inverse square root dependence of the (0–0) maximum extinction coefficient on conjugation length.

The model spectrum of each polynorbornene-capped polyene was derived empirically from Knoll's data, the preceding assumptions, and the spectra of the copolymers. The model of the absorbance A_N of an N-ene consisted of a summation of four Gaussians corresponding to the (0–0), (0–1), (0–2), and (0–3) vibronic fingers of the $\pi\pi^*$ transition:

$$A_N(\lambda) = \sum_{i=1}^{4} \epsilon_{N,i} \exp\left(-\frac{(\lambda - \lambda_{N,i})^2}{w_N}\right) \quad (8)$$

The height of the ith Gaussian peak within the summation for a specific N-ene was weighted by its relative extinction coefficient, $\epsilon_{N,i}$, at the wavelength of maximum absorption, $\lambda_{N,i}$. The width of the vibronic fingers was determined by w_N. A single value of w_N for all of the vibronic fingers of a given N-ene was found to be sufficient to fit the polyene and copolymer spectra. The values for w_4, w_5, and w_{13} were determined from the copolymer spectra. The 13(0–0) peak in the spectrum of **13t** was sufficiently distinct to permit an estimation of w_{13}. The remaining w_N were calculated from a linear fit of these values with respect to conjugation length. The $\lambda_{N,i}$ values for the $\pi\pi^*$ (0–0) transitions of all of the polyenes and the $\pi\pi^*$ (0–1) and (0–2) transitions of the polyenes shorter than 12-ene were determined directly from the spectra of the copolymers. The $\lambda_{N,i}$ values for the $\pi\pi^*$ (0–1) or $\pi\pi^*$ (0–2) transitions for the polyenes longer than 11-ene were determined from linear fits of inverse conjugation length to the energy difference between the $\pi\pi^*$ (0–1) and (0–0) transitions or between the $\pi\pi^*$ (0–2) and (0–1) transitions, respectively, in Knoll's data. The $\lambda_{N,i}$ values for the $\pi\pi^*$ (0–3) transition were determined by adding 1340 cm^{-1}, the average energy difference between the two vibronic fingers in Knoll's spectra, to the $\lambda_{N,i}$ values of the $\pi\pi^*$ (0–2) transitions.

Once the parameters were determined for the polyene model spectra, a summation of the polyene models, weighted by $c_{P,N}$, gave the copolymer absorbance A_P:

$$A_P(\lambda) = c_P \sum_{N_{min}}^{N_{max}} c_{P,N} \left(\sum_{i=1}^{4} \epsilon_{N,i} \exp\left(-\frac{(\lambda - \lambda_{N,i})^2}{w_N}\right)\right) \quad (9)$$

$c_P = m_P/\langle M_P\rangle V$, for a mass m_P of copolymer P dissolved in a volume V of solvent. A mean molecular mass $\langle M_P\rangle$ was defined as

$$\langle M_P\rangle = l_P M_{nbe} + \sum_{N_{min}}^{N_{max}} c_{P,N} 26N \quad (10)$$

where l_P is the length of the polynorbornene blocks and M_{nbe} is the mass of norbornene. The weighted summation accounted for the different polyene lengths. The simplex method[35] was used to minimize the cost function of the square of the difference between $A_P(\lambda)$ and the data, subject to the constraint

$$\sum_{N_{min}}^{N_{max}} c_{P,N} = 1 \quad (11)$$

thereby determining the values of $c_{P,N}$ and l_P.

Hydrolysis of Methylacetoin Ethyl Phosphate. Competing Pathways for Carbonyl Hydrate Participation in a Model for Biotin Carboxylation†

Scott D. Taylor and Ronald Kluger*

Contribution from the Lash Miller Laboratories, Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1. *Received August 27, 1992*

