Equilibrium Constant for the Hydrolysis of *O,O*-Diethyl Phosphofluoridate[1]

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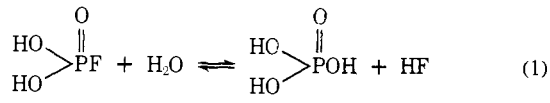
Abstract: The equilibrium constant for the hydrolysis of *O,O*-diethyl phosphofluoridate was determined in acidic solution using butyrylcholinesterase as an analytical reagent to measure the very small equilibrium concentration of *O,O*-diethyl phosphofluoridate. The value of the equilibrium constant for the hydrolysis in terms of the acid species of the reactants, and with water set at unit activity, was 1.1×10^6. This value differs markedly from the equilibrium constant for the hydrolysis of fluorophosphoric acid, 43.

Although compounds containing phosphorus–fluorine linkages are of considerable interest, there is only one compound whose equilibrium constant for hydrolysis (ECH) has been measured. The ECH of fluorophosphoric acid was first measured by Lange[4,5]



(1)

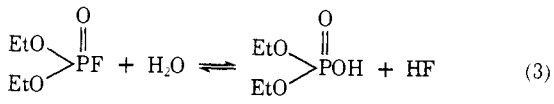
using gravimetric techniques. This work was confirmed using nmr for estimating fluorophosphoric acid.[6] The average value obtained in the latter work was

$$(H_3PO_4)(HF)/(H_2PO_3F) \simeq 43 \qquad (2)$$

but the range was rather large, 16–160.

This value for the ECH is remarkably low (considering the acidity of HF; ECH for acetyl phosphate is on the order of 10^{10}) and has led to the generalization that the P–F bond in similar compounds is exceptionally stable. This result is consistent with the high nucleophilicity of fluoride for phosphorus in phosphates, phosphonic acids, and similar compounds.

The compound *O,O*-diethyl phosphofluoridate (DEFP) is of special interest because it is a potent anticholinesterase and its ECH is necessary for evaluating certain enzymic parameters.



(3)

It might be thought, *a priori*, that the ECH for this compound would be grossly the same as for fluorophosphoric acid. We show in this report that it is in fact very different.

Experimental Section

Materials and Methods. DEFP was synthesized from *O,O*-diethyl phosphochloridate according to the method of Saunders and Stacey.[7] Diethyl phosphoric acid was purchased from the Aldrich Chemical Co. Butyrylthiocholine was purchased from Cal Biochem. Butyrylcholinesterase (horse serum) was purchased from Worthington Biochemical. All other reagents were analytical grade from various sources. Water was double distilled from a quartz still.

The equilibrium concentration of DEFP under acid conditions was determined from aliquots of solutions initially containing 1 *M* $HClO_4$ plus 0.5 *M* NaF and 0.5 *M* diethyl phosphoric acid and from the above solution with 1×10^{-3} *M* DEFP so that equilibrium could be approached from both directions. Excess DEFP hydrolyzed with a first-order rate constant of 0.087 min^{-1} at 25°. The equilibrium concentration of DEFP was many orders of magnitude too low to measure by the usual analytical techniques, but we could measure it without much difficulty by using butyrylcholinesterase. The polypropylene tubes containing the solutions were kept at 25°. Aliquots were removed at various times and diluted tenfold in 0.05 *M* Tris plus 0.2 *M* $CaCl_2$, pH 7.0. The pH was readjusted to 7.0 by quickly adding predetermined quantities of 5 *N* NaOH. The concentration of DEFP in this solution, in which DEFP is relatively stable, was determined by using the enzyme butyrylcholinesterase in two different ways.

1. Rate of Inhibition of Butyrylcholinesterase. Standard curves for the inhibition of this enzyme preparation were obtained by using known concentrations of DEFP that were at least 10 times greater than the enzyme concentration. These curves on a semilog plot were pseudo first order up to about 50% inhibition of the enzyme, and the data from this portion of the curve yielded a second-order rate constant of $8.6 \pm 0.2 \times 10^6$ M^{-1} min^{-1}.

2. Amount of Inhibition of Butyrylcholinesterase. In this method, the diluted and neutralized DEFP solution was added to an approximate twofold excess of enzyme (4.5×10^{-8} to 7.5×10^{-8} *N*) and the final per cent inhibition was determined; 10–20 min were required. Since the reaction between DEFP and enzyme goes to completion, the enzyme is a reagent for the titration of DEFP. The enzyme solution was standardized using known amounts of DEFP.

These two methods in conjunction serve as a partial identification of this anticholinesterase since together they determine the second-order rate constant for inhibition of butyrylcholinesterase by the compound.

Enzymic activity of butyrylcholinesterase was determined according to the basic method of Ellman, *et al.*,[8] but using butyrylthiocholine instead of acetylthiocholine in 0.05 *M* Tris·HCl plus 0.2 *M* $CaCl_2$, pH 8.0 at 25°. Perchloric acid serves two functions. It puts the acids in their acidic forms and it catalyzes the reaction. The diethyl phosphoric acid contained a minute amount of a potent anticholinesterase. This anticholinesterase was readily hydrolyzed in alkali. It was hydrolyzed more slowly in acid. We eliminated this anticholinesterase by allowing a solution of the acid in perchloric acid to stand for 3 days before NaF was added.

