was filtered three times through a plug of Ag-impregnated silica to remove the C_6H_5I. The yield of spectroscopically pure 1,2-propaneditriflate was 123 mg (50%). ^1H NMR ($CDCl_3$): δ 5.21 (m, 1 H), 4.56 (m, 2 H), 1.59 (d, J = 11.4 Hz). $\{^1H\}^{13}C$ ($CDCl_3$): δ 81.5, 74.9, 17.1. ^{19}F ($CDCl_3$): δ -40.7 (s), -41.4 (s) (rel to $CFCl_3$).

1 + Butadiene. A suspension of 1 (280 mg, 0.39 mmol) in 25 mL of CH_2Cl_2 was prepared in a flame-dried 50-mL Schlenk flask under N_2. As above, butadiene (0.380 mmol) was condensed into the Schlenk flask and thawed at 0 °C. Within a couple of minutes 1 dissolved and the mixture became homogeneous. $[Et_4N]Br$ (240 mg, 1.14 mmol) in 5 mL of CH_2Cl_2 was added via syringe under N_2 flow. The solution was stirred for an additional 3 h at 0 °C and filtered; after its volume was reduced to 2 mL, it was spotted on a Chromatotron plate and eluted with 15% CH_2Cl_2/hexane. Iodobenzene (R_f 0.50) and 1.4-dibromo-2-butene (R_f 0.35) were separated, yielding 45 mg (55% yield) of white crystalline 1,4-dibromo-2-butene.

1 + Butadiene. Butadiene (.023 mmol) was condensed into a 5-mm NMR tube containing 1 (17 mg, 0.023 mmol) and $CDCl_3$ (.35 mL), degassed, and sealed. After the tube was warmed to room temperature, ^1H NMR showed two strong bands at δ 6.09 and 5.02 along with iodobenzene and a trace of butadiene remaining. An ^{19}F NMR of this sample shows three absorptions at δ -41.15 (0.06), -41.24 (1.00), and -41.71 (0.06) (intensities in parentheses). Attempts to concentrate the products for isolation led to decomposition to a brown residue.

NMR Spectral Simulation. A computer program provided by IBM Instruments, Inc., known as parameter adjustment in NMR by iteration calculation (PANIC), was used for spectral simulation on an Aspect 2000 computer. Initial estimated chemical shifts and coupling constants are incorporated to calculate a spectrum, which is compared to the experimental one. An iteration process is then performed until the differences between the two are minimized.

Acknowledgment. We acknowledge Dr. Bruce Hawkins and the Colorado State University Regional NMR Center, funded by National Science Foundation Grant No. CHE-8208821, for designing the deuterium decoupler and collecting the deuterium-decoupled spectrum of *dl*-**3**-*1,2-d*$_2$; Sigrídur S. Kristjánsdóttir collected the deuterium-decoupled spectrum of *meso*-**3**-*1,2-d*$_2$. We also thank Dr. Scott Tunney for helpful suggestions and Karen Hennessy for preparing an analytically pure sample of 1. This work was funded by Department of Energy Grant DE-FG02-84ER13299.A003.

Registry No. 1, 88016-29-9; *dl*-**2**-*1,2-d*$_2$, 108868-25-3; *meso*-**2**-*1,2-d*$_2$, 108868-26-4; *dl*-**3**-*1,2-d*$_2$, 108868-27-5; *meso*-**3**-*1,2-d*$_2$, 108149-56-0; $CF_3SO_2O(CH_2)_2OSO_2CF_3$, 18928-34-2; *cis*-CHD=CHD, 2813-62-9; *trans*-CHD=CHD, 1517-53-9; triflic anhydride, 358-23-6; iodosobenzene, 536-80-1; cyclohexene, 110-83-8; *cis*-1,2-cyclohexanediol bis(trifluoromethanesulfonate), 91146-10-0; (±)-*trans*-1,2-cyclohexanediol bis(trifluoromethanesulfonate), 108868-24-2; ethylene, 74-85-1; 1,2-propanediol ditriflate, 108868-28-6; 1,3-butadiene, 106-99-0; (*E*)-1,4-dibromo-2-butene, 821-06-7; propylene, 115-07-1.

