Participation of Two Carboxyl Groups in Phosphodiester Hydrolysis. 1. Hydrolysis of Bis(2-carboxyphenyl) Phosphate

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Abstract: The energies of rotamer conformations of bis(2-carboxyphenyl) phosphate (**2**), where one *o*-carboxy group is ionized and the other is not, have been determined by semiempirical (SAM1, AM1/SM2.1) calculations in order to approximate the probability of the presence of conformations in which the *o*-CO_2^- is in position to act as a nucleophile toward phosphorus and the *o*-CO_2H function can hydrogen bond to prostereogenic oxygens of the -(PO_2^-)- (conformer **B**) or leaving phenoxide oxygen (conformer **A**). With simulated water phase, conformer **B** appears to be at the global energy minima and conformer **A** is somewhat less stable. The kinetics and the mechanism of hydrolysis of bis(2-carboxyphenyl) phosphate (**2**) have been elucidated by use of ^1H and ^{31}P NMR spectroscopy in acetonitrile/water 95:5 and D_2O at pD up to 5.0 (39 °C). The hydrolysis of **2** is initiated by nucleophilic attack of the *o*-CO_2^- upon phosphate phosphorus resulting in the elimination of salicylic acid to provide salicyloyl cyclic phosphate (**3**) with a rate constant of $1-5 \times 10^{-3}$ s^{-1} and **3** converts to salicyl monophosphate with rate constants varying between 1 and 5×10^{-4} s^{-1}. The rapidity of the formation of **3** from **2** suggests the importance of both *o*-CO_2^- and *o*-CO_2H participation {a subject of the following manuscript}.

Introduction

The hydrolysis of phosphomonoesters by P−O bond breaking takes place by a rather facile elimination reaction involving the monoanion {ROP(O)(OH)(O^-)}.[1] The timing of protonation of the leaving oxygen and the requirement to carry into the transition state a water molecule to quench the forming metaphosphate has been discussed in detail.[2] Phosphotriesters readily undergo hydrolysis by nucleophilic catalysis or nucleophilic attack of lyate species.[2] In stark contrast to phosphomonoesters and phophotriesters, phosphodiesters undergo hydrolysis with P−O bond cleavage only with great difficulty. Thus, the pseudo-first-order rate constant (25 °C) at neutrality for the hydrolysis of dimethyl phosphate with P−O bond scission has been estimated[3] as 2×10^{-14} s^{-1}. A good portion of the kinetic barrier to nucleophilic attack upon the phosphorus of phosphodiesters is due to the negative charge on the -(PO_2^-)- moiety. Removal of the negative charge by conversion of the diester to a triester or by metal ion ligation[4] or protonation increases the rate of nucleophilic attack at P by $\geq 10^4-10^5$.

The pH dependence of the rate of hydrolysis of bis(2-carboxyphenyl) phosphate has been interpreted[5] as involving nucleophilic attack of *o*-CO_2^- on phosphorus with intramolecular catalysis by the *o*-CO_2H substituent. The *o*-CO_2H group can assist nucleophilic attack by hydrogen bonding to the negative -(PO_2^-)- oxygens and/or assist in the departure of the leaving oxygen by general acid protonation. It was reported[5] that the rate enhancement due to *o*-CO_2^- nucleophilic participation was 10^4-fold while the participation of the *o*-CO_2H as a general acid catalyst was found to be "unexpectedly inefficient". If the rate-determining step involved *o*-CO_2^- nucleophilic attack and the transition state was early there would be little build up of a second negative charge on phosphorus or the leaving oxygen and the influence of *o*-CO_2H general acid catalyst would be small. Alternatively if the ground state conformation of the substrate was such that the *o*-CO_2H could not reach the negative oxygen on -(PO_2^-)-, then *o*-CO_2H could only act as a general acid catalyst for departure of the leaving group. In the case of an early transition state in *o*-CO_2^- attack, general acid transfer of a proton from *o*-CO_2H to the putative phenoxide leaving group would not be of much importance.

We now report a reinvestigation of the hydrolysis of bis(2-carboxyphenyl) phosphate. This study consists of two parts. The first describes a semiempirical study of the energies of the ground state geometries of bis(2-carboxyphenyl) phosphate when the proton of one *o*-carboxy group is dissociated and the other *o*-carboxy is not. The object of these calculations is to ascertain the probability that conformations allowing simultaneous participation of both *o*-CO_2^- and *o*-CO_2H substituents in hydrolysis are energetically favored. Semiempirical calculations of reaction trajectories, assuming *o*-CO_2^- and *o*-CO_2H participation, have been carried out in order to determine if early or late transition states would be involved. The second portion of the study involves an NMR kinetic investigation of the stepwise hydrolytic reactions.

