dried over magnesium sulfate and concentrated under reduced pressure. Vacuum distillation of the residue gave 2.45 g (50%) of the α-diketone as a clear yellow liquid, bp 55–57° (0.7 mm), lit.[20] bp 55–56° (0.5 mm), the infrared spectrum of which was superimposable with an authentic sample. The pmr spectrum (65% solution in carbon tetrachloride) showed only a 3-proton singlet at δ 2.38 and the expected 5-proton aromatic multiplet at δ 7.15–8.0.

(19) T. Cairns, G. Eglinton, and D. T. Gibson, *Spectrochim. Acta*, 20, 31 (1964).

(20) W. D. Emmons and J. P. Freeman, *J. Am. Chem. Soc.*, 77, 4415 (1955).

In the preparation of the arylglyoxals, it was noted that the presence of acids causes extensive decomposition of the glyoxal during distillation. In this preparation of the diketone, the chloroform solution was not extracted with dilute aqueous base, perhaps causing some reduction in the yield. The above procedure is a modification of the procedure used to prepare phenylglyoxal directly from the β-keto sulfoxide (procedure A, table IV), described below.

Phenylglyoxal. (Procedure A). A solution of 9.1 g (0.050 mole) of **1a** in 50 ml of water and 10 ml of 85% phosphoric acid was heated at reflux temperature and stirred vigorously for 35 hr, cooled, and extracted with five 50-ml portions of chloroform. The combined chloroform extracts were dried over magnesium sulfate and concentrated under reduced pressure. Vacuum distillation of the residue gave 4.6 g (68.6%) of anhydrous phenylglyoxal as a yellow liquid, bp 60–63° (1 mm).

Repetition of the above procedure using 75.5 g (0.42 mole) of **1a** in 620 ml of water and 62 ml of 85% phosphoric acid yielded 33.3 g (60%) of anhydrous phenylglyoxal.

Phenylglyoxal. (Procedure D). The methyl hemimercaptal of phenylglyoxal (69–74 g, 0.39–0.41 mole), prepared as described for the direct conversion of ethyl benzoate to **3a**, was dissolved in 400 ml of warm chloroform, and 60 g of finely powdered cupric acetate monohydrate (0.3 mole) was added in one portion to the well-stirred solution. The mixture was vigorously stirred at room temperature for 1 hr. The solids were removed by suction filtration and washed twice with 75-ml portions of chloroform. The combined chloroform solution was shaken with 75 ml of water in a separatory funnel. Powdered sodium carbonate (20 g) was added in small portions to the aqueous layer, and the chloroform solution was extracted with the neutralized aqueous solution (caution—carbon dioxide evolution). The aqueous solution was extracted with four 30-ml portions of chloroform. The chloroform extracts were combined and dried with magnesium sulfate, and the chloroform was removed under reduced pressure. Vacuum distillation of the residue, first at a bath temperature of about 70° to remove residual water and methyl disulfide, and then at 90–95°, gave 43–49 g (64–73% based on starting ester) of anhydrous phenylglyoxal as a yellow liquid, bp 63–65° (0.5 mm).

Studies on Sulfate Esters. I. Nucleophilic Reactions of Amines with *p*-Nitrophenyl Sulfate

Stephen J. Benkovic and Patricia A. Benkovic

Contribution from the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received August 8, 1966*

Abstract: The nucleophilic reactivity of a series of amines toward *p*-nitrophenyl sulfate exhibits a small sensitivity to the basicity of the amine ($\beta = 0.20$). The reactivity is in the order tertiary > secondary > primary amine; steric hindrance increases rapidly with α substitution. A comparison of the reactions of the same amines with *p*-nitrophenyl phosphate reveals a striking similarity in their nucleophilic reactivity and the mechanism of the displacement. Both appear to involve transition states which feature little bond formation between substrate and amine. The spontaneous hydrolysis of *p*-nitrophenyl sulfate is discussed in terms of a possible elimination of sulfur trioxide in analogy to monomeric metaphosphate formation in the hydrolysis of certain phosphate dianions.

Investigation of the chemistry of sulfate esters was prompted by several considerations: (1) the wide distribution in nature of sulfate esters, *e.g.*, mucopolysaccharides, steroidal and phenolic sulfates;[1] (2) the presence of sulfatase enzymes which catalyze the hydrolysis of such sulfate linkages;[1] (3) the possible relationship between the hydrolytic mechanisms of the sulfatases and other hydrolases (phosphatase, chymotrypsin, etc.); and (4) the hypothesis that mechanistic similarities may occur in the hydrolytic pathways of sulfate and phosphate esters.[2]

Experimental Section

Reagents. The potassium salt of *p*-nitrophenyl sulfate (Sigma Chemical, lot 124B-5110) assayed spectrophotometrically after complete acidic hydrolysis[3] proved to be of 99+% purity and

(1) J. D. Gregory and P. W. Robbins, *Ann. Rev. Biochem.*, 29, 347 (1960).

(2) E. T. Kaiser, M. Panar, and F. H. Westheimer, *J. Am. Chem. Soc.*, 85, 602 (1963).

(3) K. S. Dodgson, B. Spencer, and K. Williams, *Biochem. J.*, 64, 216 (1956).

contained less than 0.1% free *p*-nitrophenol. Amines and amine hydrochlorides (Eastman, White Label) were distilled or recrystallized prior to use and stored under nitrogen or over P_2O_5. Imidazole (Aldrich) and inorganic salts (Fisher, Reagent Grade) were used without further purification. Freshly boiled distilled water was employed to prepare all solutions. Deuterium oxide (99.9%) was furnished through the courtesy of Dr. R. A. Olofson of this department. Deuteriochloric acid was prepared from deuterium oxide and anhydrous hydrogen chloride.

Apparatus. A Gilford Model 2000 spectrophotometer equipped with a thermostated cuvette holder (±0.1°) was used for kinetic measurements. All pH determinations were made with a Radiometer Model 22 pH meter equipped with a Model PHA 630 Pa scale expander and a Radiometer G. K. 2021 B electrode. Kinetic runs of greater than 12-hr duration were carried out in Kimax (No. 45066) screw-cap tubes maintained at reaction temperature (±0.1°) by immersion in a circulating water bath. Shorter runs were conducted in thermostated, 2-cm stoppered cuvettes.

Kinetics. The liberation of *p*-nitrophenolate was monitored at 400 mμ. Reactions were initiated by the addition of 1 ml of *p*-nitrophenyl sulfate solution (0.005–0.02 *M*, μ = 1.0, KCl) to 9 ml of the reaction mixture (μ = 1.0, KCl), solutions being preequilibrated at the desired temperature. The pH of the reaction mixtures was measured at 35° upon initiation and after completion of the runs; those exhibiting pH drift greater than ±0.02 unit were discarded.