Pyridoxal-Mediated Dephosphonylation of 1-Amino Phosphonic Acids

Kim C. Calvo

Department of Chemistry, The University of Akron, Akron, Ohio 44325

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The reaction between pyridoxal and α-amino phosphonic acids is reported. Under the proper conditions, the amino phosphonates can be induced to undergo a pyridoxal-promoted cleavage of the P–C bond. The reaction requires a metal ion and a phosphonic acid that possesses a chelating heteroatom in a position β to the amino group. At 100 °C and pH 8.8, the dephosphonylation reaction is a first-order process, producing orthophosphate and an aldehyde from the amino phosphonate and pyridoxamine from the pyridoxal. The mechanism proposed for this reaction is based on an analogy with the pyridoxal-catalyzed decarboxylation of α-amino acids. The reactions reported represent the first observation of an α dephosphonylation mediated by pyridoxal.

A wide variety of phosphonic acids are known that have extremely potent biological activity. Glyphosate is one particularly well-known example in the herbicide field.[1] Of increasing interest are α-amino phosphonate analogues of natural amino acids.[2] When incorported into the proper polypeptide sequence, these analogues have proven to be extremely effective inhibitors of various serine proteases. The stability of the P–C bond in these molecules raises questions about possible mechanisms for the biodegradation of amino phosphonates. The chemistry of the P–C bond precludes nucleophilic attack at the phosphorus or carbon atom as a viable mechanism for the cleavage of this bond. To date, only a few specific examples of enzymatic reactions leading to cleavage of a P–C bond are known. Recently, Cordiero et al.[3] reported the metabolism of alkane- and alkenephosphonic acids by *Escherichia coli*. Incubation of methanephosphonate with the bacteria resulted in the formation of methane and orthophosphate, for example. These products suggest the formation of a phosphinyl radical anion as an intermediate. The P–C bond cleaves homolytically, producing monomeric metaphosphate anion and a methyl radical. The carbon radical subsequently abstracts a hydrogen atom to form methane, while the metaphosphate reacts with water to form the orthophosphate.

The mechanism for the biodegradation of phosphonoacetylaldehyde was elucidated by La Nauze[4]. The reaction is enzyme catalyzed and involves the initial formation of a Schiff base between the aldehyde carbonyl carbon and

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the side-chain primary amino group of lysine. The P–C bond is cleaved in a β elimination step, similar to a β decarboxylation. This mechanism also proposes the formation of monomeric metaphosphate as the initial phosphorus intermediate. A model reaction for the cleavage of a P–C bond by a similar β-type elimination was reported by Martel.[5] In this model reaction, 2-amino-3-phosphonopropanoic acid is reacted with a catalytic amount of pyridoxal. Elimination of monomeric metaphosphate occurs after the isomerization of the initial Schiff base to the ketimine. As is expected for a β elimination, the reaction appears quite facile.

The question addressed in this report is whether pyridoxal will catalyze the dephosphonylation of an α-amino phosphonic acid. The cleavage of a P–C bond in this class of compounds requires an α elimination. Model reactions for the pyridoxal-catalyzed decarboxylation of α-amino acids are known. However, in general, this reaction is fairly difficult to achieve.[6] This is interesting in light of the fact that pyridoxal-catalyzed amino acid α decarboxylation is a common enzyme reaction. We have identified a model system that does demonstrate the dephosphonylation of an α-amino phosphonic acid in the presence of pyridoxal. In this system, the Schiff base formed between α-amino-(2-hydroxyphenyl)methanephosphonic acid and pyridoxal can partition between two reaction pathways. The first pathway is a simple transamination followed by base cleavage of the resulting α-keto phosphonate. The second pathway is the desired dephosphonylation reaction.