Results and Discussion

Semiempirical Energies of Rotamer Conformations[6] of bis(2-carboxyphenyl) phosphate (**2**), where one *o*-carboxy group is ionized and the other is not, have been computed using SAM1. Geometry optimization calculations were carried out at every

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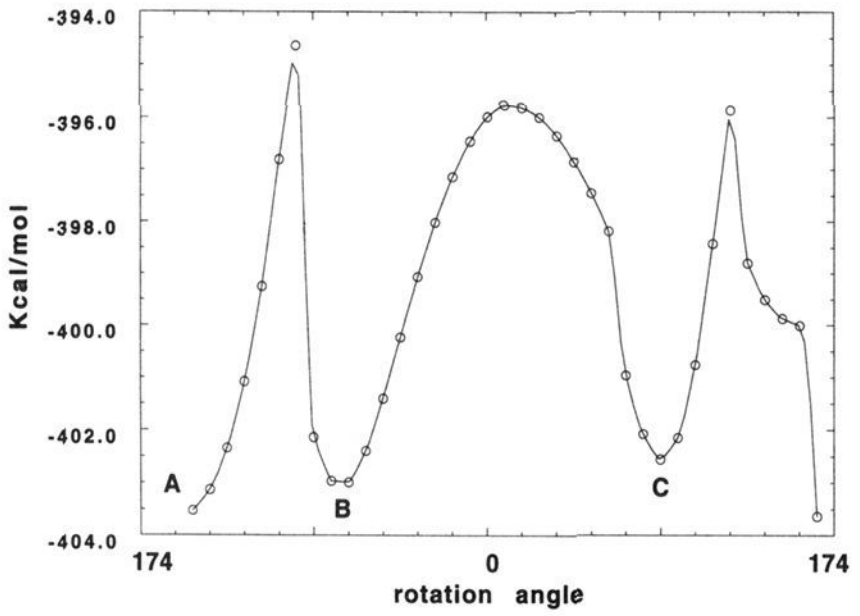
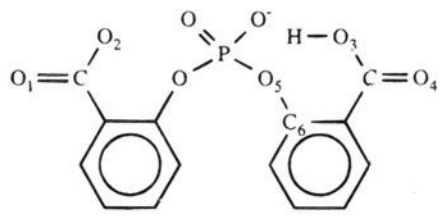


Figure 1. Energy profile for the rotation about the O5−C6 bond. SAM1 geometry optimizations were done every 10°. Letters denote the names of the structures corresponding to the particular energy.

Chart 1



10° rotation of the O(5) to C(6) torsion angle (Chart 1) with the *o*-CO_2^- oxygen to P distance held at 3.8 Å. Inspection of the plot of O(5) to C(6) torsion angle vs energy (Figure 1) shows the three gas phase minima conformations, **A**, **B**, and **C** (Figure 2). The AM1/SM2.1 water solvation model single point energies of **A**, **B**, and **C** have also been calculated (Table 1). Conformation **B**, with *o*-CO_2H in a position to hydrogen bond with the phosphate pro-stereogenic oxygens, has the lowest energy in the water solvation model (Table 1). Thus, in any instance where the *o*-CO_2^- function is in position to attack in line, the most stable conformation (**B**) has the *o*-CO_2H substituent in position such that it is allowed to act as a general acid and protonate the oxygens of the -(PO_2^-)- moiety. The next most favorable conformation (**A**) has the *o*-CO_2H adjacent to the phenoxide leaving group and away from the pro-stereogenic oxygens of the -(PO_2^-)- moiety (Figure 2). In order to assess the relationship of conformations **A** and **B** to the global

Table 1. Gas Phase and Solvated Semiempirical Energies of Stable Conformations **A**, **B**, and **C** (kcal/mol)

structure	ΔH(gas phase)	$\Delta H(H_2O)$
A	−403.6	−544.7
B	−403.0	−547.4
C	−402.6	−541.7

minima conformation, a random search was performed in Sybyl, where 10 000 iterations generated 250 conformations, and their energies were determined. Of these there could be identified 15 primary conformations. These conformations were examined using SAM1 along with the AM1/SM2.1 water solvation model, and of those conformations **B** was of lowest energy (Figure 3). On this basis conformation **B** is in essence a global minimum structure.

Calculations Dealing with the Reaction Trajectory for *o*-CO_2^- Attack on the Phosphate Ester and the Structure of the Transition States. Reaction coordinates were generated, for the nucleophilic attack of the *o*-CO_2^- upon phosphorus and elimination of phenolate oxygen, starting from structures **A** and **B** (see Experimental Section). The reaction trajectories were generated in the gas phase and transferred to water (Figure 4). There are two high points along the reaction coordinates, the first is shallow {-COO^- to P distances of 3.2 Å starting with **B** and 3.3 Å starting with **A**} and involves rotation of the *o*-CO_2^- such that the -O^- is in position to attack phosphate phosphorus and the second {-COO^- to P distances of 2.0 Å starting with **B** and 2.1 Å for **A**} is associated with the transition states for nucleophilic attack. In **A** the proton of the -COOH group is 1.6 Å from the leaving phenoxide oxygen, while in **B** the -COOH proton is 1.4 Å from a prostereogenic PO_2^- oxygen. The positions of the second transition states along the reaction coordinate are not, for our purposes, significantly different in the gas phase and water calculations. {It should be noted that in separate experiments employing 3-21G* *ab initio* computations starting with **A** the gas phase transition state was characterized by a -COO^- to P distances of 2.28 Å.} The transition state starting with conformation **A** has the leaving group oxygen 1.9 Å from the phosphorus, while with the transition state stemming from conformation **B** the leaving group is closer (1.7 Å) to the phosphorus. {Employing 3-21G* *ab initio* computations starting with **A** the gas phase transition state was characterized by the leaving group oxygen being 1.84 Å from P.} That the transition is earlier in departure of the leaving