The observed rate constants were calculated from the slopes of linear plots of optical density *vs.* time divided by the mock optical density at infinite time (OD_∞). The latter quantity at pH >9.5 was calculated from either (1) the known initial concentration of *p*-nitrophenyl sulfate and ϵ 18,320[4] for *p*-nitrophenolate or (2) complete acidic hydrolysis of an aliquot of the stock sulfate solution followed by alkaline spectrophotometric assay.[3] Rate constants obtained by either procedure were in satisfactory agreement. At pH <9.5, the OD_∞ was corrected for incomplete ionization of *p*-nitrophenol (pK_a' = 7.1) by either (1) constructing curves of optical density *vs.* concentration for a standard solution of *p*-nitrophenol[4] diluted with reaction mixture or (2) complete acidic hydrolysis of an aliquot of the stock sulfate solution, followed by dilution with the reaction mixture and spectrophotometric assay. The OD_∞ computed by both methods was within ±5%; OD_∞ values calculated for kinetic runs utilizing dimethylamine, piperidine, and methylamine were corrected for absorbance due to concomitant formation of the corresponding anilide employing ϵ 15,100, 13,500, and 16,200, respectively[5] (see Products). With other amines (C–O bond fission <25%) this effect on OD_∞ was negligible. The above method of initial rates was checked by conducting kinetic runs at higher temperatures (55 or 75°) or by runs at 35° with more reactive amines at concentrations approaching 1 *M* in amine free base. In this manner OD_∞ was actually attained. Rate constants calculated from conventional plots of log $[OD_\infty/(OD_\infty - OD_t)]$ against time agreed within ±3% with observed rate constants computed as above.

Rates of the spontaneous hydrolysis were obtained from independent measurements in solutions buffered by noncatalytic species or from intercepts of plots of the observed pseudo-first-order rate constants *vs.* amine concentration. No extraordinary precautions were taken to exclude light[6] or metal ions; nevertheless, the rates were identical with those obtained from runs conducted in the dark or in the presence of 10^{-3}–10^{-4} *M* ethylenediaminetetraacetic acid. Standardized potassium hydroxide solutions were employed at pH >12.

Second-order rate constants for the amine reactions were obtained from plots of the observed pseudo-first-order rate constants against concentration of amine free base (Figure 1). No deviations from linearity or additional dependency on pH were observed. The pK_a' values were determined by the method of half-neutralization (μ = 1.0, KCl, 35°); those of pyridine, Tris, 2,6-lutidine, and pyrazole were obtained from the literature.[7,8] Second-order rate constants for diamines were determined from plots of k_{obsd} (corrected for spontaneous hydrolysis)/(diamine H^+) against (diamine)/(diamine H^+); intercept values yield the rates associated with the diamine monocation, and slopes, the rates for diamine free base.

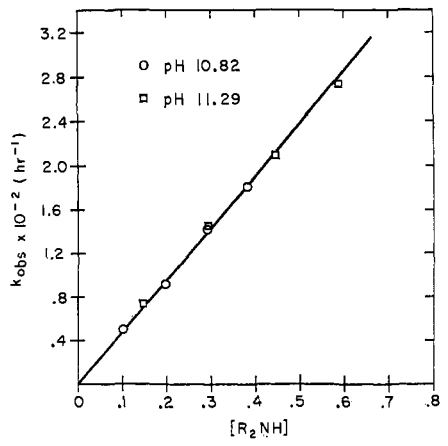

Figure 1. Plots of k_{obsd}, the pseudo-first-order rate constant, against the concentration of amine free base (35°, μ = 1.0). The slope is the composite second-order rate constant for S–O and C–O bond cleavage (*p*-nitrophenyl sulfate + $(CH_3)_2NH$).

Products. Spectrophotometric assay by the method of Kirby and Jencks[7] indicated the formation with primary or secondary amines of the corresponding *p*-nitroanilide. Actual isolation by ether extraction of precipitated N-*n*-propyl-*p*-nitroanilide (mp 65°, lit.[9] 64–65°) and N-piperidyl-*p*-nitroanilide (mp 103–104°, lit.[5,10] 102–103°, 105°) was accomplished at t_∞ for duplicate runs (reaction conditions: 0.078 *M p*-nitrophenyl sulfate, 0.78 *M* amine, 75°). No precipitation occurred during kinetic runs. Isolation and spectroscopic techniques agreed within 10%. Second-order rate constants for attack on sulfur were calculated after correction for anilide formation. Runs with tertiary amines (no absorption of cationic anilide at 400 mμ) are not susceptible to spectrophotometric assay; observed rate constants are assumed to predominantly represent attack of amine on sulfur.[11]

Detection of the sulfamate esters resulting from attack of primary and secondary amines on sulfur was complicated by concomitant hydrolysis of the sulfamate product. Control experiments with authentic samples of *n*-propyl and piperidyl sulfamate[12a,b] showed only 1–2% of anticipated product remaining at t_∞ for runs of above composition at 75°. After extraction of anilide, actual isolation of *n*-propyl and piperidyl sulfamate was achieved by removal of excess amine by evaporation to dryness of the above reaction mixture (made strongly alkaline with KOH), acidification, ether extraction (removal of *p*-nitrophenol), and evaporation to dryness at 0° followed by acetone or ethanol extraction. Melting points and infrared spectra of the products were identical with authentic samples. Runs with tertiary amines yield hydrolytically unstable amine–SO_3 adducts and preclude isolation.[13a]

The position of bond cleavage in the spontaneous hydrolysis of *p*-nitrophenyl sulfate was determined by the method of Spencer.[13b] The substrate (52 mg) was dissolved in 5 ml of water containing 6.8 at. % of O^{18}, pH 11.3 (by addition of 1.0 *N* KOH), and allowed to hydrolyze (100°) until reaction was 90% completed. Required reaction time was estimated from the activation energy. The pH was maintained by incremental additions of KOH. Infrared anal-

(4) F. Kezdy and M. L. Bender, *Biochemistry*, **1**, 1097 (1962).
(5) A. J. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, 87, 3217 (1965).
(6) E. Havinga, R. O. deJongh, and W. Dorst, *Rec. Trav. Chim.*, **75**, 378 (1956).
(7) A. J. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, 87, 3209 (1965).
(8) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley and Sons, Inc., New York, N. Y., 1962.
(9) F. K. Beilstein, "Handbuch der organischen Chemie," Vol. 12, 1933, p 351.
(10) E. Lellmann and W. Geller, *Ber.*, **21**, 2281 (1888).
(11) The second-order rate constant for the reaction of OH^- with N,N,N-trimethyl-*p*-nitroanilide is 1.1×10^{-3} l. mole^{-1} hr^{-1} at 51°, approximately 60% of the reaction yielding *p*-nitrophenol (K. R. Brower, *J. Am. Chem. Soc.*, 82, 4535 (1960)). Consequently liberation of *p*-nitrophenol by this pathway is estimated at 10^{-6}–10^{-7} min^{-1} at pH 10, being less than 10% of k_{obsd} for trimethylamine at concentrations and pH's employed. Assuming the rates of hydrolysis of other cationic anilides are similar, a similar conclusion may be drawn.
(12) (a) L. F. Audrieth, M. Sveda, H. H. Sisler, and M. J. Butler, *Chem. Rev.*, **26**, 49 (1940); (b) L. F. Audrieth and M. Sveda, *J. Org. Chem.*, **9**, 89 (1944).
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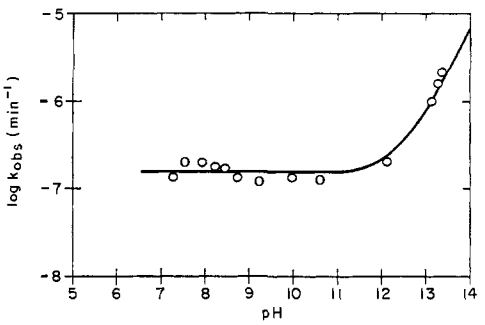


