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Hydrolysis of a Phosphate Diester by Simultaneous Carboxylate and Carboxyl Group Participation in a Rigid System with Kinetically Unfavorable Rotamers Frozen Out

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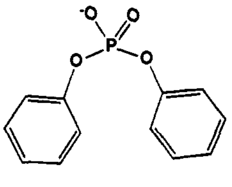
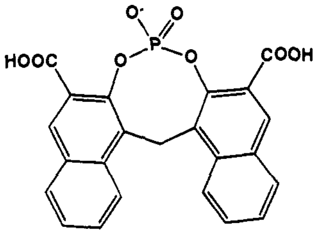
Heightened interest in biochemical and chemical processes for cleavage of the phosphodiester linkages of RNA and DNA fosters attention to catalysis of hydrolysis of phosphodiester linkages in model systems. The anions of simple phosphate diesters {RO-P(O_2^-)-OR′} are exceedingly stable toward hydrolysis in water. The rate constant for the hydrolysis of diphenyl phosphate (**I**) has been estimated to be ca. 1.2×10^{-10} s^{-1} at pH 7.0 and 100 °C ($\sim 4 \times 10^{-12}$ s^{-1} at 50 °C), corresponding to a $t_{1/2}$ of nearly 180 years.[1] We report now that the rate constant for hydrolysis of **II** exceeds that for **I** by a factor of 10^9. In the conversion of **I** to **II**, there is added an *o*-CO_2^- nucleophile and an *o*-CO_2H general acid along with a methylene bridge, etc., to hold the structure in an attack conformation.[2]



I



II

The time course for the hydrolysis of **II** was followed between pH 2.0 and 7.5 by reverse phase HPLC at 40 °C. The first peak (t_R 5.1 min) was a composite of two peaks, the second peak was the final product pamoic acid (t_R 5.5 min), and the third peak (t_R 5.7 min) was **II**. Thus, intermediate(s) are formed between **II** and pamoic acid. These were identified by following the hydrolysis of **II** by ^{31}P NMR. Aside from the major ^{31}P NMR signals of **II** (−8.45 ppm) and the minor signal of $H_2PO_4^-$ (−0.66 ppm), two other signals are observed at −11.18 ppm and at −3.22 ppm with comparable line broadenings ($\nu_{1/2} = 2$ and 4 Hz, respectively). The signal at −11.18 ppm is assigned to the cyclic six-membered acyl phosphate intermediate (**III**[4] in Scheme 1) while the signal at −3.22 ppm is assigned to the phosphate monoester intermediate, **IV** (on the basis that we observe much the same placement of peak with an authentic sample of the phosphate monoester of salicylic acid). By ^{31}P NMR, **II** disappears in a first-order process accompanied by the appearance of **III**, which then decays such that one observes **IV**, which gives way to $H_2PO_4^-$. Incorporation of ^{18}O into phosphate was observed in the course of the hydrolytic reaction as determined from the ^{31}P chemical shifts in the H_3PO_4 product [$D_2O/H_2{}^{18}O$ (1:1) at 25 °C]. The ^{31}P signal at around 0 ppm contained three peaks distanced by 0.023 and 0.015 ppm, respectively, in a ratio of peak integrals of 2:3:1 (no ^{18}O:one ^{18}O:two ^{18}O) showing incorporation of both one and two ^{18}O atoms in the H_3PO_4.[5] The ^{13}C NMR spectrum of the pamoic acid product shows no ^{18}O incorporation[8] as evidenced by a single sharp ^{13}C carboxyl peak at 178.09 ppm. The HPLC and NMR results [both ^{31}P on the partially hydrolyzed **II** and ^{31}P on the H_3PO_4 product obtained in $D_2O/H_2{}^{18}O$ (1:1)] establish the mechanism of the hydrolysis of **II** (Scheme 1).

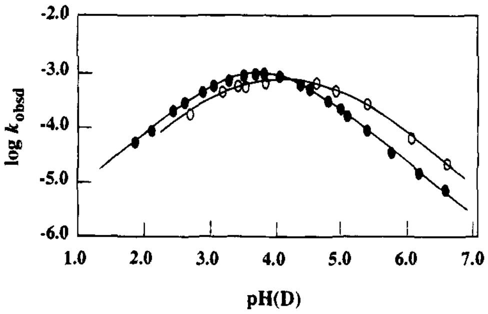
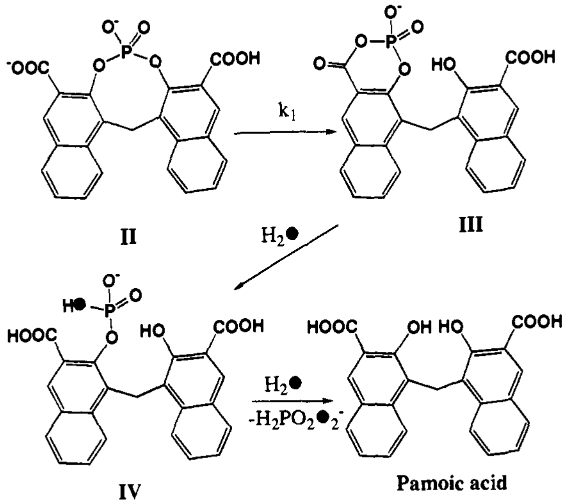


Figure 1. Plot of k_{obsd} for the hydrolysis of **II** vs pH(D), filled (open) circles, in H_2O (D_2O) (μ = 1.0) at 50 °C.

