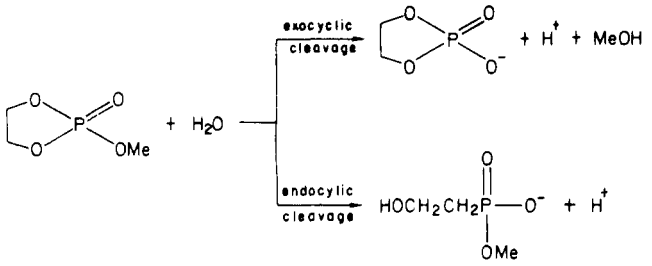
Exocyclic Cleavage in the Alkaline Hydrolysis of Methyl Ethylene Phosphate. Evidence Against the Significance of Stereoelectronic Acceleration in Reactions of Cyclic Phosphates

Ronald Kluger* and Gregory R. J. Thatcher

Contribution from Lash Miller Chemical Laboratories, Department of Chemistry, University of Toronto, Toronto, Canada M5S 1A1. *Received April 17, 1985. Revised Manuscript Received July 18, 1985*

Abstract: The hydrolysis of methyl ethylene phosphate serves as a mechanistic probe of phosphate reactivity and as a model for enzyme catalysis. Early reports, based on proton NMR and GC analysis, indicated that observable amounts of exocyclic cleavage products are obtained in the alkaline hydrolysis reaction [Kluger, R.; Covitz, F.; Dennis, E. A.; Williams, L. D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1969**, *91*, 6066]. However, later reports based on phosphorus NMR analysis of reactions in 5 M sodium hydroxide by advocates of a stereoelectronic theory indicated that, consistent with the theory, ring cleavage is the exclusive initial process [Taira, K.; Fanni, T.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106*, 1521. Taira, K.; Gorenstein, D. G. *J. Org. Chem.* **1984**, *49*, 4531]. In the present study, the products of the reaction of methyl ethylene phosphate with hydroxide under a variety of conditions were determined by proton NMR and by phosphorus NMR, with and without rapid quenching. Reactions of the products were also analyzed. The products of exocyclic cleavage, methanol and ethylene phosphate, *are* produced in the initial reaction to the extent that was reported in 1969. Subsequent reaction of the ring-cleaved products is too slow to account for the initial production of methanol. These data suggest that ring strain and apicophilicities of substituents, rather than orbital interactions, account for the substantial differences in the reactivity of phosphate esters.

Methyl ethylene phosphate reacts six orders of magnitude faster with water and hydroxide than does trimethyl phosphate.[1-3] Studies of this reactivity pattern have formed the basis for understanding the reactions of phosphates and the action of enzymes.[4-7] Methyl ethylene phosphate can rapidly undergo either



exocyclic or endocyclic ester cleavage. In dilute hydroxide, almost exclusive endocyclic cleavage is evidenced by the formation of only a very small proportion of methanol, a product of exocyclic cleavage.[2] However, at higher hydroxide concentrations, the yield of methanol from the initial reaction increases. A detailed mechanistic analysis has been presented which is consistent with the involvement of reactive pentacoordinated phosphorus intermediates which may undergo pseudorotation to cause exocyclic cleavage.[2]

Recently, Gorenstein and co-workers proposed a modified stereoelectronic theory[8-12] of reactivity of phosphate derivatives (related to a proposal of Lehn and Wipff[13]) in which exocyclic cleavage of methyl ethylene phosphate is highly disfavored (by up to 11 kcal/mol) relative to endocyclic cleavage.[14-16] They reported phosphorus NMR experiments with rapid quenching of reaction solutions[14-16] that purported to establish that "methyl ethylene phosphate hydrolyzes with complete endocyclic cleavage between pH 8 and 15"[15,16] as required by the stereoelectronic theory. Since this casts doubts on the validity of the earlier observation of exocyclic cleavage and the resulting conclusions about reaction mechanisms,[3] it is important to set the record straight.

In this paper we show that the original reports of exocyclic cleavage of methyl ethylene phosphate in alkaline solutions[2] are reproducible. Rapid quenching of the reaction gives results consistent with those without such a quench. In addition, despite reports to the contrary,[15,16] peaks corresponding to products of initial exocyclic cleavage can readily be observed by phosphorus NMR. We also report kinetic and product studies that further elucidate reaction patterns of this system.

