$3\text{-}NO_2$ group. It can be seen that **4** is far less stable than **5** and **3**, which is attributed largely to incomplete localization of the negative charge donated by $^-OCH_3$.

Experimental Section

General Procedures. Melting points are uncorrected. Elemental analyses were performed at the Microanalytical Center of Gunma University. Visible absorption spectra were measured with a Hitachi-124 UV–vis spectrophotometer. ^1H NMR spectra were taken with a Varian A-60 spectrometer. All reagents were purified by repeated recrystallization or by distillations.

Materials. The synthetic sequence for **2** is as shown in Scheme I.

4-Phenanthrol (10). Compound **10** was prepared by the sequence in Scheme I according to the method of Haworth.[17] Yields: **7**, 49%; **8**, 72%; **9**, 60%; **10**, 50%. Mp: **7**, 166.5–168.5 °C (lit.[17] mp 171–173 °C); **8**, 92.0–95.0 °C (lit.[17] mp 94–95 °C); **9**, 66.5–67.0 °C (lit.[17] mp 69 °C); **10**, 112.0–113.5 °C (lit.[17] mp 112.0–113.5 °C).

1,3-Dinitro-4-phenanthrol (11). To a stirred solution of 15 g (0.078 mol) of **10** and 900 mL of acetic acid was added dropwise 14.1 g of 70% HNO_3 (0.157 mol) at 60 °C with stirring, and the mixture was stirred for an additional 3 h. The mixture was poured onto ice water, and the precipitate was filtered, dried, and subjected to column chromatography (silica gel–benzene). When the benzene fraction was concentrated by distillation, 5.6 g (23%) of pure **11** crystallized: mp 189–190 °C (lit.[17] mp 205–208 °C).

4-Chloro-1,3-dinitrophenanthrene (12). A mixture of 5.6 g (0.02 mol) of **11**, 5.9 g (0.04 mol) of *N,N*-diethylaniline, and 3.8 g (0.02 mol) of *p*-toluenesulfonyl chloride was stirred at 80–90 °C for 8 h and then cooled to room temperature. After 300 mL of 1 N HCl was added, the mixture was stirred well, filtered, and dried. The residue was subjected to column chromatography (silica gel–benzene). After the benzene fraction was concentrated, 5.2 g (87%) of pure **12** crystallized out: mp 175.5–176.7 °C. Anal. Calcd for $C_{14}H_7ClN_2O_4$: C, 55.55; H, 2.33; N, 9.26. Found: C, 55.43; H, 2.34; N, 9.04.

1,3-Dinitro-4-phenanthryl Methyl Ether (2). A methanolic $KOCH_3$ solution (0.344 g, 0.0049 mol) was added dropwise to a 30-mL Me_2SO solution of 1 g (0.00328 mol) of **12** at room temperature; the mixture was stirred for 2 h and then poured onto 200 mL of ice water containing 2.5 mL of 2 N HCl. The precipitate was filtered, dried, and subjected to column chromatography (silica gel–benzene). After the benzene fraction was concentrated, recrystallization from ligroin yielded 0.82 g (83%) of pure **2**: mp 155.5–156 °C. Anal. Calcd for $C_{15}H_{10}N_2O_5$: C, 60.41; H, 3.38; N, 9.39. Found: C, 60.20, H, 3.36; N, 9.18.

Rate Measurement. The increase in the absorbance of **3** compared with that of **2** was measured at constant ionic strength ($NaClO_4$) with a molar excess of $NaOCH_3$ at 25 °C with a thermostated Union stopped-flow spectrophotometer RA-401 (Union Giken) in order to estimate the pseudo-first-order rate constant (k_ψ).

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[17] Haworth, R. D. *J. Chem. Soc.* **1932**, 1125.