Abstract: Methylacetoin ethyl phosphate (**1**) is a phosphodiester with a carbonyl group β to phosphorus. The phosphate ester of a carbonyl hydrate, expected to be generated from an intramolecular reaction of **1** in base, is a model for an enzymic reaction intermediate which would form from the reaction of the tetrahedral addition production of biotin and bicarbonate with ATP. The hydrolysis of **1** is 10^4–10^5 times more rapid than that of dimethyl phosphate, extrapolated to common conditions (1 M NaOH, 55 °C). Kinetic, product, and isotope labeling studies show that the hydrate of the carbonyl group of **1** serves as an intramolecular nucleophile toward phosphorus, forming the expected phosphate ester of the carbonyl hydrate through cyclic phosphorane intermediates. These adducts decompose by routes which parallel the proposed enzymic mechanism. In addition, solvent isotope incorporation reveals a route not found in reactions of carbonyl-substituted phosphotriesters: the conjugate base of the carbonyl hydrate acts an intramolecular nucleophile at the adjacent carbon atom, forming an unstable hydroxyoxirane (which becomes methylacetoin) and ethyl phosphate. An analogous reaction of ribonucleic acids (and RNAzymes) would have the 2′ hydroxyl group react at the 3′ carbon to form an oxyoxirane, cleaving the 3′ internucleotide bond.

Alkyl diesters of phosphoric acid normally are highly resistant to hydrolysis in neutral aqueous solutions. For example, the estimated half-life for hydrolysis of the phosphate ester linkage in DNA at physiological pH is on the order of millions of years.[1] This high kinetic barrier exists despite a thermodynamic situation which favors hydrolysis.[1] Thus, where catalytic pathways are available, rates of reaction can increase markedly. The primary mechanisms leading to higher spontaneous rates of hydrolysis involve nucleophilic participation at phosphorus by neighboring groups to form reactive five-membered ring intermediates.[2,3] These pathways are characterized by cleavage of the P–O ester bond. Alternative mechanisms involving C–O ester cleavage (participation at carbon with a phosphomonoester leaving group) are much less common.[4–6]

We recently reported studies of the mechanism of acceleration of phosphotriester hydrolysis by neighboring carbonyl groups.[7]

† This is paper is dedicated to Professor Frank Westheimer on the occasion of his 80th birthday.

(1) Westheimer, F. H. *Science* **1987**, *235*, 1173.
(2) Thatcher, G. R. J.; Kluger, R. *Adv. Phys. Org. Chem.* **1989**, *25*, 99.
(3) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.
(4) Cox, J. R.; Ramsay, B. *Chem. Rev.* **1964**, *64*, 317.
(5) Brown, D. M.; Magrath, D. I.; Todd, A. R. *J. Chem. Soc.* **1952**, *52*, 2708.
(6) Brown, D. M.; Usher, D. A. *Proc. Chem. Soc.* **1963**, 309.
(7) Kluger, R.; Taylor, S. D. *J. Am. Chem. Soc.* **1991**, *113*, 996.

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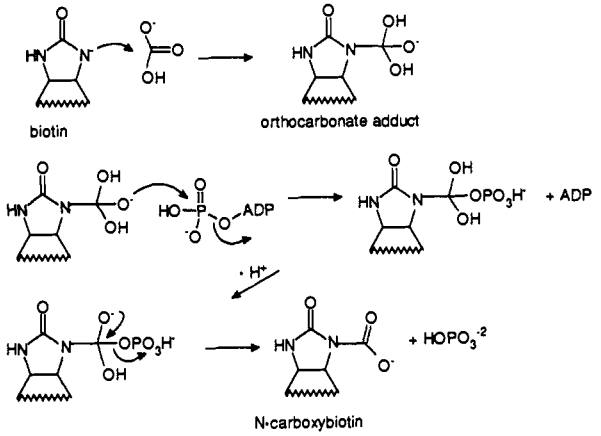
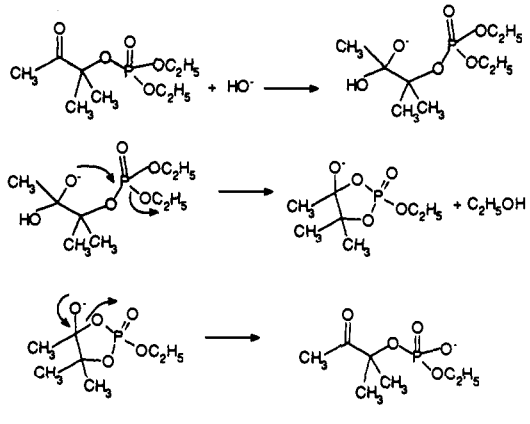
The investigation had been undertaken to test the chemical reasonableness of a proposed mechanism for the enzymic ATP-dependent formation of *N*-carboxybiotin from bicarbonate and biotin (the details of the biotin mechanism have been discussed in detail).[7,8] In the proposed biotin mechanism, the N1′ of the ureido group of biotin reacts with bicarbonate to form a tetrahedral adduct (an orthocarbonate). The hydroxyl group generated by the addition of biotin to bicarbonate reacts with ATP to form the phosphate ester of the adduct and ADP. The ester decomposes by formation of the carboxyl group of *N*-carboxybiotin with the expulsion of phosphate.