Experimental Section

Instruments. Proton NMR spectra were recorded with a Varian T-60 continuous wave spectrometer at 60 MHz. Phosphorus spectra were obtained on a Bruker WP-90 spectrometer at 36 MHz (and some were repeated with a Varian XL-200 spectrometer at 80 MHz) with fourier-transform analysis of accumulated signals and broad-band proton decoupling. All spectra were recorded for samples in 5 mm diameter NMR tubes. Chemical shifts are relative to DSS or Me_4Si for proton spectra and relative to trimethyl phosphate for phosphorus spectra. Phosphorus spectra were not used for quantitative analyses since variation in nuclear Overhauser enhancement and relaxation times between products can lead to variable sensitivity.[17,18] Thus, we rely on integrated continuous wave

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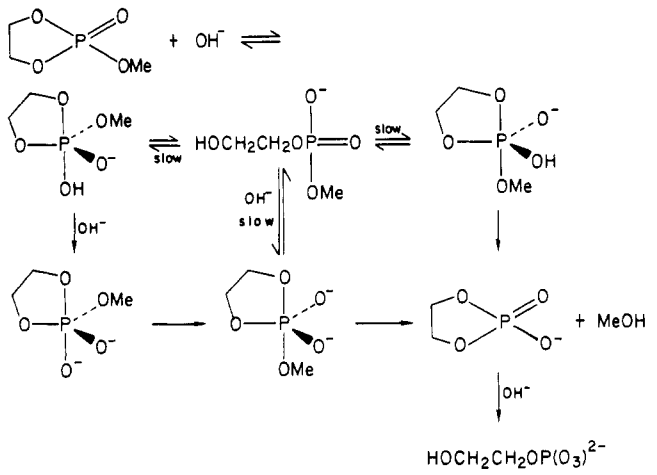
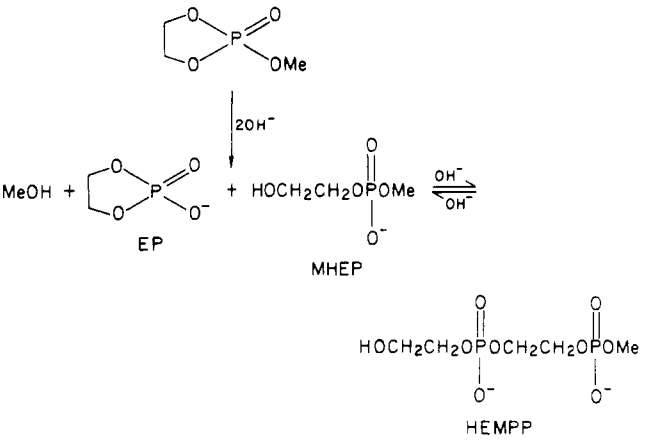
Scheme I



Scheme II



proton spectra for quantitative determinations.

Reactions. Kinetic and product analyses were based on integrated proton spectra recorded periodically. (Because NMR analysis was used, reactions were conducted in deuterated solvents. However, in general discussions of the reactions, the term "hydroxide" applies to results obtained with deuteroxide.) Reaction samples were contained in NMR tubes which were incubated in a constant-temperature bath maintained at 35 ± 0.1 °C or in the NMR spectrometer probe at the same temperature. Samples for product analysis and kinetics were mixed in the NMR tube by first adding the reaction medium to the NMR tube then layering the substrate (added from an Eppendorf mechanical micropipet) along the length of the inner wall of the tube after which the tube was capped. Reaction was commenced by vigorously shaking the tube. For "pH-jump" experiments, after the sample was dissolved, concentrated sodium deuteroxide solution was added directly from the pipet, followed by capping and shaking.

We followed the pseudo-first-order kinetics of the reaction in which methyl hydroxyethyl phosphate undergoes hydrolysis (see Scheme I). To obtain rate constants as well as to extrapolate to time of mixing we plotted the logarithm of the ratio of the integrated signal of the methanol methyl singlet to the combined integrated signals due to the methoxyl group (doublet) of methyl hydroxyethyl phosphate and the methyl group (singlet) of methanol.

Rapid Quench Procedure. In order to compare our results with those reported by Gorenstein and co-workers,[14-16] we also employed a rapid quench procedure. The method reported by those workers involves cooling the sample in a slurry of acetone and solid carbon dioxide followed by addition of concentrated sulfuric acid within 2 s of mixing. This produces a large volume of sodium sulfate precipitate and generates considerable localized heating. The solution is then treated with Tris buffer and filtered through nitrated glass wool and Chelex-100, presumably to remove paramagnetic impurities as well as solids. We were not able to reproduce this methodology reliably. We find it preferable to cool the reaction and then add concentrated HCl to bring the solution to slightly alkaline conditions.[2] No precipitates form, and spectra consist of sharp lines. However, as explained later, we find that quenching is usually unnecessary since extrapolation to time of mixing is straightforward for unquenched samples.

Materials. Deuterium oxide (99.9%) was obtained from Merck Sharp & Dohme of Canada. Sodium deuteroxide was obtained from the Aldrich Chemical Co. Potassium deuteroxide solution was prepared by dissolving reagent grade potassium hydroxide in deuterium oxide, concentrating the solution by lyophilization, and redissolving the residue in deuterium oxide. Concentrations of deuteroxide were determined by titration against standardized HCl.