Figure 2. The pH–rate profile for the hydrolysis of *p*-nitrophenyl sulfate (35°, μ = 1.0). Solid line is theoretical curve calculated from values listed in Table I.

ysis of the isolated $BaSO_4$ revealed 5.9 at. % of O^{18}, or 85% of the hydrolysis proceeded *via* S–O bond cleavage.[14]

Results

Hydrolysis. The pH–rate profile for the spontaneous hydrolysis of *p*-nitrophenyl sulfate is shown in Figure 2. The k_{obsd} values are essentially independent of pH in the range 7–12 becoming dependent on the first power of hydroxide ion concentration at higher pH values. The latter reaction apparently represents attack of hydroxide both on the aromatic ring with C–O bond cleavage and on sulfur with S–O bond fission. This contention is supported by (1) the alkaline O^{18}-tracer studies of Spencer[15] which indicate 33% of the alkaline hydrolysis of *p*-nitrophenyl sulfate proceeds *via* C–O bond cleavage and (2) the small twofold increase in the rate constant for hydroxide ion attack on *p*-nitrophenyl sulfate relative to 1-chloro-4-nitrobenzene.[5] The rate and tracer studies are not directly comparable because of differing experimental conditions.

Table I. Comparison of the Hydrolysis of *p*-Nitrophenyl Sulfate and *p*-Nitrophenyl Phosphate

p-NPS, 35°, μ = 1.0	*p*-NPP,[a] 39°, μ = 1.0
$k_0 = 1.48 \times 10^{-7}$ min^{-1} [f,h]	$k_0 = 9.3 \times 10^{-7}$ min^{-1}
$\Delta H^* = 24.6$ kcal mole^{-1} [b,c]	$\Delta H^* = 30.6$ kcal mole^{-1}
$\Delta S^* = -18.5$ eu[b,c]	$\Delta S^* = +3.5$ eu
2.1-fold decrease in rate,[d,e] 50% CH_3CN–H_2O	No effect on rate, 99% ethylene glycol–H_2O
0.8-fold decrease in rate,[d] 0.8 *M* KI	No effect on rate, added salts
1.65-fold increase in rate,[d] 0.8 *M* NaF	
1.36-fold increase in rate,[d] 0.25 *M* K_2SO_3	
$k^{H_2O}/k^{D_2O} = 1.26$[d,g]	$k^{H_2O}/k^{D_2O} = 1.12$
$k^{OH^-}/k^{H_2O} = 20.2$[i]	$k^{OH^-}/k^{H_2O} = 0.52$

[a] Data of ref 7. [b] Calculated from the equations $\Delta H^* = E_a - RT$; $\Delta S^* = (\Delta H^* - \Delta F^*)/T$; $\Delta F^* = -RT \ln k_r h/kT$ at 35°. [c] Measured in 0.18 *M* K_2CO_3 buffer, pH 9.96 at 35°. [d] At 75°. [e] $k_0 = 1.73 \times 10^{-5}$ min^{-1}, linear decrease with increasing per cent of CH_3CN. [f] Measured in 0.18 *M* Tris, K_2CO_3, and K_3PO_4 buffers; no detectable catalysis by buffer species (3-fold dilution). [g] Measured in 0.18 *M* K_2CO_3 buffer, pH 9.96 in H_2O, 35°. [h] Identical in dark and presence of 10^{-3}–10^{-4} *M* ethylenediaminetetraacetic acid. [i] $k_{OH^-} = 3.00 \times 10^{-6}$ M^{-1} min^{-1}, 35°.

(14) The above method is subject to considerable error at the per cent O^{18} level utilized. One may conclude only that the hydrolysis proceeds with predominant S–O bond cleavage but cannot rule out the possibility that some exchange may occur without hydrolysis.

(15) B. Spencer, *Biochem. J.*, 69, 155 (1958).

Kinetic studies undertaken to determine the molecularity of the plateau rate are summarized in Table I. The rate of the *p*-nitrophenyl sulfate hydrolysis is (1) slightly decreased in deuterium oxide, (2) moderately sensitive to added salts (0.8 *M* NaF and 0.25 *M* K_2SO_3 increase the rate 1.65- and 1.36-fold, respectively, while 0.8 *M* KI decreases the rate ~20%); (3) decreased linearly ~50% upon change to less aqueous media (0–50%, acetonitrile–H_2O); and (4) characterized by ΔH^* of 24.6 kcal mole^{-1} and ΔS^* of −18.5 eu.

Attack of Amines. The kinetics of the reaction of various monoamines with *p*-nitrophenyl sulfate are satisfied by the expression

$$v = k_2[RNH_2][p\text{-NPS}^-]$$

The substrate is present as the monoanion (pK_{a1} (H_2SO_4), −3[8]) at all pH's investigated in this study. No evidence was found for reactions involving $[RNH_3^+]$ or $[RNH_2]^2$ terms. Second-order rate constants for the attack of amine on sulfur, corrected for any anilide formation, are listed in Table II. Reactions at 35° and 1 *M* amine have half-lives in the order of weeks; therefore, rate constants are computed from initial rates. At higher temperatures, however, reactions can be conveniently followed to two half-lives. This reflects the high activation energies for the amine reactions, *e.g.*, ΔH^* of 23.3 and 18.9 kcal mole^{-1} for propylamine and piperidine, respectively. Evidence that the reaction represents nucleophilic attack by amines and not kinetically indistinguishable general base catalysis is given by detection of the sulfamate product and the determination of a deuterium solvent kinetic isotope effect consistent with a nucleophilic reaction.