Proton Inventory of Phosphate Monoanion Catalyzed Hydrolysis of *S*-Ethyl Trifluorothioacetate

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Dihydrogen phosphate monoanion ($H_2PO_4^-$) catalyzes the hydrolysis of *S*-ethyl trifluorothioacetate. In sodium dihydrogen phosphate solutions at pH 4.2 the only catalysts of concern are H_2O and $H_2PO_4^-$. The kinetic solvent isotope effects (k_H/k_D) on the water and $H_2PO_4^-$ catalyzed hydrolysis are 2.86 and 2.00, respectively. The observed isotope effects suggest a general base mechanism for both water and $H_2PO_4^-$ catalyzed hydrolysis. In mixtures of H_2O and D_2O, the water catalyzed hydrolysis shows a nonlinear dependence on the atom fraction of deuterium, *n*. This nonlinear dependence is consistent with a three-proton model for the transition state. The $H_2PO_4^-$ catalyzed hydrolysis shows a linear dependence on *n*. This linear dependence is consistent with a one-proton model for the transition state in which a single proton is responsible for the entire observed solvent isotope effect.

The general base catalyzed hydrolysis of *S*-ethyl trifluorothioacetate is well established. Acetate ion, imidazole, and phosphate mono- and dianions are all known to function as general bases in the above reaction.[1] In an effort to define the transition state structures for such a general base catalyzed reaction in greater detail, we have conducted a proton-inventory study of the phosphate monoanion catalyzed hydrolysis of ethyl trifluorothioacetate. The results are reported herein.

Results

The pseudo-first-order rate constants for the monoanion catalyzed hydrolysis were measured in sodium dihydrogen phosphate solutions at pH 4.2. At this pH the only catalysts we need to consider are $H_2PO_4^-$ ion and water. The water reaction is independent of pH between pH 2 and 7, and phosphate exists exclusively in the monoanionic form at this pH. The three pK_a values for H_3PO_4 are 2.12, 7.21, and 12.32, respectively.[2] Table I lists the observed first-order rate constants for different phosphate mo-

Table I. First-Order Rate Constants for the Hydrolysis of *S*-Ethyl Trifluorothioacetate in NaH_2PO_4 Solutions at pL 4.20[a] at Different Atom Fractions of Deuterium at 30 ± 0.1 °C (Ionic Strength = 1.0 M with NaCl)

n, atom fraction deuterium	10^3k_{obsd},[b] s^{-1} (NaH_2PO_4, M)
0	11.2 (0.50), 11.0 (0.46)[c], 10.5 (0.40), 10.1 (0.37),[c] 9.6 (0.30), 9.6 (0.28), 9.3 (0.20), 9.0 (0.18),[c] 8.5 (0.10), 8.5 (0.09)[c]
0.245	9.2 (0.50), 8.7 (0.40), 8.0 (0.30), 7.8 (0.20), 6.8 (0.10)
0.495	7.2 (0.50), 6.9 (0.40), 6.4 (0.30), 5.9 (0.20), 5.3 (0.10)
0.800	5.4 (0.50), 5.1 (0.40), 4.3 (0.20), 3.8 (0.10)
0.990	4.3 (0.50), 4.0 (0.40), 3.8 (0.30), 3.3 (0.20), 3.0 (0.10)

[a] pL = pH or pD; reported values are uncorrected pH meter readings. [b] Mean of 3–5 determinations, reproducible within about 3%. [c] Rate constants measured at pH 3.15.

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[1] Fedor, L. R.; Bruice, T. C. *J. Am. Chem. Soc.* **1965**, *87*, 4138.

0022-3263/85/1950-5108$01.50/0

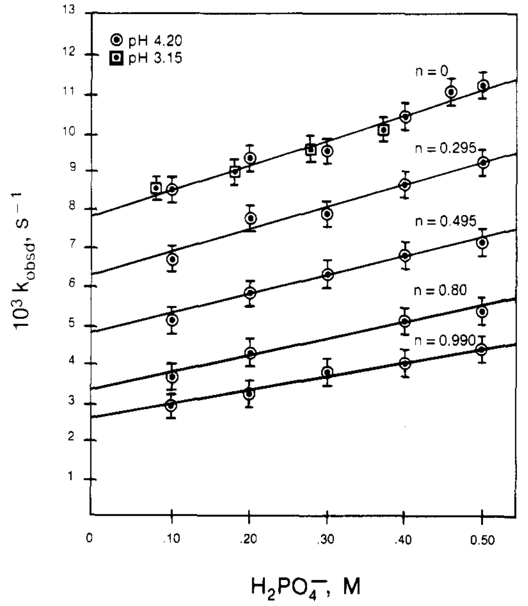

Figure 1. Linear dependence of k_{obsd} on phosphate monoanion concentration at different atom fractions of deuterium (n). Error limits are standard deviations.