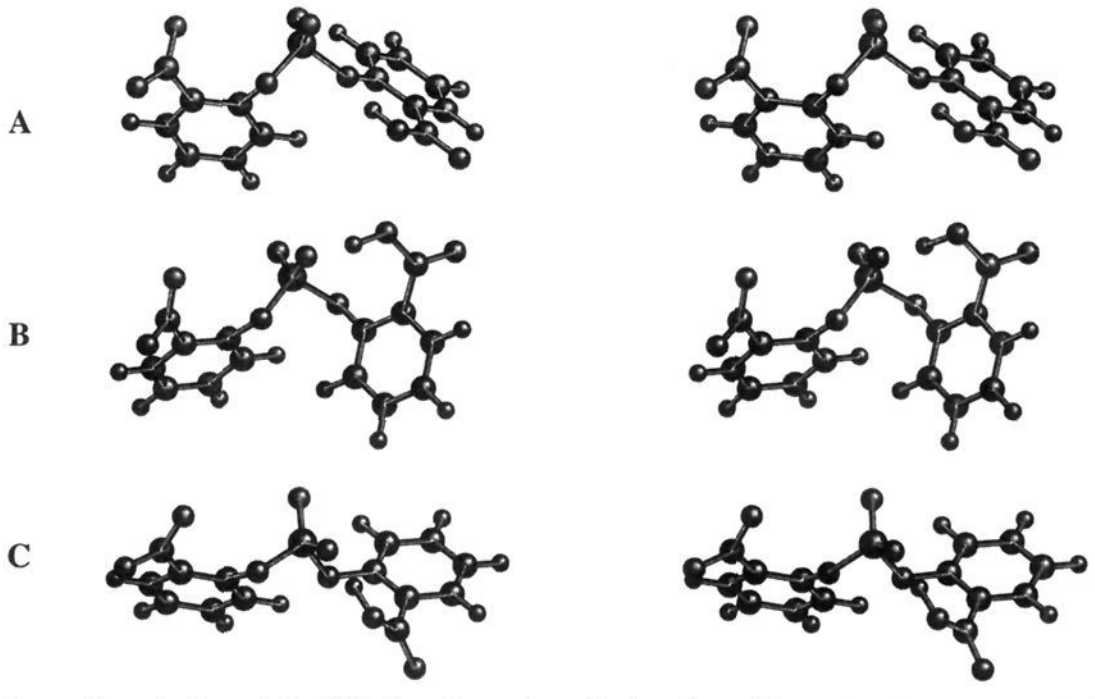


Figure 2. Stereoview of conformations **A**, **B**, and **C** of bis(2-carboxyphenyl) phosphate. These structures represent the low-energy conformations taken from the dihedral rotation study with a restraint upon the distance from *o*-CO_2^- oxygen to P of 3.8 Å. Structure **A** has the proton from the *o*-CO_2H hydrogen bonding to the putative phenoxide oxygen. Structure **B** has the *o*-CO_2H proton hydrogen bonding to one of the pro-stereogenic oxygens of the phosphate. Structure **C** is a hybrid of both **A** and **B**.

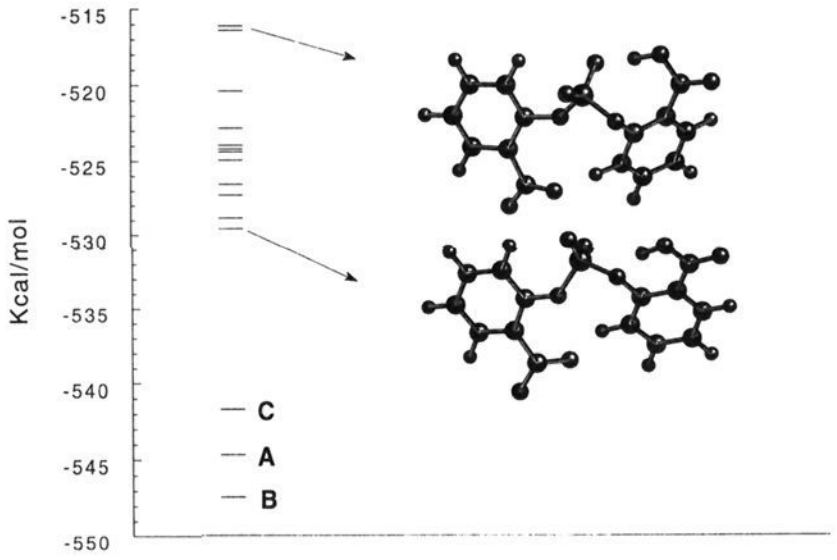


Figure 3. Selected members from the Sybyl random search and their energies. The vertical axis shows the energies of the conformations determined by SAM1//AM1/SM2.1 water solvation. The lowest energy structures are **B**, **A**, and **C**, respectively. The structure at −529 kcal/mol has the *o*-CO_2H proton hydrogen bonded to the phenoxide oxygen in a manner similar to that of **A**. The structure at −516 kcal/mol has the *o*-CO_2H proton hydrogen bonded to the pro-stereogenic oxygens of the phosphate as in **B**. Both these structures (−529 and −516 kcal/mol) have the *o*-CO_2^- rotated ca. 180° from the attack position.