Studies with several diamines at varying pH and amine concentrations indicate that both the monocation and free base species of the diamine are kinetically important.

$$v = \{k_{AH^+}[R_2\dot{N}H(CH_2)_2NR_2] + k_A[R_2N(CH_2)_2NR_2]\}[p\text{-NPS}^-]$$

The reaction of the monocation species with the sulfate is probably best described as involving monocation and sulfate anion rather than the kinetically indistinguishable representation involving diamine free base and protonated ester. Calculations assuming the pK_a' of *p*-nitrophenyl sulfate as −3 indicate the latter rate constant would approach that for a diffusion-controlled reaction. Moreover, no $[RNH_3^+]$ terms were detected for the monoamine reactions. For ethylenediamine, N,N,N′,N′-tetramethylethylenediamine, and triethylenediamine, the monocationic species is less reactive than the amine-free base. Variations in pH failed to reveal any reactivity due to hydrazine monocation.

Discussion

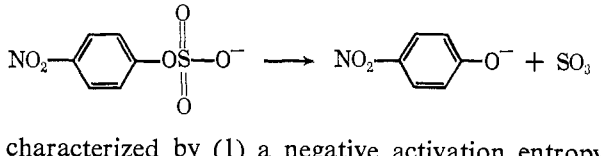
Hydrolysis. The plateau rate observed in the hydrolysis of *p*-nitrophenyl sulfate may be formulated as a unimolecular elimination mechanism or a bimolecular reaction involving water as a nucleophilic species. At first glance, the striking resemblence between the pH–rate profiles for *p*-nitrophenyl *phosphate* and sulfate might be interpreted as signifying a unimolecular elimination mechanism involving the formation of sulfur trioxide instead of monomeric metaphosphate.

Table II. Rate Constants for the Reactions of Amines with *p*-Nitrophenyl Sulfate Monoanion (35°, μ = 1.0)

Nucleophile	pH range	Concn range,[a] *M*	pK_a'[k]	No. of runs	Fraction S–O[b]	k_2, M^{-1} min^{-1}[c]
Hydrazine	8.0–8.6	0.05–0.45	8.04	12	1.0	1.25×10^{-5} [m]
Imidazole	7.0–7.6	0.20–0.90	7.05	12	1.0	7.30×10^{-7}
Piperidine	11.1–11.6	0.20–0.80	11.12	8	0.39 ± 0.04	2.15×10^{-4} [h]
Propylamine	10.55–11.4	0.19–0.95	10.56	12	0.74 ± 0.02	1.26×10^{-5} [i,j]
Morpholine	8.6–9.1	0.22–0.91	8.63	8	0.80 ± 0.01	7.24×10^{-5}
Dimethylamine	10.8–11.3	0.20–0.80	10.84	8	0.39 ± 0.02	3.05×10^{-4}
Ethylenediamine			10.02		0.84 ± 0.01	9.07×10^{-6} [m]
	7.3–10.0	0.1–0.4		12		
Monocation			7.28		0.90 ± 0.01	3.15×10^{-6}
Glycine	9.5–10.1	0.20–0.80	9.53	8	0.90 ± 0.03	1.04×10^{-5}
Methylamine	10.6–11.1	0.20–0.80	10.64	8	0.71 ± 0.01	3.05×10^{-5}
Hydroxylamine	5.9–6.5	0.20–0.80	5.95	8	1.0	5.11×10^{-6}
Trimethylamine	9.9–10.4	0.20–0.80	9.99	8		4.88×10^{-4}
N,N,N′,N′-Tetramethylethylenediamine			9.34			2.17×10^{-5} [m]
	8.9–9.8	0.18–0.50		24		
Monocation			6.22			9.50×10^{-5}
Pyridine	10.7	0.09–0.49[d]	5.1[l]	4		4.03×10^{-6}
Triethylamine	10.8	0.17–0.39	10.83			[f]
Diethylamine	11.0	0.19–0.76	11.08	4	0.84 ± 0.01	1.01×10^{-5}
Triethylenediamine			9.01			1.25×10^{-3} [m]
	6.9–11.1	0.10–0.60		16		
Monocation			3.34			8.1×10^{-5}
Ethanolamine	9.0–9.5	0.20–0.79	9.51	8	0.91 ± 0.02	9.58×10^{-6}
Pyrazole	11.1	0.10–0.50[g]	2.53[l]			[f]
2,6-Lutidine	11.1	0.05–0.16[e]	6.8[l]			[f]
Tris	8.2–8.45	0.05–0.18	8.1[l]			[f]

[a] Stoichiometric amine concentration. [b] Fraction of OD at 400 mμ due to *p*-nitrophenolate absorption. [c] For S–O bond cleavage. [d] Measured in 0.05 *M* K_3PO_4 buffer, pH 10.69, 35°. [e] Measured in 0.2 *M* K_3PO_4 buffer, pH 11.09, 35°. [f] No detectable reaction under conditions specified. [g] Measured in 0.05 *M* K_3PO_4 buffer, pH 11.09, 35°. [h] $\Delta H^* = 18.9$ kcal mole^{-1}, $\Delta S^* = -22.0$ eu (calculated from equations of Table I, footnote *b*). [i] $\Delta H^* = 23.3$ kcal mole^{-1}; $\Delta S^* = -13.5$ eu. [j] $k^{H_2O}/k^{D_2O} = 0.95$. [k] Determined at 35°, μ = 1.0, by method of half-neutralization unless otherwise specified. [l] Literature values.[7,8] [m] Statistically corrected by dividing k_2 by a factor of 2.

However, closer scrutiny of the spontaneous reaction, which proceeds predominantly *via* S–O fission, reveals a sensitivity to mechanistic probes not observed for the phosphate ester (Table I). The sulfate ester hydrolysis



is characterized by (1) a negative activation entropy, −18 eu, in contrast to small, usually slightly positive entropies observed for unimolecular reactions[16a,b] (ΔS^* for *p*-nitrophenyl phosphate = +3.0 eu); (2) the 1.3–1.7 rate accelerations produced by nucleophilic anions, fluoride and sulfite (the former, for example, being reactive toward acetyl phosphate,[17] phosphoramidate,[18] *o*-phenylene sulfite,[19] and trimethylamine–sulfur trioxide adduct[13a] and the latter toward *o*-phenylene sulfite[20] and alkyl carbon atoms);[21a] (3) a small rate decrease caused by the presence of potassium iodide which is tentatively ascribed to a negative salt effect—similar effects for unimolecular electrolytes having been observed in the spontaneous hydrolysis of cyclic and aryl sulfites;[19,21b] and (4) a linear decrease in rate as solvent composition is altered by increasing the concentration of a nonnucleophilic species. Yet, these effects are not dramatic. Although a truly appropriate reference compound is lacking, 0.8 *M* fluoride, for example, similarly increases the rate of hydrolysis of acetyl phosphate monoanion[17] ~1.4-fold; in contrast 0.08 *M* fluoride increases the rate of hydrolysis of *o*-phenylene sulfite[19] some fourfold. The former presumably hydrolyzes *via* a unimolecular mechanism;[17] the latter hydrolysis apparently involves water as a nucleophilic species.[19] Moreover, the effect of mixed solvents on the spontaneous rate is identical with that observed with acetyl phosphate dianion for which substantial evidence supports hydrolysis *via* a unimolecular elimination pathway.[22] On the other hand, the rate of hydrolysis of *o*-phenylene sulfite in 60:40 v/v dioxane–water is one-twelfth as fast as in water; solvent effects of similar magnitude are commonly observed for reactions involving water as a nucleophilic species in the hydrolyses of derivatives of inorganic and organic acids.[23] It is also necessary to point out that the data of Table I for *p*-nitrophenyl sulfate were obtained some 36° higher than that of the phosphate. Similar small effects may well be observed for the latter at higher temperatures. Equally pertinent is the deuterium solvent isotope effect of $k^{H_2O}/k^{D_2O} = 1.26$; transition states of spontaneous hydrolysis which are strongly solvated by water are apparently associated with much larger solvent deute-

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(17) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, 83, 4393 (1961).