Results

Method 1 yielded an equilibrium concentration of $2.8 \pm 0.2 \times 10^{-7}$ *M* DEFP and method 2, $3.2 \pm 0.2 \times 10^{-7}$ *M* DEFP. We have therefore used 3.0×10^{-7} *M* as the equilibrium concentration. We thus obtain

$$((EtO)_2PO_2H)(HF)/((EtO)_2POF) = 8.3 \times 10^5 \qquad (4)$$

(1) This work was supported by Grant No. NS07156, National Institutes of Health.
(2) School of Pharmacy, University of Colorado.
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(4) W. Lange, *Chem. Ber.*, **62B**, 1084 (1929).
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(7) B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 695 (1948).
(8) G. L. Ellman, K. D. Courtney, V. Andres, Jr., and R. M. Featherstone, *Biochem. Pharmacol.*, **7**, 88 (1961).

Table I

Substance	Equilibrium constant for hydrolysis, ECH Acidic species	Anal. concn[a]
O,O-Diethyl phosphofluoridate	8.3×10^5	1.2×10^{15}
O,O-Diethyl *p*-nitrophenyl-phosphate	7.4×10^8	6.7×10^{14}

[a] At pH 7. This quantity is valuable for cholinesterase studies.

(Table I) which is 2×10^4 times greater than the ECH for fluorophosphoric acid. The corresponding analytical equilibrium constant, which uses analytical concentrations rather than species concentrations calculated for pH 7.0 with the dissociation constants $K_a = 4.2 \times 10^{-2}$ for diethyl phosphoric acid[9] and 3.5×10^{-4} for hydrofluoric acid,[10] is 1.2×10^{15}. The corresponding values of ΔF° are -8.0 and -21.0 kcal/mol for ECH and the analytical ECH, respectively.

We hardly need note that the medium necessary for the facile attainment of measurable equilibrium concentrations of DEFP is not ideal and that we have used concentrations in our calculation rather than activities. This same problem applies to the previous measurements of the ECH for fluorophosphoric acid.

Controls without NaF did not produce a significant amount of an anticholinesterase.

Discussion

The ECH of *O,O*-diethyl phosphofluoridate is 2×10^4 times larger than the ECH of fluorophosphoric acid.[11] It does not appear likely that this difference could arise from electronic effects. The most evident electronic effect is the greater electron-donating power of ethyl *vs.* hydrogen. This electronic effect does not appear to be very important because *O,O*-diethyl phosphoric acid is a stronger acid ($pK_a = 1.4$) than phosphoric acid ($pK_a = 2.1$). Solvation effects are seemingly more important. Whatever the explanation may be, it is evident that the generalization of the stability in water of the "P–F bond" in these types of compounds based upon the ECH of fluorophosphoric acid may be overestimated, since fluorophosphoric acid may be unusually stable. On the other hand, it is still true that even *O,O*-diethyl phosphofluoridate is far more stable than might have been anticipated from the pK_a of HF. The only other compound for which data exist that will enable a comparison of ECH is *O,O*-diethyl *p*-nitrophenylphosphate.[12] The reaction of fluoride ion with this compound to *produce DEFP* and *p*-nitrophenolate ion has an equilibrium constant of 0.20. Using the acidic dissociation constant of 3.5×10^{-4} for hydrofluoric acid and 7×10^{-8} for *p*-nitrophenol[10] yields 8.9×10^2 for this equilibrium constant in terms of the acidic species. Finally using our value of 8.3×10^5 for ECH of DEFP yields 7.4×10^8 as the ECH for *O,O*-diethyl *p*-nitrophenylphosphate (6.7×10^{14} using analytical concentrations at pH 7.0). Thus, despite the fact that hydrofluoric acid is 3000 times stronger as an acid than *p*-nitrophenol, the ECH for DEFP is smaller by a factor of 900 than that for *O,O*-diethyl *p*-nitrophenylphosphate.

This question can be examined in a slightly different way. Bromilow, *et al.*,[13] give

$$\log K = -1.16\Delta pK_a$$

for the equilibrium constant for reactions involving a dialkylphosphoryl transfer between oxygen nucleophiles, in which the reaction is written with the nucleophiles as anions. Thus, for an oxygen nucleophile with the same pK_a as hydrofluoric acid the equilibrium constant for the reaction of the anion with *O,O*-diethyl *p*-nitrophenylphosphate would have been less than 10^{-4} rather than 0.2 as was actually found for fluoride ion. Thus the P–F linkage in DEFP is unusually stable relative to a phosphorus–oxygen bond, but the magnitude of this effect is very much smaller than in the case of fluorophosphoric acid.

The effect of alkylating the hydroxyl groups of fluorophosphoric acid is to make the P–F bond less stable toward hydrolysis by a factor of 20,000. Similarly we can say that the effect of substituting fluorine for hydroxyl in *O,O*-diethyl phosphoric acid makes the two alkyloxy groups less stable toward hydrolysis by a factor of 20,000. (This last statement becomes apparent by examining a cycle of the four reactions.)

(9) J. R. Van Wazer, "Phosphorus and Its Compounds," Vol. 1, Interscience, New York, N. Y., 1958, p 364.

(10) "Handbook of Chemistry and Physics," The Chemical Rubber Co., Cleveland, Ohio, 1970.

(11) A statistical factor of 3 is involved, corresponding to the number of hydroxyl groups available for reaction in the two compounds.

(12) Y. Ashani, S. L. Snyder, and I. B. Wilson, submitted for publication.

(13) R. H. Bromilow, S. A. Khan, and A. J. Kirby, *J. Chem. Soc. B*, 1092 (1971).