Results

Amino Phosphonates. Initial experiments used the commercially available amino phosphonates 1-aminoethanephosphonate and aminomethanephosphonate. Formation of the Schiff base between the amino phosphonates (0.1 mM) and pyridoxal (PL; 0.2 mM) was followed at 25 °C by monitoring the change in absorbance at 410 nm in phosphate buffer, pH 7–10. Schiff base formation was relatively rapid, as the A_{410} was constant within 15 min after mixing the reagents. Addition of Cu(II) (0.5 mM) shifted the maximum absorbance to 377 nm, and this peak increased intensity for about 2 h and then leveled off. It is clear that the amino phosphonates reacted to form Schiff bases with the pyridoxal and that this Schiff base was capable of complexing Cu(II), as the spectral changes described here are in accord with the observations of Langohr.[7] However, neither amino phosphonate Schiff base was able to produce orthophosphate at temperatures of 45, 65, and 100 °C (with or without added Cu(II) over the pH range 7–9.6 (amino phosphonates as buffer). Reasoning that the Cu(II) was complexing in part through the phosphonate oxygens, we synthesized (*o*-hydroxyphenyl)phosphonoglycine (OHPPG) in the hope that the phenolic oxygen would provide a competing complexing site for the Cu(II). Schiff base formation, as measured by the change in absorbance at 410 nm, was as rapid as with the less sterically hindered amino phosphonates. The complexation of the Cu(II) appeared slightly faster as the absorbance peak at 377 nm reached a maximum value within 1.5 h. Table I indicates that the Cu(II)-complexed Schiff base between PL and OHPPG was able to fragment, producing orthophosphate.

Table I. Test for Orthophosphate Formation from Amino Phosphonates and Pyridoxal

temp, °C	reactants[b]	% reaction[a,d] at pH 7.0[c]	8.0	8.8	9.6
65	AMP + PL + Cu	nr	nr	nr	nr
65	AEP + PL + Cu	nr	nr	nr	nr
65	OHPPG	nr	nr	nr	nr
65	OHPPG + PL	nr	nr	nr	nr
65	OHPPG + PL + Cu	1	5	10	7
30	OHPPG + PL + Cu			nr	
45	OHPPG + PL + Cu			1	
80	OHPPG + PL + Cu			27	
100	OHPPG + PL + Cu			40	

[a] Percent reaction = (mole phosphate/mole amino phosphonate) × 100. Phosphate was determined by the procedure of Ames.[12] nr = no reaction detected. For the reactions at 30, 45, and 65 °C the reaction time was 8 h, and at 80 and 100 °C the reaction time was 4 h. [b] AMP = aminomethanephosphonate, AEP = 1-aminoethanephosphonate, PL = pyridoxal OHPPG = (*o*-hydroxyphenyl)phosphonoglycine, Cu as $CuSO_4$. [c] Initial pH of solution. [d] For pH 7 and 8 the buffering component was the amino phosphonate, and for pH 8.8 and 9.6 the buffer was 0.05 M borate.

Reaction Requirements. Having observed orthophosphate formation from the Cu(II) chelate of the Schiff base between OHPPG and PL, we investigated the conditions required for the reaction. Table I indicates the results of several reactions. It is apparent from the table that phosphate formation requires the presence of all three components, (*o*-hydroxyphenyl)phosphonoglycine, pyridoxal, and Cu(II), over the range pH 7–9.6. By our standard assay conditions for phosphate formation, we could detect as little as 0.5 nmol of phosphate (0.01% reaction). Under the standard reaction conditions, 65 °C for 8 h, the amount of phosphate produced was maximal at pH 8.8. The temperature dependence for phosphate formation indicates that measurable reaction occurs only at temperatures exceeding about 45 °C, and the amount of phosphate produced in 8 h increases steadily as the temperature increases above this value.

Product Distribution from the Cleavage of Keto Phosphonate. The pyridoxal-catalyzed transamination of α-amino carboxylates[8] and phosphonates[9] is known. This reaction would produce an α-keto phosphonate from an α-amino phosphonate. The P–C bond in α-keto phosphonates is fairly labile. Berlin[10] has characterized the mechanism for cleavage of this class of compounds. In basic solution, the products of the reaction are the carboxylic acid and phosphorous acid dianion. We considered the possibility of this type of mechanism, accounting for the cleavage of the P–C bond in OHPPG. The keto phosphonate is relatively stable at 100 °C, pH 8.8. Phosphorus NMR analysis indicates about 15% decomposition in 1 h. The phosphorus products were phosphorous acid and orthophosphate in a ratio of 14 to 1 and unreacted keto phosphonate. The orthophosphate apparently arises from oxidation of the phosphorous acid by the small amount of dissolved oxygen present in the solution. In the presence of Cu(II), the decomposition was about 2-fold greater under the same conditions; 32% of the keto phosphonate was converted to phosphorous acid in 1 h at 100 °C. The extent of the decomposition was inhibited if the keto phosphonate was preincubated for 15 min with pyridoxamine prior to the addition of the Cu(II).