Table II. Second-Order Rate Constants for the $H_2PO_4^-$ Catalyzed Hydrolysis of S-Ethyl Trifluorothioacetate as a Function of Atom Fraction of Deuterium (n) at 30 ± 0.1 °C

n	k_2, $M^{-1}\ s^{-1}$	k_2, $M^{-1}\ s^{-1}$ (calcd)[a]
0	6.60 ± 0.20	6.60
0.245	5.70 ± 0.16	5.79
0.495	4.80 ± 0.20	4.97
0.800	4.00 ± 0.15	3.96
0.990	3.25 ± 0.15	3.33

[a] Based on a one-proton model with ϕ = 0.50.

noanion concentrations as a function of atom fraction of deuterium, n, in the solvent. The observed first-order rate constants vary linearly with phosphate monoanion concentration (Figure 1). Such linear variation is consistent with eq 1 where k_0 is the rate constant for the water catalyzed hydrolysis and k_2 is the second-order rate constant for the monoanion catalyzed hydrolysis.

$$k_{obsd} = k_0 + k_2(H_2PO_4^-) \qquad (1)$$

Figure 1 also depicts the linear variation of the observed pseudo-first-order rate constants with the monoanion concentration in 100% D_2O and in different mixtures of H_2O and D_2O. Equation 1 is thus obeyed in solutions containing different atom fractions of deuterium, n. The second-order rate constant k_2 for the phosphate monoanion catalyzed hydrolysis is 0.0066 $M^{-1}\ s^{-1}$. The value for k_2 reported by Fedor and Bruice is 0.0083 $M^{-1}\ s^{-1}$. In view of this minor discrepancy between our value and Fedor's value, we determined k_2 at a second pH value. The value of k_2 at pH 3.15 was found to be 0.0066 $M^{-1}\ s^{-1}$. The k_{obsd} values obtained at pH 3.15 fit the same line that describes the variation of k_{obsd} with $[H_2PO_4^-]$ at pH 4.2 as shown in Figure 1. The solvent isotope effect on k_2 is 2.00. The first-order rate constants k_n for the water catalyzed hydrolysis in solvents containing different atom fractions of deuterium are not identical with the ones reported in literature,[3] but compare favorably with them. Table II lists k_2, the second-order rate constant for the phosphate monoanion catalysis, as a function of n. Figure 2 shows the variation of k_2 with n. It is apparent from Figure 2 that k_2 varies linearly with n.

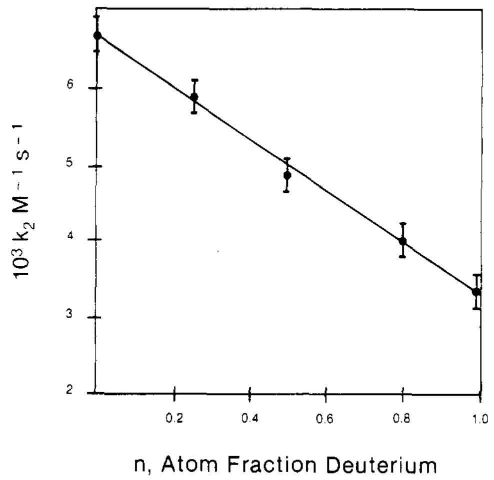

Figure 2. Linear dependence of k_2, the rate constant for phosphate monoanion catalyzed hydrolysis, on atom fraction of deuterium (n). The solid line is generated for a one-proton model, $k_n = k_0(1 - n + 0.50n)$. Error limits are standard deviations.