Methyl ethylene phosphate was prepared by dropwise addition of 7.4 g of methyl dichloro phosphate (prepared by the reaction of phosphorus oxychloride with methanol followed by distillation) in 25 mL of dry benzene to 3.1 g of dry ethylene glycol in 50 mL, of dry benzene containing 5 g of 2,6-lutidine at 0 °C.[2,19] The lower temperature and shorter reaction time compared to the original preparations avoid decomposition of the product. After addition was complete, the solution was brought to room temperature and filtered to remove lutidine hydrochloride. The filter cake was washed thoroughly with benzene. The filtrate and washings were rotary evaporated and distilled under vacuum (yield 50%). The material was pure by both proton and phosphorus NMR analysis.[2,16] The liquid became a crystalline solid[20] when immersed in a low-temperature bath. Samples were stored as solids at −5 °C.

We also prepared methyl ethylene phosphate by oxidation of 15 mL of methyl ethylene phosphite[21] in 200 mL of methylene chloride at −10 °C with 1% ozone generated in a stream of dry oxygen for 20 h (until a downstream solution of KI darkened).[22] The reaction solution was concentrated under vacuum and distilled twice (bp 75 °C, 0.05 torr). The yield was 40% based on methyl ethylene phosphite. This material gave the same extent of exocyclic cleavage by proton NMR analysis as material prepared by the other procedure (at 5 M NaOD) and also gave identical phosphorus NMR results. The fact that comparable results were obtained with products from very different sources greatly reduces the possibility of our observations being due to an artifact of our synthesis.

Sodium ethylene phosphate was prepared by refluxing methyl ethylene phosphate in dry acetone containing 2 equiv of sodium iodide for 48 h. It is preferable but not necessary to remove methyl iodide formed dry the reaction by keeping the temperature of the condenser above that of the boiling point of methyl iodide. The precipitated product was recrystallized from ethanol.[22,23] The material showed a single peak in the proton-decoupled phosphorus NMR at 15.9 ppm downfield from trimethyl phosphate in deuterium oxide. The proton NMR spectrum in deuterium oxide consisted of the expected doublet at 4.2 ppm.[2]

Side Reactions. The products of the initial hydrolysis of methyl ethylene phosphate can react with one another: methyl hydroxyethyl phosphate may react with ethylene phosphate in analogy to the reactions derived from ethyl ethylene phosphate which were studied by Mathis and co-workers.[24] High concentrations and nonaqueous solvents promote this reaction and other polymerization processes.[24-26] We prepared the addition product of methyl hydroxyethyl phosphate and ethylene phosphate, 2-hydroxyethyl 2-(ethyl methyl phosphate) phosphate [HEMPP in Scheme II], by adding ethylene phosphate to a basic solution of methyl hydroxyethyl phosphate (phosphorus NMR chemical shifts in deuterium oxide: −1.2 and −2.2 ppm; the negative signs indicate that the peaks are upfield of trimethyl phosphate).

Validity of the Analytical Method. Under conditions where the hydrolysis of methyl ethylene phosphate is slow (pH 5), it has been reported that the hydrolysis is partially autocatalytic when high (0.2 M) substrate concentrations are used.[2] In dilute solutions (0.004–0.008 M), autocatalysis is not observed. At higher pH, where reaction with hydroxide is fast, autocatalysis does not compete. Thus, under conditions where reaction with water is slow, products should be analyzed for dilute solutions (avoiding product formation from the autocatalytic process). Thus, Kluger et al. used GC analysis of quenched reactions done in dilute solutions to determine methanol production.[2] This was in addition to the more convenient NMR analysis. The results for reactions conducted in acid or base (and thus reaction with solvent is fast) should be in close

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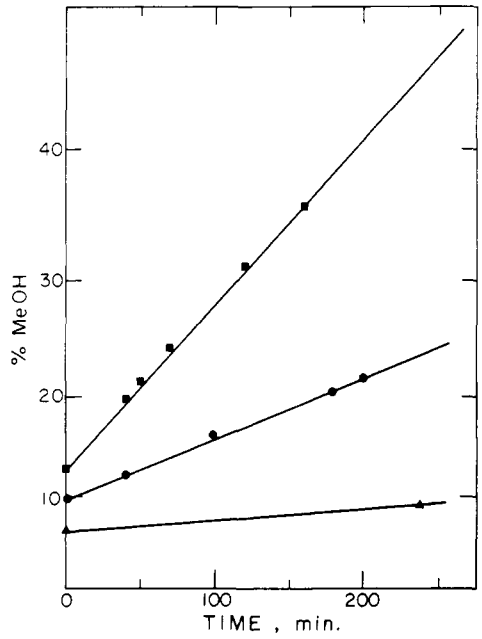

Figure 1. Typical time courses of methanol production (as percentage of methoxyl signal from methanol compared to total methoxyl concentration, determined by proton NMR) from methyl ethylene phosphate (0.3 M) and NaOD (1.4 M, ▲; 4.6 M, ●; 7.0 M, ■) in D_2O (35 °C). Further data (not shown) were obtained in each case. Rates at other base concentrations are shown in Figure 3. Data are plotted according to the integrated first order rate law with logarithmic ordinate.