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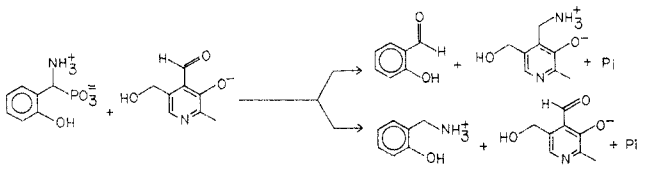
Scheme I. Possible Products of the Dephosphonylation Reaction



In this situation, 12% of the keto phosphonate was converted to phosphorous acid in 1 h at 100 °C. In each case, HPLC analysis of the products of these reactions demonstrated the formation of salicylic acid and the presence of unreacted keto phosphonate in a ratio consistent with the ^{31}P NMR analysis. When the reaction mixture contained the keto phosphonate, pyridoxamine, and Cu(II), three other minor peaks appeared in the HPLC elution profile. Two of these peaks were due to the formation of OHPPG and pyridoxal. These products apparently arise from a transamination reaction and account for about 5% of the loss of the keto phosphonate. The third new peak in the HPLC trace (retention time 18.9 min) was unidentified. On the basis of peak area analysis of the amount of keto phosphonate remaining and the amount of OHPPG formed, the third product peak could have been formed from no more than 2% of the keto phosphonate.

Product Distribution from the Cleavage of the Amino Phosphonate. The products of the pyridoxal-catalyzed dephosphonylation reaction were determined by HPLC. We considered, in analogy with pyridoxal-catalyzed decarboxylations,[11] the formation of either *o*-hydroxybenzylamine plus pyridoxal or salicylaldehyde plus pyridoxamine from the cleavage of the P–C bond (Scheme I). The pair of products formed would depend upon which carbanion was the most stable, the carbanion α to the pyridine ring or the carbanion α to the benzene ring. It was clear from the elution profile of reaction products obtained by incubation for 4.0 h at 100 °C in borate buffer (pH 8.8) that salicylaldehyde and pyridoxamine were formed. It was also clear that transamination was a competing reaction. In all cases, reaction products demonstrated the same retention times (±0.05 min) as the corresponding synthetic compounds. We followed the rate of formation of salicylaldehyde and orthophosphate as a function of time. HPLC analysis indicated salicylaldehyde was formed according to first-order kinetics, as was orthophosphate. The rate constant for salicylaldehyde formation was 1.20×10^{-2} min^{-1} ($\pm 1 \times 10^{-3}$) and the rate of formation of orthophosphate was 1.30×10^{-2} min^{-1} ($\pm 2 \times 10^{-3}$) as determined from the average of two measurements. Product and kinetic analyses indicated that about 40% of the OHPPG reacts to form salicylaldehyde, pyridoxamine, and orthophosphate, while the other 60% reacted to form the keto phosphonate and pyridoxamine. At 60°C approximately 20% of the OHPPG reacts to form salicylaldehyde, pyridoxamine, and orthophosphate, and the remaining material partitions to form the keto phosphonate. The rate of salicylaldehyde formation at this temperature was 3.3×10^{-4} min^{-1}. It is interesting to contrast these results with the analogous reaction of (*o*-hydroxyphenyl)glycine (OHPG), the carboxylate analogue of the amino phosphonate we investigated. In this case, OHPG did not decarboxylate. Rather, pyridoxal-catalyzed transamination, producing the α-keto carboxylate, was the only reaction detected.