(18) M. Halmann, A. Lapidot, and D. Samuel, *J. Chem., Soc.*, 1299 (1963).

(19) C. A. Bunton and G. Schwerin, *J. Org. Chem.*, 31, 842 (1966).

(20) P. B. D. de la Mare, J. G. Tillett, and H. F. van Woerden, *J. Chem. Soc.*, 4888 (1962).

(21) (a) C. G. Swain and C. B. Scott, *J. Am. Chem. Soc.*, 75, 141 (1953); (b) J. G. Tillett, *J. Chem. Soc.*, 5138 (1960).

(22) G. Di Sabato and W. P. Jencks, *J. Am. Chem. Soc.*, 83, 4400 (1961).

(23) See ref 19 and references therein.

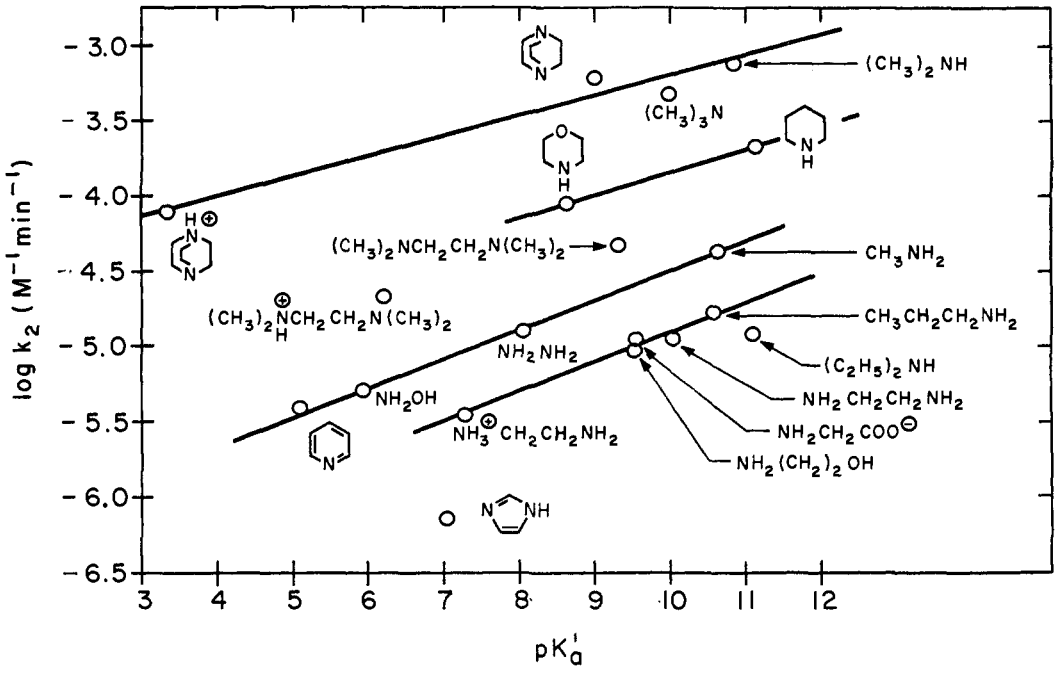


Figure 3. Plot of the logarithm of composite second-order rate constant (M^{-1} min^{-1}, 35°, μ = 1.0) *vs.* pK_a'. Rate constants for diamines are statistically corrected.

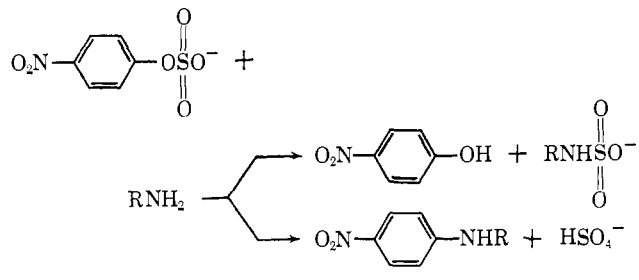
rium isotope effects.[24,25] The observed solvent isotope effect more closely resembles those observed in the hydrolyses of alkyl halides for which the small magnitude of the isotope effect apparently implies a transition state in which very little bond formation has occurred between solvent and substrate[26] and those solvent isotope effects observed in reactions postulated as being unimolecular.[7,22] It is more difficult, however, to rationalize the negative entropy term as evidence for other than a bimolecular reaction although unimolecular solvolyses are known for which ΔS^* is -10 to -17 eu.[21a] Thus the results do not clearly define a particular hydrolysis mechanism, but, if viewed as involving participation of water as a nucleophilic species, the data imply a small degree of bond formation between solvent and ester.

It is possible that a better leaving group may clearly demonstrate a unimolecular elimination mechanism anticipating that a unimolecular pathway should be more sensitive to the nature of the leaving group.[7] An attractive possibility is 3-phosphoadenosine-5-phosphosulfate (p$K_{a_2}' < 7$ but dependent on the state of ionization of 5-phosphate) which functions as the sulfate donor in the synthesis of various biologically important sulfate esters.[27,28] It is tempting to speculate that its role as sulfating agent may be through the elimination of sulfur trioxide (see ref[29] for the possibility that this may be acid catalyzed), although a bimolecular displacement mechanism offers a simpler means of directing the selectivity of the sulfation reaction.

(24) C. A. Bunton, N. A. Fuller, S. G. Perry and V. J. Shiner, *J. Chem. Soc.*, 2918 (1963).
(25) C. A. Bunton and V. J. Shiner, *J. Am. Chem. Soc.*, 83, 3207 (1961).
(26) C. A. Bunton, "Nucleophilic Substitution at a Saturated Carbon Atom," Elsevier Publishing Co., London, 1963.
(27) P. W. Robbins and F. Lipmann, *J. Biol. Chem.*, 229, 837 (1957).
(28) J. D. Gregory and F. Lipmann, *ibid.*, 229, 1081 (1957).
(29) S. J. Benkovic, *J. Am. Chem. Soc.*, 88, 5511 (1966).