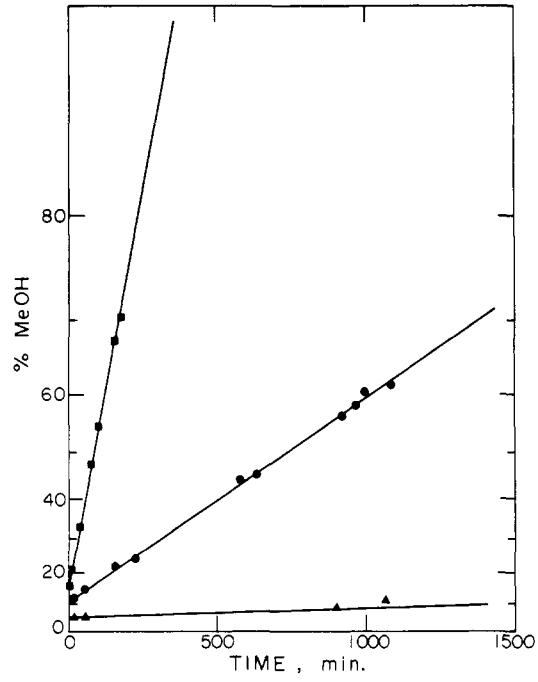

Figure 2. Typical time courses of methanol production (as percentage of methoxyl signal from methanol compared to total methoxyl concentration, determined by proton NMR) from methyl ethylene phosphate and KOD (1.6 M, ▲; 5.4 M ●; 10.9 M, ■) in D_2O (35 °C). Further data (not shown) were obtained in each case. Rates at other base concentrations are shown in Figure 3.

agreement by both methods and in that paper the agreement is noted. Therefore, proton NMR analysis is also valid in the present study.

Results

Proton NMR Analysis. Methyl ethylene phosphate was added to 0.3 mL of NaOD in D_2O by methods described in the Experimental Section. These samples were immediately analyzed by proton NMR. In addition to the signals due to phosphate esters, the methyl singlet of methanol (3.55 ppm; confirmed by addition of a genuine sample of methanol) appeared immediately. The methanol signal was integrated and compared to that of the methoxyl proton signal of methyl hydroxyethyl phosphate (Scheme I). The accuracy of the integration method was tested for reproducibility. The absolute uncertainty is about 2% (such as 10 ± 2% exocyclic cleavage) based on averages of three measurements. In the paper of Kluger et al.[2] uncertainties of "perhaps 10% of these values" were cited, meaning, for example, 10 ± 1% exocyclic cleavage. Unfortunately, this has been cited as an *absolute error* of ±5%[15,16] (that is 10 ± 5%).

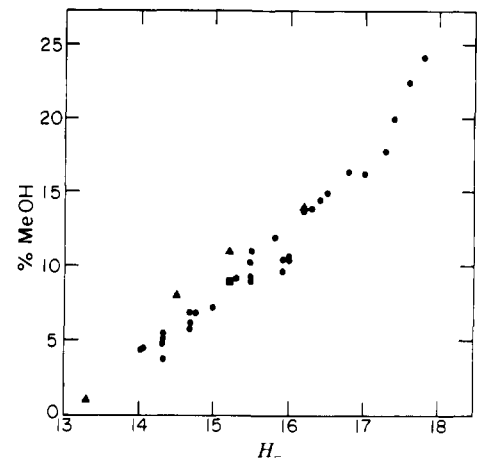

Figure 3. Extent of initial exocyclic cleavage of methyl ethylene phosphate in deuterium oxide as a function of H_- value of comparable undeuterated solutions. Points from this study are indicated as ●. The data were obtained from quench and/or kinetic extrapolation procedures, using NaOD or KOD and substrate concentrations ranging from 0.2 to 1.0 M. Points from ref 2 are included for comparison. Those which were obtained by GC analysis are indicated as ■ and those from proton NMR analysis are ▲.

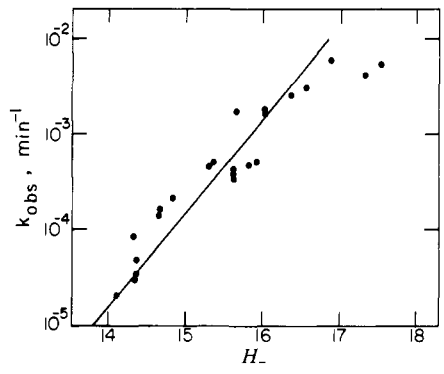

Figure 4. Hydrolysis of methyl hydroxyethyl phosphate at 35 °C as a function of H_- values of comparable undeuterated solutions. Data are compiled for catalysis by both KOD and NaOD. The rate constant for deuteroxide-catalyzed hydrolysis of methyl hydroxyethyl phosphate is approximately 2×10^{-5} min M^{-1}. The line is drawn with unit slope but has not been statistically fitted.