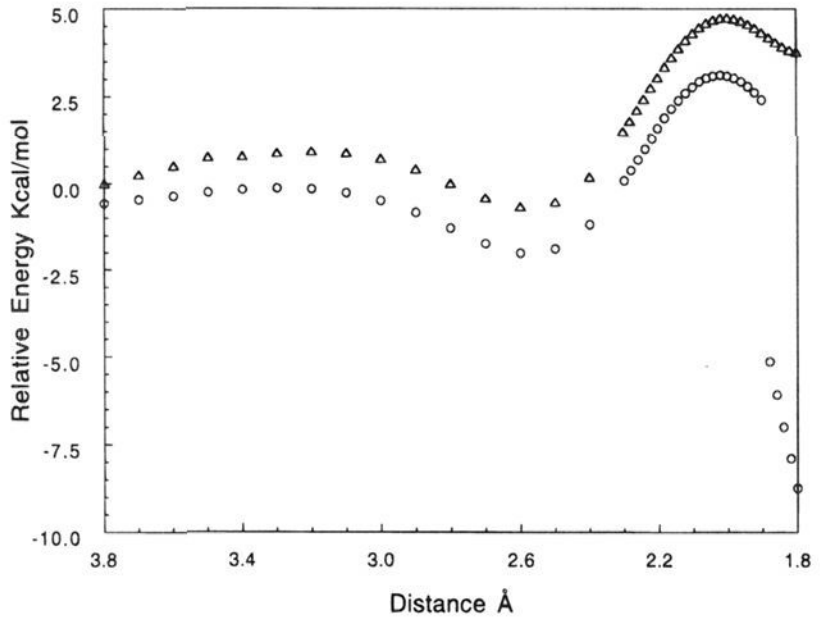
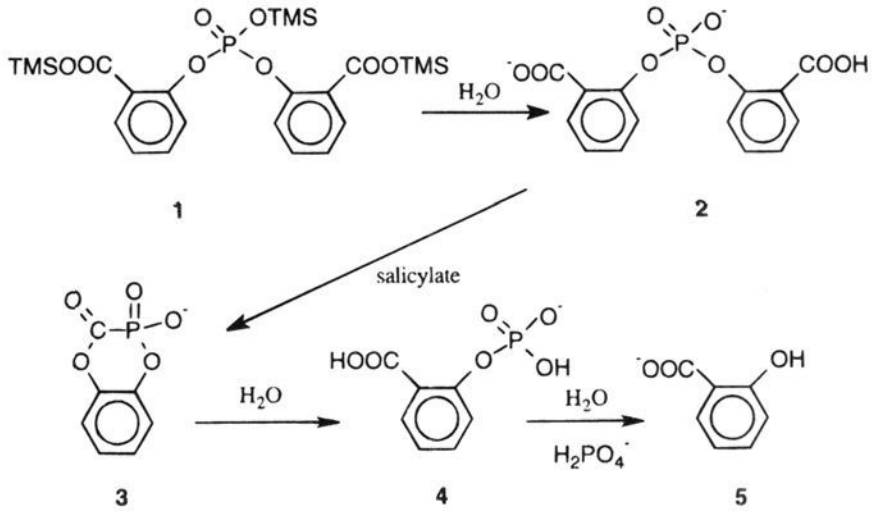


Figure 4. SAM1 gas phase reaction coordinates of **A** (○) and **B** (△) going to acyl phosphate and salicylate. The vertical axis shows the energy relative to the starting energy of structure **B**.

group starting with **B** may relate to *o*-CO_2H general acid assistance of departure.

NMR Spectroscopy. In the preparation of bis(2-carboxyphenyl) phosphate (**2**) we followed the synthetic procedure reported earlier by Abell and Kirby.[5] The literature procedure for the synthesis of **2** from **1** (Scheme 1) involves dissolving **1** in acetonitrile/water (95:5) and after 20 min at room temperature Rotavap removal of solvent to give **1** as a residue. By ^{1}H NMR we observed this procedure to yield a mixture of **3**, **4**, and **5**. This led us to undertake a kinetic study of the hydrolysis of bis(2-(trimethylsilyloxycarbonyl)phenyl) trimethylsilyl phosphate (**1**) commencing immediately on its dissolution in acetonitrile/water 95:5. In the previous investigation[5] Abell and Kirby followed the ester exchange from bis(2-(benzyloxycarbonyl)phenyl) methyl phosphate to bis(2-(trimethylsilyloxycarbonyl)phenyl) trimethylsilyl phosphate (**1**) by ^{1}H NMR spectroscopy. We have, in addition, characterized **1** by ^{1}H NMR and APcI mass spectrometry. In what follows we will discuss the hydrolysis of **1** under three different solvent conditions: these are (i) acetonitrile:water 95:5, (ii) acetonitrile:water 95:5 containing 0.01 M DCl, and (iii) D_2O solutions at various pD values.

Scheme 1



(1) ^{1}H NMR. The hydrolysis of **1** was followed under the same solvent conditions as previously reported[5] (acetonitrile: water 95:5 at 25 °C). Our use of ^{1}H and ^{31}P NMR spectroscopy to follow the solvolysis of **1** has led to the reaction sequence depicted in Scheme 1. A stacked plot of the hydrolysis of **1** in the 7.45−8.12 ppm region is shown in Figure 5. The aromatic protons of **1** resonate at 7.985, 7.635, 7.389, and 7.255 ppm in CD_3CN. In the aromatic region we saw small signals of salicyloyl cyclic phosphate (**3**), monosalicyl phosphate (**4**), and salicylic acid (**5**) (*vide infra*). The aliphatic region has a relatively broad signal ($\nu_{1/2}$ = 15 Hz) at 0.290 ppm (the protecting TMS group) and a sharp signal at 0.079 ppm. Two minutes after addition of 5% D_2O, the latter signals of **1** collapsed and the aromatic region exhibited new major signals at 7.868, 7.558, 7.413, and 7.297 ppm, which were assigned to bis(2-carboxyphenyl) phosphate **2**. The signal at 0.290 ppm collapses also, while the signal at 0.067 ppm changed to 0.059 ppm. We assigned this signal to $(CH_3)_3SiOH$ (Figure S1, supporting information).