It is noteworthy that the attack of hydroxide on the sulfate ester is mainly on sulfur rather than the aromatic ring as found in the related phosphate. This evidently indicates in part the diminishing electrostatic barrier although k_{OH^-} is only of the order of 3.00×10^{-6} l. mole^{-1} min^{-1} (35°, μ = 1.0). However, k_{OH^-} is similar in magnitude to the rate of attack of several neutral amine species on sulfur; therefore, coulombic repulsions may not be the main factor in the observed low nucleophilic reactivity. It is not known whether the reaction pathway involving hydroxide ion proceeds *via* formation of a metastable pentacovalent intermediate; a related species was not detected by means of O^{18} back incorporation in the hydrolysis of ethylene sulfite or dialkyl sulfites.[30a,b]

Nucleophilic Reactivity. The reaction of various amines with *p*-nitrophenyl sulfate may occur *via* attack on the aromatic ring leading to C–O bond cleavage and anilide formation or attack on sulfur leading to S–O bond fission and amine sulfamate. Second-order rate constants for the over-all process are plotted against



pK_a' in Figure 3. No kinetic evidence was found to indicate the existence of an intermediate adduct in the nucleophilic aromatic displacement reactions as ob-

(30) (a) C. A. Bunton, P. B. D. de la Mare, P. M. Greaseley, D. R. Llewellyn, N. H. Pratt, and J. G. Tillett, *J. Chem. Soc.*, 4751 (1958); (b) J. G. Tillett, *ibid.*, 37 (1960).

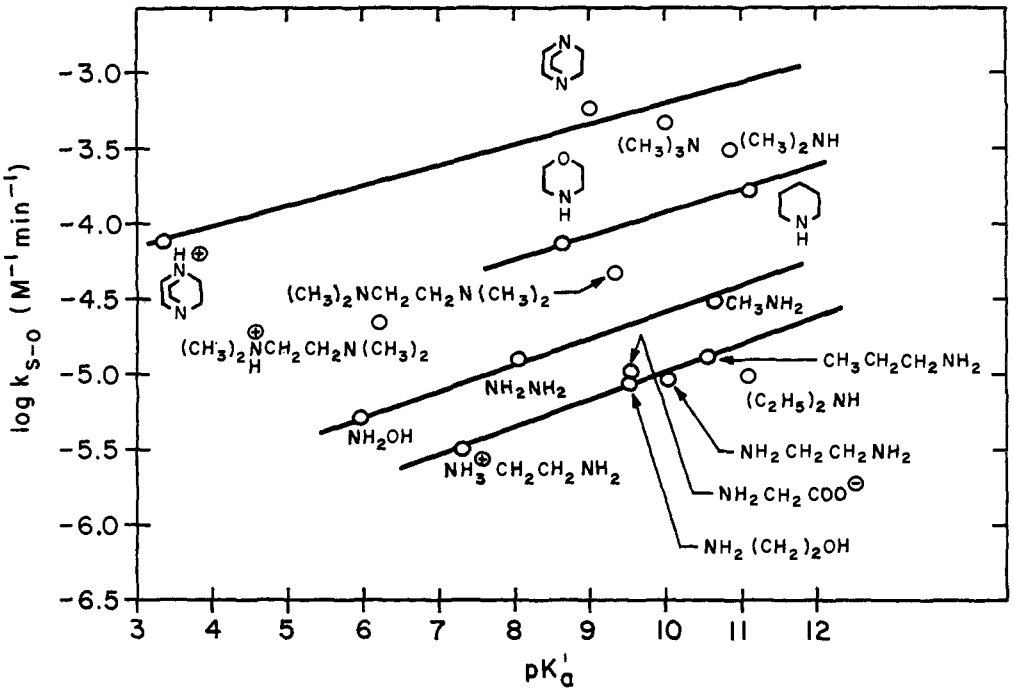


Figure 4. Plot of the logarithm of the second-order rate constant (M^{-1} min^{-1}, 35°, μ = 1.0) for S–O bond cleavage *vs.* pK_a'. Rate k_{S-O} for tertiary amines assumed equal to calculated second-order rate constant. (See ref 11.) Rate constants for diamines statistically corrected.

served in other systems;[5,31] this is expected on the basis of the low pK_a' of SO_4^{2-} and the improbability that its expulsion would become rate determining. Our primary interest, however, is focused on the amine reactions that lead to S–O bond cleavage. The rate constants for the latter reaction are plotted in the Brønsted fashion in Figure 4.[32] The Brønsted slopes do not differ significantly from Figure 3 due to compensation arising from product composition.

The observed order of reactivity—tertiary > secondary > primary amines—is similar to that observed in other nucleophilic reactions, *e.g.*, *p*-nitrophenyl phosphate. The separation of reactivity is a reflection of the inadequacy of correlating nucleophilicity with thermodynamic basicity, the latter quantity being greatly sensitive to the type of amine due to their varying ability to hydrogen bond with the solvent.[33] This factor which tends to increase the pK_a of primary amines would be of diminished importance in altering nucleophilicity.

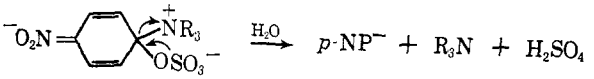
Several experiments were designed to test the electrostatic effect of the negatively charged sulfate on nucleophilic reactivity. As implied by the reactivity of hydroxide and fluoride anions these electrostatic repulsive forces are penetrable both for sulfate monoanions and phosphate dianions.[7] The identical reactivity of glycine anion and ethanolamine toward *p*-nitrophenyl sulfate may indicate the rather short-range nature of these forces in amine–sulfate transition states. Experimental efforts to unambiguously demonstrate electrostatic acceleration as might be possible for the monocations of ethylenediamine, N,N,N′,N′-tetramethylethylenediamine, and triethylenediamine did not reveal monocation reactivity greater than the diamine free base species nor any significant deviation from values predicted from log k_2 *vs.* pK_a' plots. In contrast the monocation of N,N,N′,N′-tetramethylethylenediamine is more reactive than the free-base species against *p*-nitrophenyl phosphate.[7] Thus it appears that electrostatic acceleration is not a significant factor in amine–sulfate ester reactions, a result anticipated because of the net reduction of one negative charge in proceeding from the phosphate to the sulfate ester. Moreover, the significant reactivity of the monocation species of triethylenediamine, for which hydrogen-bonded assistance of the nucleophilic attack is sterically prevented, effectively rules against similar catalysis in other diamines.

The reactivity of nucleophilic reagents toward *p*-nitrophenyl sulfate is somewhat sensitive to steric effects although the latter are not dominant—the order of reactivity being tertiary > secondary > primary amines. Substitution α to the nitrogen retards reactivity in the order dimethylamine > piperidine > diethylamine (the latter actually falling below the line for primary amines). Similarly methylamine is more reactive than *n*-propylamine while Tris is unreactive. Trimethylamine exhibits high reactivity; triethylamine is unreactive. It is of interest to note that hydroxylamine and hydrazine, amines which exhibit the "α-effect"[34a] and generally are 10^1–10^2-fold more reactive than predicted on basis of their pK_a,[34b] are only 2–4-fold more reactive toward the sulfate ester. The nucleophilic site of hydroxylamine was not determined.