The methanol signal was integrated and compared to that of the methoxyl proton signal of methyl hydroxyethyl phosphate (Scheme I). The accuracy of the integration method was tested for reproducibility. The absolute uncertainty is about 2% (such as 10 ± 2% exocyclic cleavage) based on averages of three measurements. In the paper of Kluger et al.[2] uncertainties of "perhaps 10% of these values" were cited, meaning, for example, 10 ± 1% exocyclic cleavage. Unfortunately, this has been cited as an *absolute error* of ±5%[15,16] (that is 10 ± 5%).

The amount of methanol present after the initial reaction of methyl ethylene phosphate with base increases very slowly (see Figures 1 and 2) due to the hydrolysis of initially formed methyl hydroxyethyl phosphate (Scheme I). Selected samples were quenched by cooling and neutralizing with concentrated HCl, since it had been implied that rapid quenching is necessary for accurate analysis of the products of the initial reaction of methyl ethylene phosphate.[14-16] Samples in dilute base were not quenched since the product itself is an acid and neutralizes the base sufficiently to slow the reaction. In all cases, the results obtained by kinetic extrapolation and by quenching (Figure 3 and Table I) are in agreement.

The extent of initial rapid methanol production increases with deuteroxide concentration, approximately following H_-[27] (Figure

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Table I. Peaks Observed by Phosphorus NMR for Solutions (0.3 mL) in Deuterium Oxide (unless otherwise stated) after Addition of Samples to Solutions Described[a]

substrate	μL	[NaOD] or [KOD] initial	[NaOD] or [KOD] final	products:[b] P NMR shift MEP	EP	HEP	MHEP	initial exocyclic cleavage[c]
EP	20[d]	0	0		15.90			
MEP	15			17.72[e]				
MEP	20	7	0.2			1.69	−0.99	10.3
MEP	15	5.6	0.1		15.90	1.62	−0.90	9.2
MEP	17	1.0	0.35		15.87	1.32	−1.03	4.4
MEP	20	1.0	<0.2		15.90		−0.99	4.4
MEP	10	4.2	0.1		15.87		−0.99	8.0

[a] Freeze–acid quench procedure is described in the text. All post-quench solutions were alkaline to pH paper. Chemical shifts are ppm downfield from trimethyl phosphate in deuterium oxide. Percentage of exocyclic cleavage is based on amount of methanol produced as determined by proton NMR integration. In addition, peaks due to HEMPP are observed, as discussed in the text. Further data were obtained at other concentrations of base and substrate. The amount of EP relative to HEP depends upon the final concentration of deuteroxide since EP undergoes base-catalyzed hydrolysis. [b] Abbreviations: sodium ethylene phosphate (EP), methyl ethylene phosphate (MEP), 2-hydroxyethyl phosphate (HEP), methyl 2-hydroxyethyl phosphate (MHEP). The structure of HEMPP is in Scheme II. [c] Quantities determined by proton NMR analysis of methyl signals. [d] Milligrams of solid. [e] In anhydrous acetonitrile-d_3.

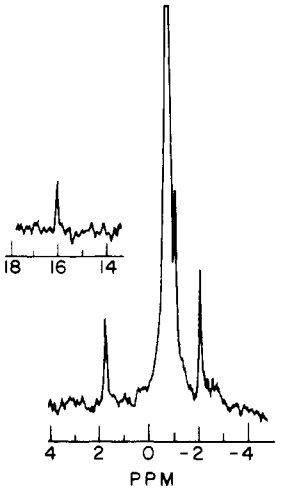

Figure 5. Phosphorus NMR spectrum after reaction of methyl ethylene phosphate in 5.6 M KOD followed by quenching with HCl. Peak assignments are indicated in Table I.

3). The rate of hydrolysis of methyl hydroxyethyl phosphate also increases as deuteroxide concentration increases (Figure 4). The rate is about 10000 times that of dimethyl phosphate and 1/100 that of ethylene phosphate[23] under comparable conditions. These results are consistent with a mechanism involving intramolecular nucleophilic catalyis by the hydroxyl group (effective molarity, 10^4 M) forming ethylene phosphate as an intermediate (Scheme I).

Phosphorus NMR Analysis. Solutions were cooled and quenched with concentrated HCl when concentrated base was used since the analytical method is relatively slow. For more dilute base solutions, no acid was added since the product itself neutralizes the base sufficiently. In all cases, a large peak due to methyl hydroxyethyl phosphate appears. However, contrary to the reports of Gorenstein and co-workers,[15,16] peaks corresponding to hydroxyethyl phosphate and ethylene phosphate are apparent (Table I and Figure 5). We confirmed the peak assignment for ethylene phosphate by adding a genuine sample and obtaining a coincident peak.

Reactions between Products. As discussed earlier, the expected reaction product of ethylene phosphate and methyl hydroxyethyl phosphate (HEMPP, for the reaction and other abbreviations see Scheme II) is formed to a significant extent.[24] Genuine samples of HEMPP were prepared in situ by adding ethylene phosphate to methyl hydroxyethyl phosphate in basic solutions. Prior to making the solution basic, no phosphorus NMR peaks other than those of the reactants were observed. The new peaks in the phosphorus NMR coincided with the highest field peaks that

(28) Covitz, F.; Westheimer, F. H. *J. Am. Chem. Soc.* **1963**, *85*, 1773.