Discussion

The formation for the Schiff base between amino phosphonates and pyridoxal is readily demonstrated by the absorption changes near 410 nm. This peak is characteristic of the Schiff base between pyridoxal and amines and has been demonstrated by several workers using amino acids[7-9,16] and amino phosphonic acids.[7,9] As demonstrated by Langohr,[7] Schiff base formation is not complete, and the extent of formation is highly pH dependent. The maximum Schiff base formation occurs near the pK_a of the amino group undergoing condensation. The addition of Cu(II) causes a shift in the absorption peak consistent with chelation of the Schiff base. The formation of the chelate at room temperature is relatively slow, even when excess pyridoxal and Cu(II) are present. At the higher temperatures required for the dephosphonylation reaction the rate of chelate formation must increase considerably. Nevertheless, chelate formation is evident with all the α-amino phosphonates used in this study. Under the proper conditions of concentrations and pH, we can show complete chelate formation within 2 h at 25 °C. This is much faster than the rate of chelate formation observed by Langohr.[7] In that study, equal molar concentrations of all species were used. An earlier study by Cassaigne,[9] under conditions similar to those used in this work, demonstrated chelate formation on the same time scale as we report. The chelation of Cu(II) is expected to drive Schiff base formation to completion, as the logarithm of the formation constant for this type of chelate has been estimated as 15 for α-amino phosphonates[7] and α-amino carboxylates.[17]

Our inability to observe any orthophosphate formation from the Cu(II) chelate of aminomethanephosphonic acid is apparently due to the involvement of the phosphonate oxygens in the chelation ring. This chelation would result in electron withdrawal from the phosphonate group. Electron flow would therefore occur in the wrong direction for dephosphonylation. The incorporation of a second potential chelation site, via the ortho phenolic oxygen of OHPPG (Scheme II), apparently alleviates this problem to some extent. However, we have not examined the extent to which the phenolic oxygen and the phosphonate oxygens compete in the Cu(II) chelate of the Schiff base between OHPPG and PL. Although it is difficult to find appropriate model systems in the literature, we can compare the stability constants for Cu(II) complexed by 2-aminoethanephosphonate ($\log K = 8.5$[18]) and pyridoxamine ($\log K = 10.2$[19]). It is probable, given the similarity in the chelating ability of these two types of oxygen (the ratio of the two stability constants is 50), that both chelate forms exist in solution. Given this, it is not surprising that we can identify two reaction pathways. The reaction pathway that predominates at all temperatures examined is the one that results in α proton removal from the OHPPG moiety. This results in the transamination of the amino phosphonate (Scheme II, upper path). This reaction can occur from either chelate structure. The second reaction is cleavage of the P–C bond in a reaction similar to a de-

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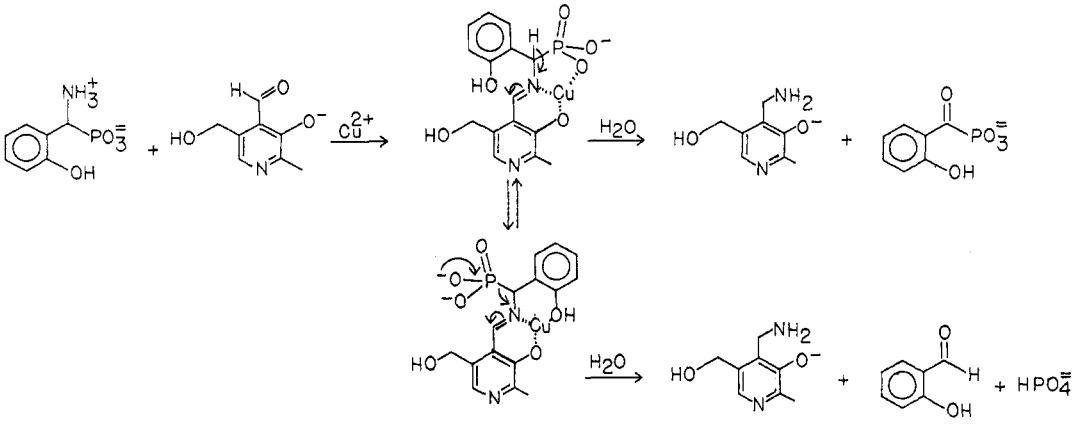
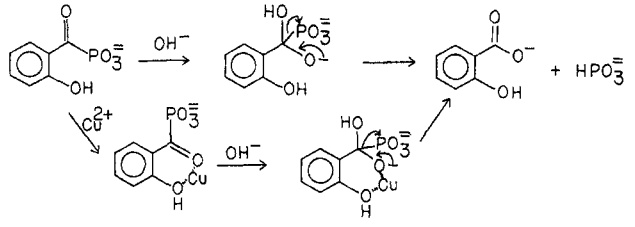
Scheme II. Mechanism of the Dephosphonylation Reaction



Scheme III. Mechanism for the Dephosphonylation of the α-Keto Phosphonate



carboxylation (Scheme II, lower path). This pathway is apparently not possible when the phosphonate oxygens are involved in the chelation of the Cu(II) (vide infra from the reaction of aminomethanephosphonic acid).