Scheme 1



The pseudo-first-order rate constants (k_{obsd}, s^{-1}) for the disappearance of **II** in both H_2O and D_2O, at given values of constant pH(D) (between 2 and 7), were determined by spectrophotometric measurements of the change in absorbance at 250 nm with time (50 °C, μ = 1.0 with KCl). The plots of $k_{obsd}{}^H$ and $k_{obsd}{}^D$ *vs* pH = pD (Figure 1) are quantitatively fitted by eq 1, where $a_{H(D)}$ represent the activities of H_3O^+ and D_3O^+ and K_{a1} and K_{a2} are the kinetically apparent first and second carboxylic acid dissociations of **II**. Equation 1 was derived from

$$k_{obsd} = \frac{k_1 K_{a1} a_H}{K_{a1} K_{a2} + K_{a1} a_H + a_H{}^2} \qquad (1)$$

a material balance in the ionic species of **II** and the assumption that the reactive species is characterized by *o*-CO_2^- and *o*-CO_2H.

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(1) Kirby, A. J.; Younas, M. J. *J. Chem. Soc. B* **1970**, 510.

(2) We have recently shown that the $\Delta G^{\ddagger}$ for intramolecular nucleophilic attack is directly proportional to the logarithm of the probability that the compound exists in an "attack conformation" (see ref 3).

(3) Lightstone, F. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 10789.

(4) Six-membered cyclic phosphates exhibit ^{31}P chemical shifts between −4 and −14 ppm (see refs 5–7).

(5) Gorenstein, D. G. In *Phosphorus-31 NMR*; Gorenstein, D. G., Ed.; Academic Press: New York, 1984; pp 7–36.

(6) Gallagher, M. J. In *Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis*; Verkade, G., Quin, L. D., Eds.; VCH: Deerfield Beach, FL, 1987; pp 297–329.

(7) Munoz, A.; Gallagher, M.; Klaebe, A.; Wolf, R. *Tetrahedron Lett.* **1976**, *9*, 673.

(8) A value of $\Delta\delta$ = 0.02–0.03 ppm is common for the ^{13}C chemical shifts of carboxylic acids containing an ^{18}O (see ref 9).

(9) O'Leary, M. H.; Hermes, J. D. *Anal. Biochem.* **1987**, *162*, 358.

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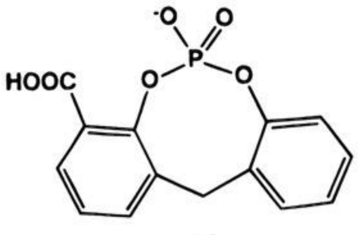
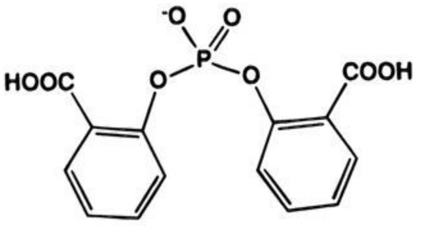
Values of k_{obsd} at various pH levels were found to be insensitive to changes in the concentrations of buffers employed [H_3O^+/H_2O, HCO_2H/HCO_2^-, $(CH_3)_2As(O)OH/(CH_3)_2As(O)O^-$, $(HOCH_2)_3CNH_3^+/(HOCH_2)_3CNH_2$]. The deuterium solvent kinetic isotope effect (k_1^H/k_1^D) is <2, and the value of ΔS° [−3.9 eu] is in accord with a rate-determining unimolecular reaction.

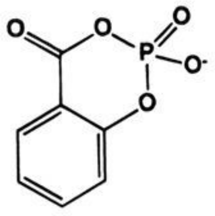
The rate constant (k_1) for the hydrolysis of **II**, with one *o*-carboxyl group ionized and the other not [1.8×10^{-3} s^{-1} at 50 °C], exceeds that for diphenyl phosphate (**I**) by $\sim 10^9$ and **V** by 10^4. [In a previous study with **VI**, it was stated[10] that the second carboxyl group is responsible for an only 4-fold rate enhancement (see ref 11)]. The unique role of both *o*-CO_2^- and *o*-CO_2H *functionalities in the hydrolysis of* **II** *cannot be* questioned. The o-CO_2^- functionality undoubtedly acts as an intramolecular nucleophile. The role of the *o*-CO_2H must be that of a general acid. The possibilities are as follows: (i) protonation of the oxygen of $-(PO_2^-)-$ if nucleophilic attack is important and departure of the leaving group is not (**VIII**);[12] (ii) protonation of the leaving oxygen if nucleophilic attack is advanced and departure of the leaving group is rate limiting (**IX**);[12] and (iii) formation of a bifurcated hydrogen bond between the *o*-CO_2H hydrogen and an oxygen of $-(PO_2^-)-$ as well as the leaving oxygen if nucleophilic attack and leaving-group departure are well coordinated. Cancellation of the negative charge of the diester $RO(PO_2^-)OR'$ by protonation or ligation has been predicted[13,14] (and shown[15]) to increase the rate constant for nucleophilic attack on phosphorus by $>10^4$.