Table II. Effects of Variation of Reaction Conditions on Percentage Exocyclic Cleavage of Methyl Ethylene Phosphate [MEP] in Deuterium Oxide

base, M	H_-[a]	MEP, M	%
NaOD, 9.2	16.0	0.3	10.5
NaOD, 9.2	16.0	0.6	10.5
NaOD, 3.1	14.7	0.33	5.8
NaOD, 3.1	14.7	0.66	6.2
KOD, 1.6	14.4	0.33	5.5
KOD, 1.6	14.4	0.66	4.8
NaOD, 8.4	15.8	0.9	12.0
NaOD, 8.4	6% CD_3CN[b]	0.23	9.0

[a] H_- values are for nondeuterated solutions from ref 27 since values for deuterated solutions are unavailable. [b] Solution was cloudy. H_- value unknown.

appear during the course of hydrolysis of high concentrations of methyl ethylene phosphate in basic solutions. The intensity of these peaks relative to those of other products varies as described below.

On the basis of the mechanism in Scheme II, the following predictions of product patterns were made and verified: (a) Increasing substrate concentration increases the amount of HEMPP relative to methyl hydroxyethyl phosphate. (b) Increasing base concentration increases the amount of HEMPP relative to methyl hydroxyethyl phosphate. (c) The percentage of methanol produced initially is independent of substrate concentration (Table II).

These statements are reflected in the following rate laws based on Scheme II.

$$d[HEMPP]/dt = k[EP][MHEP][OH^-] \quad (1)$$

At a constant hydroxide concentration

$$d[HEMPP]/dt = \alpha[MEP]^2 \quad (2)$$

$$d[EP]/dt = \beta[MEP] \quad (3)$$

At constant substrate level and varying base concentration

$$d[MHEP]/dT = \gamma[OH^-] \quad (4)$$

$$d[EP]/dt = \delta[OH^-]^2 \quad (5)$$

The total amount of ethylene phosphate produced initially should equal the sum of ethylene phosphate, HEMPP, and hydroxyethyl phosphate observed in the quenched solution. Since methanol does not undergo further reaction, the sum of these concentrations should equal the concentration of methanol produced initially. Integrations in our phosphorus NMR spectra follow this trend.

Other possible combinations of reactants do not give the observed results. For example, if production of HEMPP were the result of a reaction of methyl ethylene phosphate and methyl hydroxyethyl phosphate, the amount of HEMPP relative to methyl

Table III. "pH Jump": Percent Exocyclic Cleavage 2 min after Addition of Base to Partly Buffered Solutions (pD 7) of MEP

buffer, M	MEP, M	final OD^-, M	%
phosphate, 0.05	0.3	6.3	5
phosphate, 0.05	0.3	6.7	6
phosphate, 0.05	0.3	not added	no reaction[a]
2,6-lutidine, 0.3	0.3	6.9	6
2,6-lutidine, 0.3	0.3	6.9	8
2,6-lutidine, 0.3	0.3	not added	no reaction[a]

[a] Control experiment.

hydroxyethyl phosphate would not increase with hydroxide concentration. Although the addition of methyl hydroxyethyl phosphate to methyl ethylene phosphate is base catalyzed, the addition of hydroxide to methyl ethylene phosphate will also increase in rate and thus no differential increase in HEMPP would be observed. However, since the yield of HEMPP increases with hydroxide concentration, a secondary reaction of ethylene phosphate is implicated, since ethylene phosphate itself is produced in higher proportion as hydroxide concentration increases.

Ruling Out Improbabilities. Various alternative explanations for the production of methanol and ethylene phosphate were tested by the product studies above. In addition we did the following.

Predilution pH Jump. Could a complex series of reactions occur in undiluted methyl ethylene phosphate as it is dispersed into water which lead to anomalous production of methanol? Since the relative amount of methanol produced is independent of substrate concentration, this explanation requires that undetected interfacial phenomena occur during the dissolution process. Although our methods of mixing were designed to avoid this possibility, we performed control experiments (Table III). We predissolved methyl ethylene phosphate in buffered neutral solution in an NMR tube and then immediately added sodium deuteroxide solutions from a pipet and capped and shook the sample tube. Control samples in the same buffer did not react during the time of the analysis as observed by proton NMR. (The hydrolysis of methyl ethylene phosphate eventually generates sufficient acid to overcome the buffer but this takes several minutes. Excess buffer was avoided since buffers catalyze hydrolysis.[28]) The sample in the alkaline solution was immediately analyzed for the production of methanol by NMR. From this analysis, we ascertain that the relative amount of exocyclic cleavage was between 5% and 6%. This amount is expectedly less than the amount observed when the sample is added directly since the base must mix with the buffered solution. The reaction occurs in a less alkaline environment than the final solution since mixing must be completed. Since 1% or less exocyclic cleavage products result at pH 13 or less and the expected half-life at pH 13 is about 0.1 s,[2] the amount of exocyclic cleavage we observe is consistent with the results from direct addition. Reactions in droplets could not have occurred yet exocyclic cleavage is observed.