The model we propose for the pyridoxal-catalyzed dephosphonylation reaction is depicted in Scheme II. Dephosphonylation occurs from the Cu(II) chelate of pyridoxal and OHPPG. The rate of formation of this chelate must be greater than the rate of the dephosphonylation, as salicylaldehyde is formed by a first-order kinetic step. The dephosphonylation presumably results in the initial formation of metaphosphate and the Schiff base between salicylaldehyde and pyridoxamine. For our HPLC product analysis, this Schiff base is hydrolyzed by the addition of acetic acid, allowing us to confirm the identity of the aromatic products. The metaphosphate subsequently reacts with water in a very rapid step, producing the product orthophosphate. As expected by this scheme, the rate of phosphate formation is the same as the rate of salicyaldehyde formation (within experimental error). HPLC analysis also confirms the occurrence of the upper reaction pathway of Scheme II. We were able to show the formation of the α-keto phosphonate depicted (after acid hydrolysis of the Schiff base). The chelated product indicated in the upper pathway is apparently fairly stable, as salicylic acid is only a very minor product of this reaction (<4%). The salicylic acid would arise from the base-catalyzed hydrolysis of the keto phosphonate. This can be contrasted to the solution reactivity of the keto phosphonate itself (Scheme III). The base-catalyzed hydrolysis of the keto phosphonate is fairly rapid (the estimated rate constant is 2.6×10^{-3} min^{-1}). The Cu(II) ion apparently accelerates this rate 2-fold. This catalytic effect may result from chelation of Cu(II) by the ketone oxygen and the phenolic hydroxyl group. This structure would presumably lower the energy for formation of the anionic tetrahedral intermediate. As we have shown by both ^{31}P NMR and HPLC analysis, this reaction of the keto phosphonate cannot account for any of the major products observed in the dephosphonylation of OHPPG. The slightly larger rate of formation of orthophosphate during the dephosphonylation of OHPPG may be due, in part, to this side reaction. Cassaigne et al.[9] also reported orthophosphate formation when α-amino phosphonates were incubated with pyridoxal and Cu(II). They attributed this to the decomposition of the corresponding keto phosphonate. As we show, this explanation cannot be correct. The keto phosphonates produce phosphorous acid. Unfortunately, the French workers did not look for the formation of the carboxylates expected for the cleavage of the keto phosphonates. It is unclear whether they observed dephosphonylation of the Schiff base between their amino phosphonates and pyridoxal or whether the phosphate arose from the air oxidation of the phosphorous acid produced from the cleavage of the keto phosphonates.

The reaction of OHPPG can be compared with the reaction of the corresponding carboxylic acid compound. When (*o*-hydroxyphenyl)glycine is reacted with PL and Cu(II), only transamination results. This difference in reactivity can be accounted for by the larger negative charge density of the phosphonic acid relative to the carboxylate. Since the α proton of the Schiff base is either removed by the negatively charged borate anion or by hydroxide ion, electrostatically we expect this reaction to be slower in the phosphonic acid case than in that of the carboxylate. This unfortunate circumstance prohibits us from being able to compare the rate of dephosphonylation with the rate of decarboxylaton.

The fact that pyridoxal can catalyze decarboxylation of amino acids and amino phosphonates indicates that it should certainly be possible for an enzyme to catalyze the dephosphonylation of an α-amino phosphonate using pyridoxal phosphate as the active-site catalyst. Such an enzyme-catalyzed reaction has yet to be observed. Although the exact nature of the initial phosphorus product for the dephosphonylation reaction is not known, based on analogy with the Conant–Swan fragmentation,[20] we expect metaphosphate or a metaphosphate-like species to be formed.[21] The reactivity of metaphosphate is such that if it were formed at the active site of an enzyme it would be expected to react with any nucleophile available, resulting in the phosphorylation of the enzyme. Since the enzyme would probably not be able to catalyze the hydrolysis of such a foreign species, we would expect the enzyme to be irreversibly inhibited. It is possible that under the proper conditions α-amino phosphonates could be developed as active-site-directd mechanism-based inhibitors of enzymes.