The most remarkable feature of the reactions of

(31) J. F. Bunnett and R. H. Garst, *J. Am. Chem. Soc.*, 87, 3875, 3879 (1965); J. F. Bunnett and C. Bernasconi, *ibid.*, 87, 5209 (1965).

(32) Product isolation experiments do not rule out the possibility that nucleophilic-catalyzed hydrolysis may arise *via* breakdown of an amine–sulfate ester adduct in the fashion



However, this is unlikely if pK_a' is an index of leaving group qualities.

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(34) (a) J. O. Edwards and R. G. Pearson, *ibid.*, 84, 16 (1962); (b) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, Inc., New York, N. Y., 1966, Chapter 1.

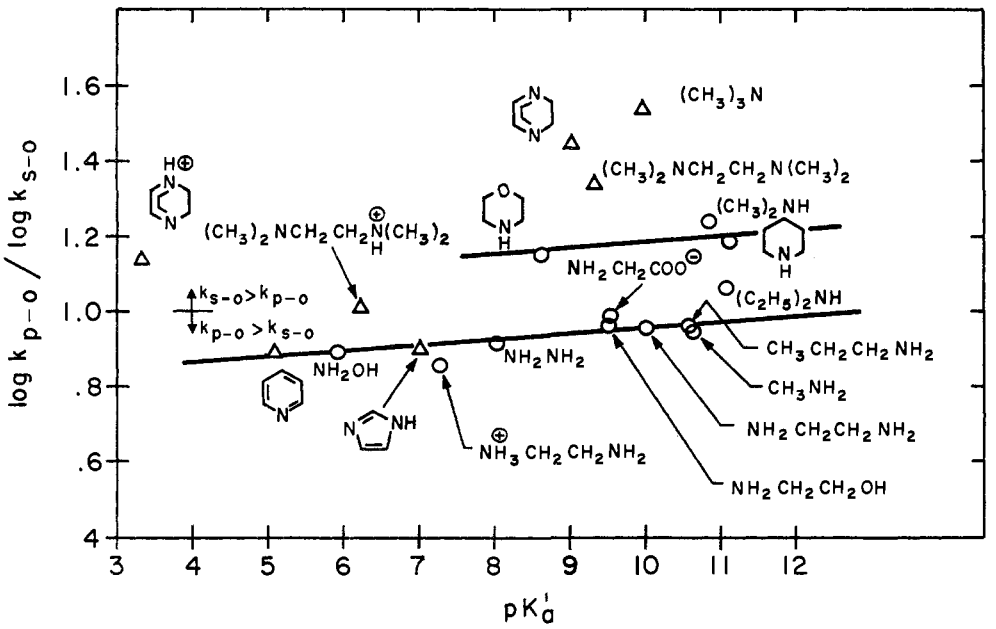

Figure 5. Plot of the ratio of the logarithms of the second-order rate constants (M^{-1} min^{-1}) for P–O (μ = 1.0, 39°)[7] and S–O (μ = 1.0, 35°) bond cleavage *vs.* pK_a'. Rate constants for diamines statistically corrected. Esters are *p*-nitrophenyl phosphate dianion and sulfate monoanion, respectively.

amines with *p*-nitrophenyl sulfate is the lack of sensitivity of the reaction rates for S–O fission to the basicity of the nucleophile (β = 0.20). Similar small dependencies on basicity have been established for the reaction of substituted pyridines with phosphoramidate (β = 0.22),[35a,b] allyl bromide (β = 0.37),[36] and ethyl methanesulfonate (β = 0.11),[37] and reactions of amines with *p*-nitrophenyl phosphate (β = 0.13).[7] In contrast, reactions of nucleophiles with acyl groups or phosphate triesters exhibit a marked dependency on basicity (β = 0.80).[34b,38] The small values of β have been associated with transition states involving little bond formation between the incoming nucleophile and substrate. Such a transition state rationalizes, for example, the high reactivity of trimethylamine which in general is sterically hindered in nucleophilic displacement reactions[34b] and the negligible effect of charge on the nucleophilicity of glycine anion. Consequently the sulfate–amine reactions may derive much of their driving force from redistribution of electron density from the sulfate to the *p*-nitrophenolic moiety; indeed reaction of dimethylamine (0.8 *M*, 35°) with phenyl sulfate was not detectable.[39] Assuming the maximum detectable second-order rate constant under our conditions is $\sim 10^{-7}$ M^{-1} min^{-1}, a minimum ρ value of *ca.* +4 may be calculated. It is quite probable that low β and high ρ values will be distinguishing characteristics of reactions kinetically bimolecular but possessing some features of unimolecular reactions. A similar state of affairs has been postulated by Jencks as prevailing in nucleophilic reactions of phosphoramidate and phosphate monoester monoanions.[7,40] Westheimer[41] has noted an identical situation in nucleophilic reactions with N,N,N′,N′-tetramethylphosphorodiamidic chloride. It is doubtful, therefore, that nucleophilic groups will function as effective catalysts in the sulfatase enzymes without enzyme-mediated alterations on the activation energy of the nucleophilic pathway.

Implied in the above discussion is the close resemblance between nucleophilic reactions of phosphate monoester dianions and sulfate monoester monoanions. The striking near identity of the second-order rate constants for nucleophilic attack by amine on phosphorus or sulfur (*p*-nitrophenyl esters) and the insensitivity of reactivity to pK_a' is graphically illustrated in Figure 5. The plot is essentially a ratio of free energies of activation for the two systems (phosphate reactions: 39°, μ = 1; sulfate reactions: 35°, μ = 1) against pK_a'. Primary amines, amines capable of exhibiting an "α-effect," pyridine, and imidazole fall close to values of unity. The small positive slope indicates a slightly greater sensitivity of reactivity to basicity for the sulfate–amine reactions, a conclusion that may be deduced from the above β values. Electrostatic acceleration which is of some importance in the phosphate–amine reactions is revealed by points for diamine monocations being less than unity. Secondary and tertiary amines appear to be more reactive toward *p*-nitrophenyl sulfate; this reflects the more limited range of reactivity for amines toward *p*-nitrophenyl phosphate. The exact reasons for this behavior are not clear; sulfate has a greater degree of π bonding than phosphate,[42,43] but why this (if this is the rational) should be reflected in the Brønsted intercepts and not the slopes is not known. Moreover, if propylamine and piperidine are assumed to be representative examples, the rate acceleration observed for the former is mainly caused by an entropy effect while the latter arises from