Cations, Trace Catalysts, and Impurities. Could exocyclic cleavage be due to an impurity in the sodium deuteroxide? To test this, we used potassium deuteroxide in place of sodium deuteroxide and also observed exocyclic cleavage (Figures 1–3). Since H_- for comparable concentrations is higher for potassium ion than sodium ion,[27] we expect and observe higher amounts of exocyclic cleavage when comparable concentrations of the two are used. This rules out an unknown catalytic impurity being present in the deuteroxide. We also synthesized the substrate by two independent routes and obtained the same results.

Cosolvents. The use of these was avoided because they are unnecessary and are expected to promote reactions of methyl ethylene phosphate with itself.[24] However, since recent studies used dioxane as a cosolvent, we tested this methodology.[15,16] Since dioxane will interfere with proton NMR analysis, we first tried adding the substrate in deuterated acetonitrile to a solution of sodium deuteroxide. This caused considerable cloudiness and affected the product composition (Table II). We also observed that dioxane does not dissolve in 5 M NaOH solution but forms a suspension. Since the reaction solutions are high in ionic strength, cosolvents may cause localized concentration of the substrate and create artifacts.[24] It is noteworthy that calorimetric studies have utilized direct addition of methyl ethylene phosphate to aqueous solutions. The substrate was observed to be well-behaved.[20]

Discussion

Our results indicate that methyl ethylene phosphate undergoes reaction to give both exocyclic and endocyclic cleavage products, consistent with the mechanism in Scheme I. The formation of ethylene phosphate and methanol in an initial reaction is demonstrated by the observation by proton NMR of methanol in samples that were quenched with acid as well as those that were not. Furthermore, we clearly see peaks in the phosphorus NMR corresponding to ethylene phosphate and 2-hydroxyethyl phosphate. The absence of such peaks had been reported by Gorenstein and co-workers to implicate an important manifestation of stereoelectronic effects in these systems.[15,16] On the basis of our results, we must conclude that an experimental deficiency in the procedures used by those workers to analyze their samples led to their observation. Possible sources of error include lack of calibration for phosphorus NMR analysis, selective product trapping during their quench-filtration procedure, or artifacts due to the use of cosolvent. Whatever the case, our results are completely in accord with the earlier work from Westheimer's group:[2] *methyl ethylene phosphate undergoes exocyclic cleavage in alkaline solution. The extent of exocyclic cleavage increases with added base.*

Reaction Scheme. The minimal amount of exocyclic cleavage of methyl ethylene phosphate in dilute base indicates that the initial monoanionic pentavalent intermediate in Scheme I undergoes ring opening faster than pseudorotation.[2] To explain the rise with alkalinity, a mechanism was proposed in which proton removal from the intermediate competes with ring opening, generating the conjugate base which rapidly pseudorotates[2] or which pseudorotates as the proton is removed (Scheme I). Can we ignore stereoelectronic effects and still account for our results?

As base concentration is increased, the *rate* of proton removal relative to the rate of ring opening of the monoanion increases. Since the intermediate should be a very weak acid,[2] transfer of a proton to hydroxide will be endergonic. We see that the yield of methanol increases with deuteroxide concentration. The mechanism in Scheme I requires that direct opening of the monoanionic intermediate continues to compete with proton removal and pseudorotation. If the dianion loses methoxide at the same rate at which its ring opens, then the amount of exocyclic cleavage reflects half the amount of reaction occurring via the dianion. At the base concentration at which 25% exocyclic cleavage would be observed, the rates of proton transfer and ring opening of the monoanion are equal. Where ring opening of the monoanion is three times the rate of dianion formation, we would expect about 12% exocyclic cleavage. This roughly corresponds to the conditions at 9 M NaOD. We do not know the upper limit of exocyclic cleavage, but in 12.8 M potassium deuteroxide (H_- = 17.6) we observe 22.5% initial exocyclic cleavage. In saturated sodium deuteroxide (18.5 M, H_- = 17.8), we observe 24% initial exocyclic cleavage. Therefore, this simple model appears to be valid.

Hexavalent Intermediates. The increase in exocyclic cleavage with base concentration in an overall reaction that is base catalyzed indicates that product determination is second order in base.[2] The mechanism we have presented involves base-promoted ionization of an intermediate which forces pseudorotation, permitting methoxide to be expelled (Scheme I). The possibility of addition of hydroxide to the pentavalent intermediate followed by expulsion of methoxide is kinetically equivalent.[2] Gorenstein and co-workers recognized that the use of O-18 water and its effect on phosphorus NMR chemical shifts can resolve this question.[14-16] However, they examined only methyl hydroxyethyl phosphate which is produced primarily by the route involving 1 equiv of hydroxide (Scheme I). Formation of ethylene phosphate and its decomposition products occurs exclusively via the process which is second order in hydroxide. Therefore, it is important that the products of exocyclic cleavage be examined. We are currently performing

such studies and they will be reported later.