Reactions were followed by recording NMR spectra every 5 min. As the signals of **2** decrease, those of salicyloyl cyclic phosphate (**3**) (8.027, 7.750, 7.335, and 7.249 ppm) and those of the salicylic acid (**5**) (7.852, 7.500, 6.994, and 6.922 ppm) increase (Figure 5). After 30 min the signals of **3** decreased when **2** had almost disappeared. Concurrently, the signals of the salicyl monophosphate (**4**) (7.852, 7.574, 7.350, and 7.300 ppm) increased slowly. The chemical shifts of **4** and **5**, from the reaction mixture, are consistent with those determined from authentic samples. In the conversion of the phosphate diester **2** to the cyclic phosphate ester **3** there is produced salicylic acid (**5**). In the hydrolysis of **3**, the concentration of salicylic acid remained constant (Figure S2, supporting information). This shows that hydrolysis of **3** must occur with ring opening and the only product can be salicyl monophosphate (**4**). The time dependence of the concentration of **1**, **2**, **3**, **4**, and **5** is shown in Figure 6. The lines which fit the points of Figure 6 were computer generated from the sequential first-order reaction sequence of Scheme 2 using the rate constant for hydrolysis of salicyl monophosphate[5] of $k_4 = 10^{-5}$ s^{-1} (Experimental Section). The computed rates are $k_1 = 7.2 \times 10^{-2}$ s^{-1}, $k_2 = 1.4 \times 10^{-3}$ s^{-1}, $k_3 = 4.4 \times 10^{-4}$ s^{-1} (see also ref 7).

The hydrolysis of **2** was also followed in D_2O solution at different pD values (Table 2) using both ^{1}H and ^{31}P NMR spectroscopy. In these experiments the disappearance of **1** is too rapid to allow the determination of the rate constant k_1 by NMR spectroscopy. In CD_3CN/D_2O 95:5 containing 0.01 M DCl, we saw (after 5 min of mixing) the completion of the disappearance of **1** to provide **2** whose disappearance was then observed. In neat D_2O, at various pD values, the disappearance of **1** was to rapid to observe but the disappearance of **2** could

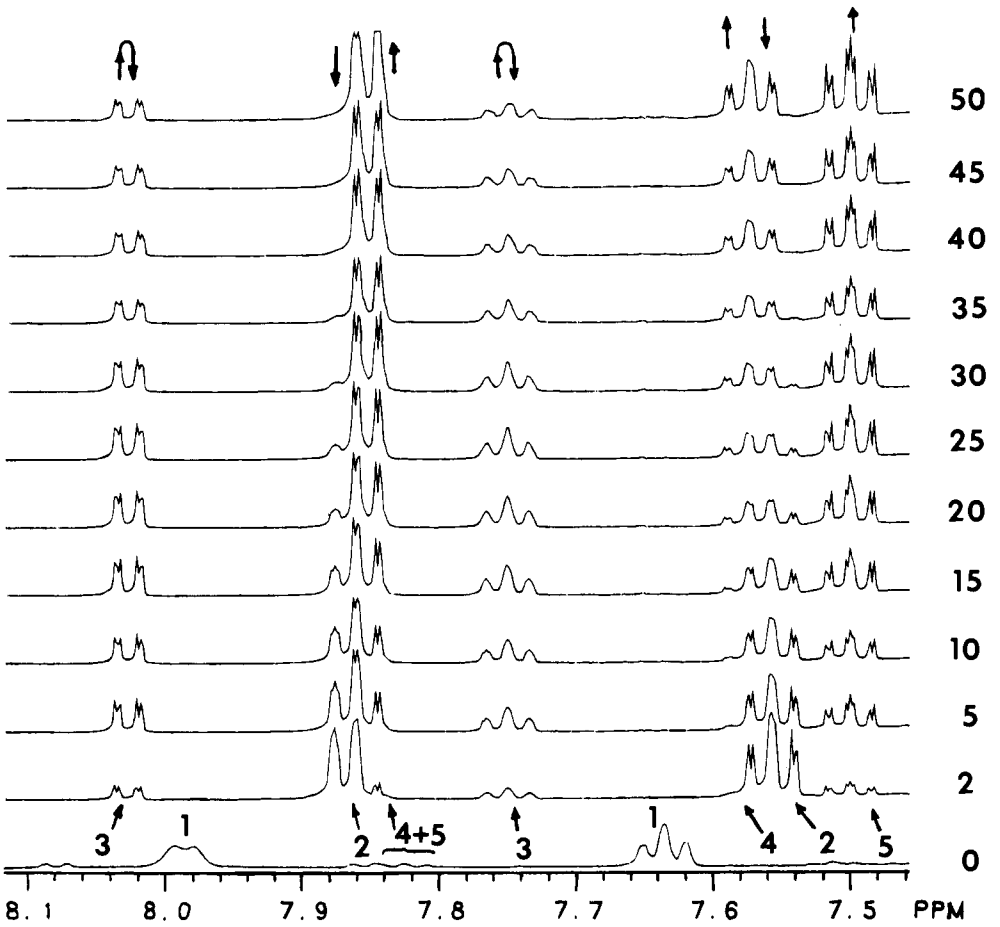


Figure 5. Stacked plot of the ^{1}H NMR spectra of the hydrolysis of **1** (0.01 M) in CD_3CN/D_2O 95:5 at 25 °C at the indicated reaction times (min).