The two critical steps in the mechanism are the formal transfer of HPO_3^- from ATP to the orthocarbonate adduct of biotin and the expulsion of HPO_4^{2-} from the adduct, by which process the orthocarboxylate becomes a carboxylate (overall, there is a net transfer of oxygen from bicarbonate to the terminal phosphate of ATP).

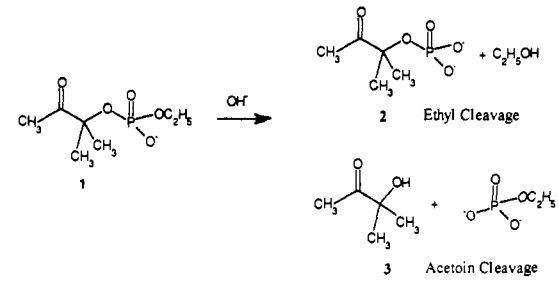
In the case of the phosphotriester used to model this process, the reaction involves initial formation of the carbonyl hydrate.[7] The resulting hydroxyl group serves as an intramolecular nucleophile at phosphorus, generating a reactive cyclic ester intermediate derived from the carbonyl hydrate. The carbonyl group is regenerated by expulsion of the phosphate ester, thus paralleling both steps in the proposed biotin mechanism.

An alternative mechanism for carbonyl hydrate participation involving intramolecular attack by a hydroxyl group at carbon to form an oxirane with expulsion of the phosphodiester[9] (as is observed in the case of hydroxyalkyl esters[5,6]) was ruled out by product and labeling studies. This mechanism would not have been relevant to the biotin mechanism proposals. The triester, however, is a neutral species, and the proposed biotin mechanism involves attack on an anionic phosphate. Therefore, we investigated the reaction of a diester, which is anionic, as a closer analogue. We find that a similar participation mechanism occurs, but additional mechanisms also arise which are unique to the phosphodiester.

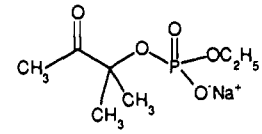


(8) Kluger, R. *Bioorg. Chem.* **1989**, *17*, 287.
(9) Witzel, H.; Botta, A.; Dimroth, K. *Chem. Ber.* **1965**, *98*, 1465.

Scheme I



Methylacetoin ethyl phosphate (**1**) is a phosphodiester which contains a β-carbonyl group. We find that the rate of ester



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hydrolysis is highly accelerated relative to that of an analogue lacking the carbonyl group. In this case, product and labeling studies reveal the importance of two competing pathways. One parallels that for the triester and is a model for the proposed biotin mechanism.[7,8] A second route involves an intermediate oxirane, a pathway which is not observed in the hydrolysis of the related triester.

Experimental Section

Materials. All chemicals used in syntheses were purchased from the Aldrich Chemical Company and distilled before use. Solvents were purchased from Caledon Laboratories Ltd. and BDH Chemicals. Deuterium oxide (99.97%) was a gift from Ontario Hydro, Toronto, Canada. Concentrated sodium hydroxide solutions were prepared from reagent grade NaOH pellets obtained from BDH Chemicals. Concentrated sodium deuteroxide solutions and ^{18}O-enriched water (97% ^{18}O) were obtained from Aldrich Chemical Company. Dilute solutions of sodium deuteroxide and sodium hydroxide were titrated with standardized hydrochloric acid. Sodium methylacetoin ethyl phosphate (**1**) was prepared from methylacetoin diethyl phosphate.[8]