Discussion

A proton-inventory experiment involves rate measurements in H_2O, D_2O, and mixtures of H_2O and D_2O. This technique can be used to obtain information on transition state structures in both enzyme model and actual enzymic reactions.[4] Several examples of the application of this technique to elucidate transition state structures are reported in literature.[5] An abridged version of the theory is given below. The measured rate constant k_n, in a solution of atom fraction of deuterium n, is related to the rate constant k_0 in pure H_2O by eq 2.

$$k_n = k_0 \prod_i^{TS}(1 - n + n\phi^*_i) / \prod_j^{RS}(1 - n + n\phi_j) \qquad (2)$$

In eq 2, ϕ^* and ϕ are the isotopic-fractionation factors for each exchangeable hydrogenic site present in the transition state and the reactant state respectively. The isotopic-fractionation factor ϕ for any given site is simply the ratio of the preference of the site for deuterium compared to protium relative to the preference of a solvent site for deuterium compared to protium. This is mathematically expressed by eq 3.

$$\phi_k = [(D)/(H)]k/[(D)/(H)]_{solvent} \qquad (3)$$

For sites that show a greater preference for protium than deuterium relative to the solvent, the fractionation factor will be less than unity. If all reactant-state fractionation factors are equal to unity then eq 1 simplifies to eq 4.

$$k_n = k_0 \prod_i^{TS}(1 - n + n\phi^*_i) \qquad (4)$$

Under these conditions the entire solvent isotope effect arises from transition-state hydrogenic sites only. Furthermore, k_n changes linearly with n if only one transition state proton contributes to the entire solvent isotope effect except under some very special situations.[6] If more than

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[2] Bjerrum; Schwarzenbach; Sillen, "Stability Constants of Metal-Ion Complexes", Inorganic Ligands; Chemical Society: London, 1957; Part II.

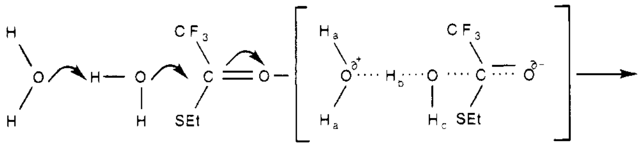
[3] Venkatasubban, K. S.; Davis, K. R.; Hogg, J. L. *J. Am. Chem. Soc.* **1978**, *100*, 6125.

[4] Venkatasubban, K. S.; Schowen, R. L. *CRC Crit. Rev. Biochem.* **1984**, *17*, 1 and references therein. See also: Gapalakrishnan, G.; Hogg, J. L. *J. Org. Chem.* **1984**, *49*, 3161 and references therein.

[5] Schowen, K. B.; Schowen, R. L. *Methods Enzymol.* **1982**, *87C*, 551.

[6] Kresge, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 3065.

Scheme I



one transition-state proton contributes to the overall isotope effect, then a nonlinear "bulging down" plot for k_n vs. n results.

It is apparent from Figure 1 that two proton inventories can be constructed from our data, one for the water catalyzed hydrolysis k_0, first-order rate constant, and one for $[H_2PO_4^-]$ catalyzed hydrolysis k_2, a second-order rate constant. The two inventories are discussed separately below.

Water-Catalyzed Hydrolysis. The proton inventory for the water catalyzed hydrolysis of ethyl trifluorothioacetate has been studied a few years ago by Venkatasubban, Davis, and Hogg in 10^{-3} M HCl/DCl solutions.[3] The data obtained in this study (not shown) is consistent with a three-proton model that was proposed in the above study. This three-proton model is described by eq 5. An appropriate structure for this three-proton transition state is shown in Scheme I.

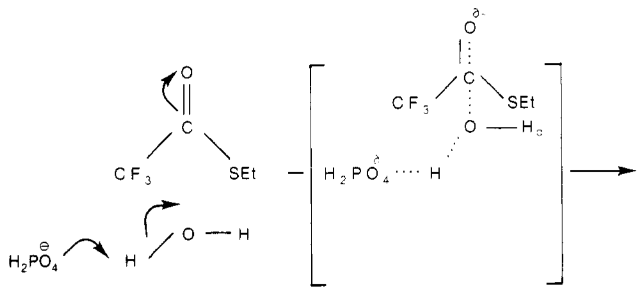
$$(k_n/k_0) = (1 - n + 0.48n)\,(1 - n + 0.86n)^2 \qquad (5)$$

In Scheme I the transition-state proton H_a undergoes minimal bonding changes, proton H_b undergoes major bonding changes, and proton H_c undergoes no bonding changes. Consistent with this, the fractionation factors for H_a, H_b, and H_c protons are 0.86, 0.48, and 1, respectively. The nice fit of our current data to a transition-state model proposed earlier further enhances our confidence in our k_2 values.