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Experimental Section

General Procedures. All reagents were of the highest purity commercially available and were used as received unless otherwise specified. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^1H NMR were obtained on a Varian EM-360 or Varian FT-80A instrument and are referenced to tetramethylsilane. ^{13}C and ^{31}P NMR were obtained on the Varian FT-80A instrument using a broad-band probe and are referenced to Me_4Si and 85% phosphoric acid, respectively. Microanalysis was performed by Galbraith Laboratories. UV/vis spectra were obtained on a Beckman Model 25 spectrophotometer. Pyridoxal hydrochloride, pyridoxamine dihydrochloride, and aminomethanephosphonic acid were obtained from Sigma. The 1-aminoethanephosphonic acid was from Fluka, and the (*o*-hydroxyphenyl)glycine was purchased from Alfa.

Methods. Standard reactions were performed in 0.05 M borate buffer, pH 8.8, or at pH 8.8 with the amino phosphonates as the buffering component. In all cases, the pyridoxal, pyridoxamine, and amino phosphonate solutions were adjusted to pH 8.8 prior to addition to the reaction. Reaction mixtures contained either 4.0 or 30 mM pyridoxal, 5.0 or 50 mM cupric sulfate, and 3.3 or 20 mM amino phosphonate. These concentrations were obtained by adding the appropriate volumes of 30 or 200 mM stock solutions of each. Pyridoxal solutions were used wihin 3 days. It is best to mix the pyridoxal and amino phosphonate, allowing them to react for about 5 min, and then add the solution of the copper salt. The buffer, if any, is added about 10 min later. This sequence avoids precipitation when borate buffers are used. In all cases where orthophosphate was produced, a greenish precipitate developed in the reaction vials. This was apparently due to precipitation of the copper salt of borate or phosphate. All reaction mixtures and stock solutions were purged with nitrogen for 10 min to minimize oxidation of the pyridoxal at the elevated temperatures required for the chemical reaction. Reactions were performed in total volumes of 1 mL in Teflon-lined screw cap vials. Temperature was maintained by a Thermolyne dry-block heater. Aliquots (0.1 mL) were removed as a function of time and diluted 3-fold with 1 M acetic acid to solubilize any precipitate and hydrolyze the Schiff base. Phosphate was determined by the procedure of Ames.[12] All glassware was washed with sulfuric acid and KOH solution and rinsed with distilled water to avoid extraneous phosphate contamination.

HPLC separations were performed with a Perkin-Elmer LC-4 and an Alltech Econosphere C-18 (5-μm) column (4.6 × 250 mm). A linear gradient, 3–20%, of 0.4 M phosphoric acid in acetonitrile/water (60/40, v/v) was used to elute the products from the column. Detection was at 254 nm (OHPPG, salicylaldehyde) or 300 nm (pyridoxal, keto phosphonate, pyridoxamine) on a Perkin-Elmer LC-90 UV detector. Peak integrations were obtained with a Perkin-Elmer LCI-100 apparatus. Peak areas were strictly linear with the amount of the various compounds injected onto the column. The acetic acid diluted aliquots were filtered through disposable 0.45-μm filters prior to injection into the HPLC.

The analysis of the rate constant for the reaction was performed by averaging the data from two separate runs and fitting the concentration vs. time data by a nonlinear curve-fitting routine. The data were fit to a first-order equation and a second-order equation. The sum of the residuals squared indicated that the first-order equation was better able to describe the rate data. For the second-order fit, the concentration of pyridoxal at each time was determined from the HPLC analysis in the same way as the concentration of salicylaldehyde.

In some cases ^{31}P NMR was used to confirm the identity of phosphorus-containing products. If the reaction contained Cu(II), the metal was removed by passing the reaction mixture through a 10-mL bed volume column of Biorad Chelex 100. Elution was accomplished with 0.01 M borate, pH 9. The eluate was concentrated and dissolved in deuterium oxide containing 1 mM EDTA. The phosphorus NMR spectrum was recorded under proton-coupled and -uncoupled conditions. This allowed phosphorous acid to be identified by the shift due to the large (600-Hz) H–P coupling constant.