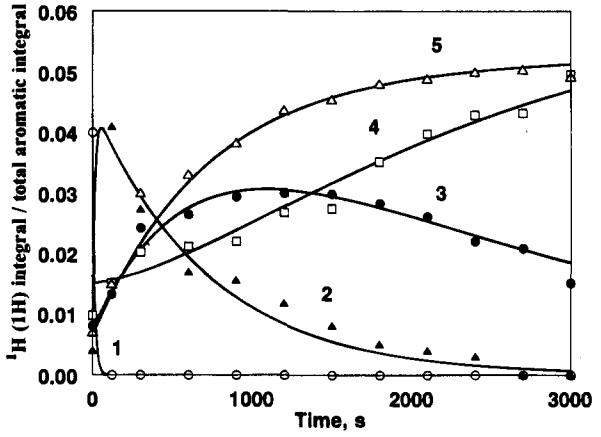
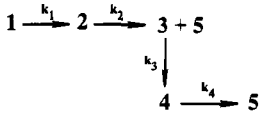


Figure 6. Hydrolysis of **1** in CD_3CN/D_2O 95:5 at 25 °C followed by ^{1}H NMR spectroscopy. (○) TMS protected bis(2-carboxyphenyl) phosphate (**1**); (▲) bis(2-carboxyphenyl) phosphate (**2**); (●) salicyloyl cyclic phosphate (**3**); (□) monosalicyl phosphate (**4**); (△) salicylic acid (**5**).

Scheme 2



be followed. Except for CD_3CN/D_2O 95:5, k_2 was not measurable and k_3 was constant with pD variation (Table 2).

(2) ^{31}P NMR. In CD_3CN at 25 °C the sample employed of partially hydrolyzed **1** exhibits three ^{31}P signals (Figure S3, supporting information). At −6 ppm there is a broad signal attributed to the monoester **3** ($\nu_{1/2}$ = 800 Hz), a relatively sharp signal at −13 ppm ($\nu_{1/2}$ = 300 Hz) with a broad shoulder at −15 ppm, and another relatively sharp signal at −22 ppm ($\nu_{1/2}$ = 300 Hz) with a broad shoulder at −25 ppm. On decreasing the temperature to −40 °C, all signals sharpen. There is then observed five signals (Figure 7) at −6.0 ppm ($\nu_{1/2}$ = 89 Hz), −13.3 ppm ($\nu_{1/2}$ = 76 Hz), −15.4 ppm ($\nu_{1/2}$ = 145 Hz), −22.2 ppm ($\nu_{1/2}$ = 70 Hz), and −25.4 ppm ($\nu_{1/2}$ = 133 Hz). Upon addition of 1% D_2O, the signals at −22.2 and −25.4 ppm collapsed. This instability led us to the assignment of these signals to the trimethylsilyl-protected bis(2-carboxyphenyl) phosphate which we propose to exist as two conformational isomers. In 1% D_2O, the remaining signals sharpen further ($\nu_{1/2}$ = 20−30 Hz at CD_3CN/D_2O 99:1 and $\nu_{1/2}$ = 5−10 Hz at CD_3CN/D_2O 95:5) due to faster equilibriation of the various conformational isomers created by the bulkiness of the protecting groups on the carboxyl substituents of the phosphate esters. The signal at −15.4 ppm decreases when the D_2O content in CD_3CN was increased from 1 to 5%. We assigned this signal to the diester **2** and that at −13.3 ppm to salicyloyl cyclic phosphate (**3**). The signal at −13.3 ppm shifts to −12.2 ppm

Table 2. Hydrolysis of **2** and **3** Followed by ^{1}H and ^{31}P NMR Spectroscopy[a]

solvent	pD	T, °C	k_2 (s^{-1})	k_3 (s^{-1})
CD_3CN/D_2O 95:5,	0.01 M DCl	39	5×10^{-3}	5.0×10^{-4}
CD_3CN/D_2O 95:5[b]		39		2.0×10^{-4}
DCl/D_2O[c]	1.20	39		2.2×10^{-4}
DCl/D_2O	2.52	39		1.0×10^{-4}
DCl/D_2O	3.12	39		1.5×10^{-4}
acetate buffer/D_2O	5.00	40		1.2×10^{-4}

[a] Recorded after 5 min of the D_2O addition. [b] ^{31}P NMR recorded after 10 min of the D_2O addition. [c] In D_2O solution the diester was almost collapsed when the first spectrum was taken.