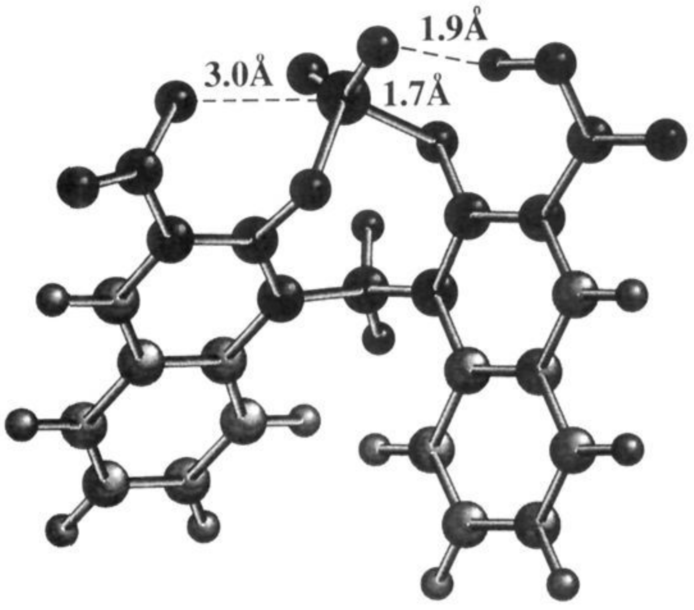
V



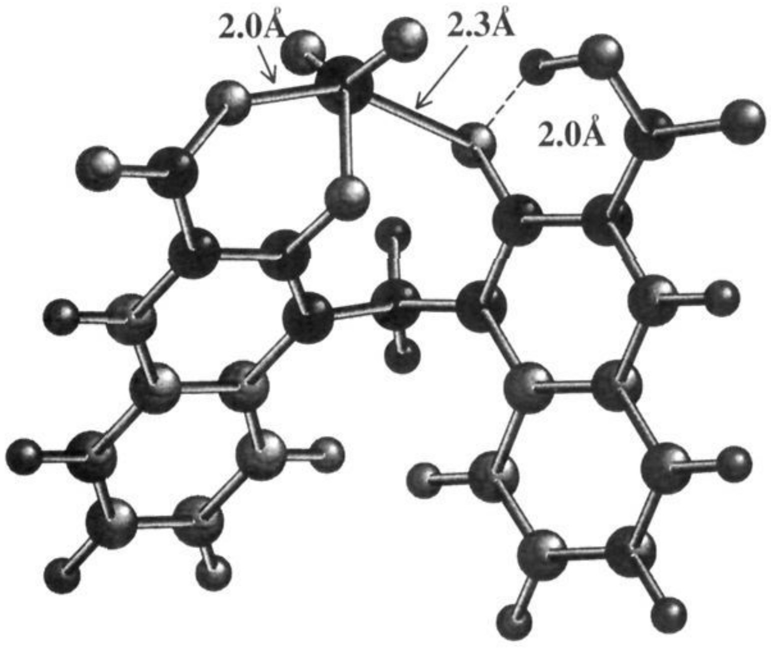
VI



VII



VIII



IX

Acknowledgment. This work was supported by the National Institutes of Health and the National Science Foundation. We are most appreciative of the support of our computational facility by the Office of Naval Research.

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[10] Abell, K. W. Y.; Kirby, A. J. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1171.

[11] The hydrolysis of bis(salicylic acid) phosphate **VI** has been suggested to take place by simultaneous intramolecular nucleophilic (*o*-CO_2^-) attack assisted by the *o*-CO_2H group. The kinetic contribution of the *o*-CO_2H has been reported (ref 10) as a 4-fold rate enhancement. The comparison of this 4-fold rate enhancement in the hydrolysis of **VI** to the 10^4-fold rate enhancement in the hydrolysis **II** provided by the *o*-CO_2H is the subject of a future report.

[12] Structures **VIII** and **IX** have been taken from an AM1-derived reaction coordinate trajectory. *Ab-initio* calculations at the 3-21G* level are currently in progress.

[13] Westheimer, F. H. In *Phosphorus Chemistry, Developments in American Science*; Walsh, E. N., Griffith, E. J., Parry, R. W., Quin, L. D., Eds.; ACS Symposium Series; American Chemistry Society: New York, 1992.

[14] Dalby, K. N.; Holifelder, F.; Kirby, A. J. *J. Chem. Soc., Chem. Commun.* **1992**, 1770.

[15] Dempcy, R. O.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 4511.