Materials. 2-(Aminomethyl)phenol (*o*-hydroxybenzylamine) was obtained by reduction of the oxime of salicylaldehyde with 2.5% sodium amalgam:[13] mp 128 °C (lit.[14] mp 129 °C; ^{13}C NMR (D_2O, 0.5 N NaOH) δ 61.04, 137.07, 140.66, 142.06, 152.43, 152.55, 176.26; ^1H NMR (D_2O, 0.5 N NaOH) δ 6.70–7.30 (m, 4 H), 4.00 (s, 2 H).

Bis(cyclohexylammonium) 1-(2-Hydroxyphenyl)-1-oxomethanephosphonate (Keto Phosphonate). Trimethyl phosphite (3.15 g) in 5 mL of dichloromethane was added to an ice-cold, stirred solution of acetylsalicyloyl chloride (5 g) in dichloromethane (10 mL). The reaction was allowed to warm to room temperature. After 12 h the solvent was evaporated and the residual yellow oil distilled, yielding dimethyl 1-(2-acetoxyphenyl)-1-oxomethanephosphonate: 5.4 g, 85%; bp 142–145 °C (0.1 mm); ^{31}P NMR ($CDCl_3$) δ −1.60 (s); ^1H NMR ($CDCl_3$) δ 8.30–8.10 (m, 1 H), 7.65–6.85 (m, 3 H), 3.77 (d, J = 11 Hz, 6 H), 2.27 (s, 3 H). This α-keto phosphonic diester (1 g) in 10 mL of dry chloroform was treated with bromotrimethylsilane (1.22 g, 1.1 mL). After 12 h at room temperature, the solvent was removed and the residual clear oil treated with 25 mL of methanol containing 1.2 g of cyclohexylamine. After concentration in vacuo the residue was dissolved in acetone/water (20/4, v/v). The yellowish crystals that separated were recrystallized from a minimal amount of water, yielding fine colorless needles: 0.52 g, 33%; ^{31}P NMR (D_2O) δ 1.13 (s); ^1H NMR (D_2O) δ 8.45–8.30 (m, 1 H), 7.65–6.85 (m, 3 H), 3.10 (br s, 2 H), 2.00–1.00 (m, 20 H). Anal. Calcd for $C_{19}H_{33}N_2O_5P\cdot H_2O$: C, 54.53; H, 8.43; N, 6.69. Found: C, 54.13; H, 8.42; N, 6.38.

Monohydrogen 1-Ammonio-1-(2-hydroxyphenyl)methanephosphonic Acid [(*o*-Hydroxyphenyl)phosphonoglycine, OHPPG] was synthesized by a slight modification of the general procedure of Kudzin.[15] Salicylaldehyde (2.44 g), triphenyl phosphite (6.21 g), and *N*-phenylthiourea (3.04 g) in 25 mL of glacial acetic acid were stirred at room temperature for 20 min and then at 80 °C for 2 h. Concentrated HCl was added and the mixture refluxed overnight. The solvent was evaporated and 20 mL of water added, and then the mixture was evaporated again. The residue was dissolved in 50 mL of ethanol and treated with small portions of propylene oxide until the precipitate stopped forming. The pinkish solid was filtered and recrystallized from water. The solid was then dried at 60 °C (0.5 mm) in a drying pistol: 2.43 g, 60%; mp >250 °C; ^1H NMR (D_2O, 2 N NaOD) δ 7.45–6.90 (m, 4 H), 4.71 (d, J = 16.5 Hz, 1 H); ^{31}P NMR (D_2O, 2 N NaOD) δ 21.79 (s); ^1H-coupled ^{31}P NMR (D_2O, 2 N NaOD) δ 21.79 (d, J = 15 Hz); ^{13}C NMR (D_2O, 2 N NaOD) δ 47.66 (d, J = 136 Hz), 114.53, 119.01, 127.94, 128.78, 131.57, 163.68. Anal. Calcd for $C_7H_{10}NO_4P$: C, 41.39; H, 4.96; N, 6.89. Found: C, 41.63; H, 5.35; N, 6.47.

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