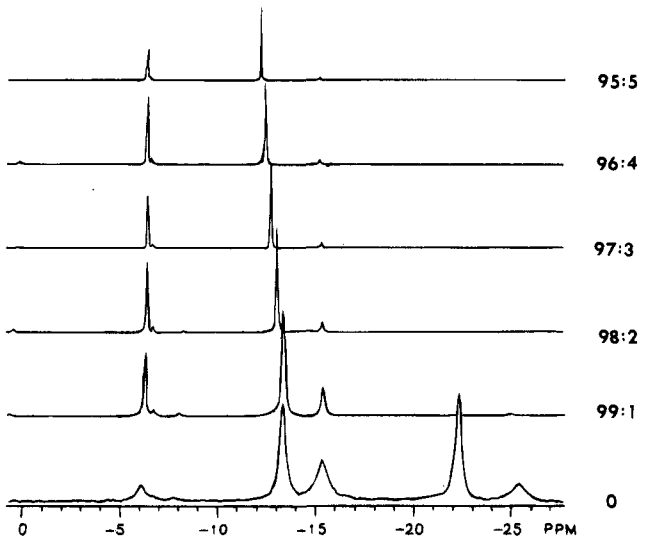


Figure 7. Stacked plot of the ^{31}P NMR spectra of the partially hydrolyzed **1** at −40 °C at the indicated CD_3CN/D_2O ratios.

when the D_2O content is increased from 0 to 5%. In CD_3CN/D_2O 95:5 and 25 °C, the monoester ^{31}P signal is at −5.696 ppm, the salicyloil cyclic phosphate at −11.008 ppm, and the bis(2-carboxyphenyl) phosphate at −11.867 ppm (*vs* external H_3PO_4).

Conclusions

Semiempirical energies of rotamer conformations of bis(2-carboxyphenyl) phosphate (**2**) establish that the rotamer (**B**) in which *o*-CO_2^- and *o*-CO_2H functions are in positions to act as nucleophile and hydrogen-bonding general acid toward the prostereogenic oxygens of the phosphate, respectively, is likely the global minima conformation. The conformation **A** in which *o*-CO_2^- and *o*-CO_2H functions are in positions to act as nucleophile and hydrogen bonding general acid toward the leaving phenoxide oxygen is only somewhat less favored. Both carboxyl functions of conformations **A** and **B** are, therefore, in position to participate in the hydrolysis of **2**. Qualitative semiempirical evaluations of the transition states leading from conformations **A** and **B** suggest that participation of both functions are to be expected.

The hydrolysis of **2** has been shown to be very rapid and to proceed (Scheme I) *via* formation of salicyloyl cyclic phosphate (**3**) which ring opens to give salicyl monophosphate (**4**) and finally salicylic acid (**5**). Under the conditions employed, phosphodiester **2** eliminates salicylic acid in becoming salicyloyl cyclic phosphate (**3**) with a rate constant of $1-5 \times 10^{-3}$ s^{-1} and **3** converts to salicyl monophosphate with rate constants varying between 1 and 5×10^{-4} s^{-1}. The results of this study are complementary[7] to those reported by Abell and Kirby.[5]

Experimental Section

Materials. The salicyl monophosphate was synthesized as previously described[8] and salicylic acid was commercial (Aldrich). In the synthesis of bis(2-(trimethylsilyloxycarbonyl)phenyl) trimethylsilyl phosphate (**1**), we followed the synthetic procedure earlier reported.[5]

Bis(2-(benzoyloxycarbonyl)phenyl) Methyl Phosphate. Benzyl salicylate (19.7 g, 0.086 mol) in 10 mL of THF was added dropwise to a suspension of NaH (90%, 2.249 g, 0.084 mol) in 20 mL of THF at room temperature with stirring during 1 h under an argon atmosphere. The brown-green NaH suspension changed to a green liquid. To this solution was added 4 mL of methyl phosphorochloridate (97%, 0.04 mol) during 15 min at room temperature with stirring. The reaction was stirred overnight, the solvent removed (Rotavap), and the residue resuspended in ethyl ether. The etheric solution was washed three times with water, the ethereal extract was dried over anhydrous Na_2SO_4, and finally the solvent was removed under reduced pressure. The crude product was purified by column chromatography (20% petroleum ether, 80% ethyl ether) to obtain the mixed triester as a colorless oil in 80% yield. HR mass spectrum: M^+ 532.128918, calcd 532.128707. ^1H NMR (CD_3CN, 25 °C) δ (^3J, ^4J): 7.887 dd (9, 1) 1H, 7.531 td (8.5, 1.5) 1H, 7.351 td (7, 1.5) 1H, 7.398 dd (8.5, 1) 1H, 5.288 s 4H $BzCH_2$; 3.851 d ($^3J_{P-H}$ = 12 Hz) 3H POMe. *^{31}P NMR* (CD_3CN, 25 °C) δ: 11.31 ppm.

Bis(2-(trimethylsilyloxycarbonyl)phenyl) Trimethylsilyl Phosphate (1). To bis(2-(benzoyloxycarbonyl)phenyl) methyl phosphate (0.39 g, 7.32×10^{-4} mol) was added 6 equiv, 0.6 mL (0.88 g, 4.39×10^{-3} mol), of iodotrimethylsilane (Lancaster) under an argon atmosphere at room temperature. ^{31}P NMR (CD_3CN, 25 °C, *vs* external H_3PO_4) shows instantaneous silylation of the POMe group (δ = −28.56 ppm) when the spectra was recorded after 5 min of mixing. After 10 min a new peak appeared at −29.92 ppm and a small, but well defined, peak at −30.92 ppm. The peak at −28.56 ppm disappered after 2 h, and the peak at −29.92 ppm increased and slowly decreased. After 10 h the peak at −30.92 ppm increased and shifted to −32.05 ppm. We assigned the peak at −28.56 ppm to the monosilylated compound (POTMS), that at −29.92 to the bissilylated compound (POTMS and one COOTMS), and that at −30.92 ppm to the tris-TMS-protected bis-(2-carboxyphenyl) phosphate. The reaction mixture was allowed to stay for 3 days in these conditions for completion, and the excess TMSI was removed under reduced pressure. The brown reddish oil was further used in the NMR kinetic studies.