$H_2PO_4^-$ Catalyzed Hydrolysis. Figure 2 shows the variation of the second-order rate constant k_2 for dihydrogen phosphate monoanion catalyzed hydrolysis of *S*-ethyl trifluorothioacetate with n. The plot of k_2 vs. n is linear with some scatter. Such a linear plot suggsts that the dihydrogen phosphate catalysis of ethyl trifluorothioacetate hydrolysis involves one proton which is isotopically distinct from water in the transition state. This transition state proton produces an isotope effect $k_H/k_D = 2.00$. A possible transition state structure involving a single proton transfer is shown in Scheme II.

This means that H_d proton in Scheme II has a fractionation factor of unity. This is consistent with a smooth conversion of H_d from a reactant water proton ($\phi = 1$) to a hydroxyl proton ($\phi = 1$) in the tetrahedral intermediate. Such a one-proton transition state has been established for the intramolecular carboxylate catalysis of *o*-(dichloroacetyl)salicylic acid anion hydrolysis[7] and imidazole catalyzed hydrolysis of acetylimidazole and ethyl trifluorothioacetate.[8] The above analysis of our data assumes a unit fractionation factor for $H_2PO_4^-$ ion. This is a reasonable assumption in view of the fact that the fractionation factor for all neutral hydroxyl functional groups like OH and –COOH is 1. We do not expect any extensive solvation for the weakly basic dihydrogen phosphate monoanion and thus there should be no contribution to the fractionation factor from water molecules in the solvation shell like the one observed for hydroxide ion.[9]

Scheme II



We conclude that dihydrogen phosphate monoanion catalyzed hydrolysis of ethyl trifluorothioacetate involves a one-proton solvation bridge as shown in Scheme II.

Experimental Section

Materials. Ethyl trifluorothioacetate was purchased from Aldrich Chemical Co. and was distilled under vacuum before use. NaH_2PO_4 was oven dried before use. Deuterium oxide (99.8%) was purchased from Sigma and was used as obtained. The deuterium content of "100% D_2O" solutions was determined by NMR as described by Schowen.[10] Stock buffer solutions containing 0.5 M sodium dihydrogen phosphate were made up in H_2O and D_2O. The pH (L = H or D) of these solutions was 4.2. The pL values reported here are uncorrected pH meter readings. Ionic strength was maintained at 1.0 M with NaCl.

Kinetics. Sodium dihydrogen phosphate solution was thermostatted in the constant-temperature cell holder of a DMS 90 spectrophotometer at 30 ± 0.1 °C. To initiate the reaction 20 μL of a stock solution of substrate in acetonitrile were injected with an Eppendor pipet. The decrease in the absorbance of ethyl trifluorothioacetate at 243 nm was monitored. The reaction was allowed to proceed for five to ten half-lives. The first-order rate constants were calculated by plotting ln $(A_T - A_\infty)$ vs. time, using a linear least-squares program on an Apple IIe computer.

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Registry No. NaH_2PO_4, 7558-80-7; *S*-ethyl trifluorothioacetate, 383-64-2; deuterium, 7782-39-0.

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(7) Minor, S. S.; Schowen, R. L. *J. Am. Chem. Soc.* **1973**, *95*, 2279. See also: Venkatasubban, K. S.; Hogg, J. L. *Tetrahedron Lett.* **1979**, 4355.

(8) Patterson, J. F.; Huskey, W. P.; Venkatasubban, K. S.; Hogg, J. L. *J. Org. Chem.* **1978**, *43*, 4935.

(9) Gold, V.; Grist, S. *J. Chem. Soc., Perkin Trans. 2* **1972**, *89*.

(10) Schowen, K. B. In "Transition States of Biochemical Processes"; Gandour, R. D., Schowen, R. L., Eds. Plenum: New York, 1978.