Hydrolysis of Methyl Hydroxyethyl Phosphate. The reaction almost certainly involves neighboring group participation to form ethylene phosphate and methanol (Scheme I). The pentavalent intermediate which forms in the initial intramolecular addition is a stereoisomer of the adduct formed by addition of lyate ion to methyl ethylene phosphate. In the case of the intermediate derived from methyl hydroxyethyl phosphate, the methoxyl group is axial and can leave directly to produce ethylene phosphate.[2,3] The intermediate derived from methyl ethylene phosphate must pseudorotate to give the same products, and the barrier to this is larger than the barrier to expulsion of methoxide.[2] However, since anionic ligands preferentially occupy equatorial positions, formation of the conjugate base of the intermediate will cause a rapid pseudorotation. Thus, the monoanionic intermediates from the two substrates are isomers which do not interconvert, but their conjugate bases do arrive at a common structure.

The Importance of Strain. The rate constant for the hydroxide-catalyzed hydrolysis of methyl ethylene phosphate is 10^6 times that for an acyclic analogue, trimethyl phosphate.[2] This factor is consistent with relief of enthalpic strain in the reaction of the cyclic ester of about 8 kcal/mol. Measurements of the enthalpic strain of methyl ethylene phosphate in 0.1 M NaOH yielded values of 7 to 9 kcal/mol.[1] A later report gave a lower value (5.5 kcal/mol),[20] but the acidity of the solution is unspecified. If significant exocyclic cleavage occurred under conditions in which the ethylene phosphate produced initially is slow to react, then a significant underestimation of enthalpy could have resulted. A more recent report suggests that the strain may indeed be as high as 9 kcal/mol.[7] Thus, the acceleration of hydrolysis in the triester is likely to result almost entirely from the relief of ring strain in the pentavalent transition state. Product patterns result from the apicophilicities of substituents[29] and the inherent energetics of pentacovalent intermediates.[30]

Conclusion

We have shown that methyl ethylene phosphate undergoes significant amounts of exocyclic cleavage in alkaline solution and that reports to the contrary are in error. A stereoelectronic theory, emphasizing the importance of antiperiplanar orbital interactions, strongly predicts that exocyclic cleavage of methyl ethylene phosphate will be highly disfavored relative to endocyclic cleavage. Therefore, the applicability of antiperiplanar lone-pair hypotheses to the reactions of cyclic phosphates appears to be doubtful.

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Structure of Trichorzianine A IIIc, an Antifungal Peptide from *Trichoderma harzianum*[1]

Bernard Bodo,*† Sylvie Rebuffat,† Mohamed El Hajji,† and Daniel Davoust‡

Contribution from the Laboratoire de Chimie du Muséum National d'Histoire Naturelle, 75231 Paris Cedex 05, France, and the Laboratoire de Chimie Organique Structurale de l'Université Pierre et Marie Curie, 75230 Paris Cedex 05, France. *Received February 4, 1985*

Abstract: Trichorzianine A IIIc is the main component of a peptide mixture isolated from a sporulated culture of *Trichoderma harzianum* that inhibits the growth of some fungal plant pathogens. This novel peptide has been shown to consit of one acetylated N-terminal residue, 18 amino acids, and a tryptophanol C-terminal amino alcohol. Sequence assignment was based on positive-ion FAB mass spectrometry and high-field NMR data using two-dimensional NMR assignment techniques and proton NOE difference studies. Information on the conformation was derived from $J_{NH-C^{\alpha}H}$ coupling constants, solvent and temperature NH chemical shift dependence, and transfer of solvent saturation experiments. These data show the N-terminal part of the peptide to be ordered in a helix, the first turn of which is of the 3_{10} type.

Trichoderma are widespread soil fungi, some of which exhibit antagonistic properties against other microscopic fungi.[2] The remarkable antagonism of *T. harzanium* against the plant pathogen *Botrytis cinerea* has been shown to be due to diffusible chemical compounds by in vitro experiments.[3] We have studied the antifungal substances produced by this *Trichoderma* and have isolated a complex peptide mixture, containing what we call trichorzianines, that explains partly the observed activity.

Trichorzianines consist of two closely related groups of peptides: a main, neutral component, trichorzianine A (TA), and a minor acidic component, trichorzianine B (TB).

Since the ninhydrin test was negative and no methylation occurred upon treatment with diazomethane, TA is likely to have neither the N nor the C terminal free. The N-terminal group is acetyl protected and the C-terminal residue is an amino alcohol. Moreover, since trichorzianine A contains a high proportion of α-aminoisobutyric acid (Aib) and has a molecular weight near 2000, it belongs to the class of peptaibols.[4,5] This special class of antibiotic peptides includes alamethicin[6-8] and suzukacillin,[9,10]

† Laboratoire de Chimie, UA 401 CNRS, Muséum National d'Histoire Naturelle.
‡ Laboratoire de Chimie Organique Structurale, UA 455 CNRS, Université Pierre et Marie Curie.

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