(35) (a) J. D. Chanley and E. Feageson, *J. Am. Chem. Soc.*, 85, 1181 (1963); (b) W. P. Jencks and M. Gilchrist, *ibid.*, 87, 3199 (1965).
(36) K. Clarke and K. Rothwell, *J. Chem. Soc.*, 1885 (1960).
(37) R. F. Hudson and R. J. Withey, *ibid.*, 3513 (1964).
(38) See ref 34b, Vol. II, Chapter 6.
(39) S. J. Benkovic, unpublished results.
(40) W. P. Jencks and M. Gilchrist, *J. Am. Chem.. Soc.*, 86, 1410 (1964).
(41) P. S. Traylor and F. H. Westheimer, *ibid.*, 87, 553 (1965).
(42) L. Pauling, *J. Phys. Chem.*, 56, 361 (1952).
(43) E. A. Lucken and M. A. Whitehead, *J. Chem. Soc.*, 2459 (1961).

an enthalpy effect. Thus there appears a tendency toward compensation, demanding that solvation effects must be considered in explaining the phenomena.[34b]

The similarity between phosphate and sulfate esters as revealed in this study does not allow one to speculate on biological mechanisms. Nevertheless, it is intriguing to ask whether there is any dual functionality in the sulfatase and phosphatase hydrolytic enzymes; if none, what mechanistic differences prevail in the enzymic reactions? Of equal importance are model studies directed at uncovering such differences. Our future research is in both directions.

Acknowledgment. This investigation was supported by Public Health Research Grant GM 13306-01.

Studies on Sulfate Esters. II. Carboxyl Group Catalysis in the Hydrolysis of Salicyl Sulfate

Stephen J. Benkovic

Contribution from the Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802. *Received August 8, 1966*

Abstract: The pH–rate profile for the hydrolysis of salicyl sulfate reveals hydronium ion and intramolecular carboxyl group catalysis. In contrast the rate of hydrolysis of *p*-carboxyphenyl sulfate is accelerated only by hydronium ion. The acid-catalyzed hydrolysis of aromatic sulfates is viewed as an A1 mechanism on the basis of activation entropies and D_2O solvent isotope and substituent effects. Intramolecular carboxyl group participation is discussed in terms of general acid or specific acid nucleophilic catalysis and compared to other examples involving similar behavior including salicyl phosphate.

In our continuing investigations of the hydrolytic chemistry of sulfate esters it became desirable to investigate a possible intramolecular system since such would more closely resemble an enzyme–substrate complex and experimentally might provide rates of greater magnitude. Suitable juxtaposition of a possible catalytic group might then allow observation of general acid–base catalysis or its kinetic equivalent, even though such behavior was not observed in bimolecular systems.[1] Moreover, it is of interest to determine the boundaries on the apparent analogies between the hydrolytic mechanisms of phosphate and sulfate esters.

Experimental Section

Materials. The *o*- and *p*-carboxyphenyl sulfates were prepared by the method of Burkhardt, *et al.*,[2a,b] modified to yield the dipotassium salt.[3] Kinetic solutions were prepared from freshly boiled distilled water. Reagent grade salts and acids (Fisher, Baker) were used without further purification. Deuterium oxide (99.9%) was obtained through the courtesy of Dr. R. A. Olofson of this department. Deuteriochloric acid was prepared from deuterium oxide and anhydrous hydrogen chloride.

Apparatus. All instrumentation was identical with that previously described.[4] Kinetic runs of greater than 12-hr duration were carried out in Kimax (No. 45066) screw-cap tubes maintained at reaction temperature by immersion in a circulating water bath. Shorter runs were conducted in thermostated, 2-cm stoppered cuvettes.

Kinetics. The hydrolysis of salicyl sulfate was monitored at 296 mμ following the increase in absorption due to salicylic acid formation. Reactions were initiated by the addition of 1 ml of salicyl sulfate solution ($3–7 \times 10^{-4}$ *M*; μ = 1.0, KCl) to 9 ml of the buffer solution (0.2 *M* in total buffer species; μ = 1.0, KCl), solutions having been preequilibrated at the desired temperature. Buffers employed were acetate (pH 3.6–5.3) and formate (pH 2.9–3.6); hydrochloric acid was used to prepare solutions of pH <2.5. No buffer effects (tenfold dilution) were noted. The pH of the kinetic runs was measured at 35° upon initiation and after completion of the runs; those exhibiting pH drift greater than ±0.02 unit were discarded.[5a] Identical techniques were employed for the investigation of the hydrolysis of *p*-carboxyphenyl sulfate with *p*-hydroxybenzoic acid formation being monitored at 282 mμ.

The observed first-order rate constants for hydrolysis of salicyl sulfate were calculated from slopes of plots of $\log [OD_\infty/(OD_\infty - OD_t)]$ against time for solutions of pH <4.3 followed to at least one half-life (Figure 1). Kinetic runs of pH <2.8 were followed to OD_∞; OD_∞ for those of pH >2.8 were computed from OD_∞ values obtained for reaction solutions at pH <1.0 related by means of standard curves of OD_{296} *vs.* salicylic acid concentration at the corresponding pH values. Rate constants for the hydrolysis of salicyl sulfate at pH >4.3 were obtained from slopes of OD_∞ *vs.* time divided by the computed OD_∞. Duplicate runs agreed within ±5% at pH >2; ±3%, pH <2.

The observed first-order rate constants for the hydrolysis of *p*-carboxyphenyl sulfate were calculated from slopes of plots of $\log [(OD_\infty - OD_t)/(OD_\infty - OD_t)]$ (due to initial absorbance by substrate) against time for kinetic runs of pH <1.6; those at higher pH were calculated as initial rates by the method discussed above.

The observed first-order rate constants for the hydrolysis of salicyl and *p*-carboxyphenyl sulfates, calculated as above, were determined in deuterium oxide (35°, μ = 1.0, KCl), the desired pD being obtained through addition of standardized DCl; the corrected pD was calculated from the formula of Fife and Bruice.[5b] Precautions were taken that the solutions remained anhydrous.

Products. Spectrophotometric scanning (225–335 mμ) of salicyl sulfate kinetic solutions at pH's where a greater percentage of the reaction proceeds *via* carboxyl-group catalysis failed to disclose accumulation of any reaction intermediates. The ultraviolet spectra at OD_∞ is identical with that of salicylic acid. The absence of reaction intermediates at significant concentrations is also implied by the lack of any observable lag phase in the kinetics.

Preparative runs (0.06 and 0.1 *M* in salicyl and *p*-carboxyphenyl

(1) J. L. Kurz, *J. Phys. Chem.*, 66, 2239 (1962).
(2) (a) G. N. Burkhardt and A. Lapworth, *J. Chem. Soc.*, 684 (1926); (b) G. N. Burkhardt, C. Horrex, and D. I. Jenkins, *ibid.*, 1649 (1936).
(3) M. Loeper, J. Cottet, and J. Parrod, *Compt. Rend. Soc. Biol.*, 135, 917 (1941).
(4) S. J. Benkovic and P. A. Benkovic, *J. Am. Chem. Soc.*, 88, 5504 (1966).
(5) (a) No correction was applied for the small error incurred in reading pH values between pH 0 and 1. (b) T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, 65, 1079 (1961).