Mass spectrum (APcI): $(M + H)^+$ 555.2, calcd for $C_{23}H_{35}O_8PSi_3$ 554.14. ^1H NMR (CD_3CN, 25 °C) δ (^3J, ^4J): 7.985 d (7) 1H, 7.635 t (8, 1) 1H, 7.389 d (8) 1H, 7.255 t (7.5) 1H, 0.079 s 18H COOTMS, 0.290 bs 9H POTMS.

^1H chemical shifts were reported for the signal of CD_2HCN = 1.940 ppm and ^{31}P chemical shifts were reported for the signal of H_3PO_4 = 0 ppm.

Kinetic Procedures. In the quantitation of **3** and **5** we used the resonances at 8.027 and 6.992 ppm where the signals were well separated. The disappearance of **2** was followed at 7.868 ppm as a half-doublet, separated from the overlapped resonances of **4** and **5** close to this region. The appearance of **4** was followed at 7.574 ppm quantitating one-fourth of its triplet at 7.585 ppm which was separated from the diester signal at 7.558 ppm. The kinetic simulation was performed using KINSIM[9] Version 3.4 PC 900819 (Dr. Bryce V. Palpp, University of Iowa). The time parameters were delta time = 3 s, iteration/point = 1, run time = 3240 s, Y_{max} = 0.06, flux tolerance = 0.1, integral tolerance = 0.001. The rate constant k_4 was fixed to 10^{-5} s^{-1} (considering our experimental conditions as acidic), consistent with the previously determined rate constant[5] for the hydrolysis of monosalicyl phosphate (**4**) at pH 1.18 of 7.5×10^{-6} s^{-1}.

Theoretical Calculations. All semiempirical calculations were performed with the AMPAC 5.0[9] package using SAM1[10] and AM1/SM2.1[11] for solvation model calculations. SAM1 was chosen for these calculations because it is a method which includes d orbitals explicitly, which may make its performance for phosphorus-containing compounds superior to that of AM1, PM3, MNDO, and other semiemperical methods. Spartan 3.1[12] was employed for 3-21G* *ab initio* calculations.

(7) Abell and Kirby reported that **2** hydrolyzes with *o*-CO_2^- participation but report no experimental evidence for the existence of salicyloyl cyclic phosphate intermediate (**3**). We found by ^1H NMR that their procedure for preparation of **2** yields a mixture of **3**, **4**, and **5**. Also, we find the rate of the hydrolysis of **1** in acetonitrile:water 95:5 at 25 °C (leading to **2**, ^1H NMR) to be 7.2×10^{-2} s^{-1}, while the rate constant for hydrolysis of the wanted **2** is 1.4×10^{-3} s^{-1}; $t_{1/2}$ = 500 s^{-1}. Thus, the time they allowed (1200 s) for the preparation of **2** by solvolysis of **1** was, in our hands, sufficient to hydrolyze ~80% of **2**. Perhaps, in the colder laboratory rooms at Cambridge the rate constants would be sufficiently different to allow the preparation of **2**.

(8) Chanley, J. D.; Gindler, E. M.; Sobotka, H. *J. Am. Chem. Soc.* **1952**, *74*, 4347.

(9) AMPAC 5.0, 1994, Semichem, 7128 Summit, Shawnee, KS 66216.

(10) Dewar, M. J. S.; Jie, C. X.; Yu, J. G. *Tetrahedron* **1993**, *49*, 5003.

(11) Cramer, C. J.; Truhlar, D. G. *J. Comput. Aided Molec. Des.* **1992**, *6*, 629.

(12) Spartan 3.1, Wavefunction, Inc., Irvine, CA.

(13) A software product of Tripos Associates, Inc., St Louis, MO.

The reaction profiles for nucleophillic attack were started from the ground state optimized structures of bis(2-carboxyphenyl) phosphate. The points comprising the gas phase reaction coordinate were obtained through geometry optimization, using SAM1, without any constraints save the given value of O2−P bond distance. The O2−P bond was decreased from its optimized starting distance of 3.8 Å to a final distance of 1.8 Å. Transfer of the reaction coordinate to water was accomplished by single-point calculations using the structures for each point of the gas phase calculation with the AM1/SM2.1 solvation model. The dihedral drive resulting in rotating around the O5−C6 bond provided structures every 10° that were refined by geometry optimization. An AM1/SM2.1 single-point calculation was done on the three structures with the lowest energy to obtain the solvation energy. The random search was implemented on Sybyl[13] utilizing a 10 000 iteration search and then minimizing each structure using the Sybyl force field.

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Supporting Information Available: Figure S1, stacked plot of the 0−5.6 ppm region in the hydrolysis of **1** in CD_3CN/D_2O 95:5 at 25 °C; Figure S2, ^1H NMR kinetic plot for the hydrolysis of **3** (8.02 ppm) and **5** (6.92 ppm) in 0.01M DCl at 39 °C; Figure S3, ^{31}P NMR of the partially hydrolyzed **1** in CD_3CN at 25 °C (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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