Instrumentation. ^1H NMR and proton-decoupled ^{13}C NMR spectra were obtained with a Varian Gemini-200 spectrometer. Chemical shifts for ^1H NMR spectra of chloroform-*d* solutions are relative to internal TMS. ^{13}C chemical shifts are relative to the central peak of chloroform-*d* at δ 77. For deuterium oxide solutions, ^1H chemical shifts are relative to the residual HDO peak at δ 4.68 and ^{13}C chemical shifts are relative to internal dioxane at δ 67.4. Proton-decoupled ^{31}P NMR spectra were recorded on a Varian XL-200 spectrometer with chemical shifts relative to external 85% phosphoric acid. Chemical ionization–gas chromatography (CIGC) mass spectra were recorded on a VG Analytical 70-250S with a Hewlett-Packard 5890 gas chromatograph equipped with a 20-m DB-5 column (diameter 25 μm). Isobutane was used as the ionizing agent.

Product Distribution. The product distributions for the hydrolysis of methylacetoin ethyl phosphate in basic solutions were determined using ^{31}P and ^1H NMR spectroscopy. For reactions conducted in NaOD solutions of 2 M concentration or higher, 7.5 mg (3.2×10^{-5} mol) of methylacetoin ethyl phosphate was added to a base-resistant vial containing 0.5 mL of the NaOD solution at 55 °C. The vial was shaken, and then the mixture was incubated at 55 °C. When the reaction was complete, 0.25 mL of deuterium oxide was added, and the solution was transferred to an NMR tube. A spectrum was immediately recorded. Reactions conducted in solutions of lower alkalinity were conducted in an NMR tube.

The hydrolysis of methylacetoin ethyl phosphate in basic solution yields four products in two sets of two. One set is ethanol and methylacetoin phosphate (**2**, in Scheme I) (the ethyl cleavage products: see results section). The second set is methylacetoin (**3**, in Scheme I) and ethyl phosphate (the acetoin cleavage products). The relative amounts of the sets of products depends upon the reaction conditions. The fraction of phosphorane products was determined by measuring the ratio of the integrated signal due to the α-methyl protons of methylacetoin phosphate (δ 1.19) to the combined integrated signals due to the α-methyl protons of methylacetoin phosphate and the *gem*-dimethyl protons of methyl-

Table I. Observed First-Order Rate Constants for the Basic Hydrolysis of Methylacetoin Ethyl Phosphate at 55 °C

conditions	k, s^{-1} [a]	k', s^{-1} [b]	k'', s^{-1} [c]
1.0 M NaOD, 3.0 M NaCl	6.5×10^{-5}	4.48×10^{-5}	2.02×10^{-5}
1.0 M NaOD	2.8×10^{-5}	1.39×10^{-5}	1.40×10^{-5}

[a] Overall first-order rate constant. [b] Rate of formation of ethyl cleavage products determined from product ratio. [c] Rate of formation of acetoin cleavage products determined from product ratio.

acetoin (δ 1.11). The relative amount of this product was also determined using ^{31}P NMR by measuring the ratio of the intensity of the signal due to methylacetoin phosphate (δ 3.8) to the combined signal intensities due to ethyl phosphate (δ 0.6) and methylacetoin phosphate. The relative amounts of products determined by ^{31}P NMR agreed with those obtained by ^1H NMR.

In order to use ^{31}P for quantitative analysis, a relaxation delay at least twice the relaxation time (T_1) of the phosphate products was employed to prevent partial saturation of the signals. Quantitative analysis using ^{31}P NMR may also be further complicated by differential nuclear Overhauser effects (NOE) for the two phosphate products.[10] However, since the values obtained by ^{31}P NMR agreed with those obtained by ^1H NMR, it is likely that the NOE in the two phosphate products is approximately the same.

Kinetic Measurements. The rate of hydrolysis of methylacetoin ethyl phosphate in basic solution was measured using ^1H NMR. Methylacetoin ethyl phosphate (25 mg, 1.1×10^{-4} mol) was added to a vial containing 5 mL of NaOD solution which had been preincubated at 55 °C. The mixture in the vial was incubated in a water bath that was maintained at 55 °C, samples were withdrawn at various time intervals and cooled to 5 °C, and spectra were immediately recorded. For reactions conducted in solutions more basic than 1 M NaOD, samples were quenched with deuterium chloride to bring the concentration of NaOD to 1 M before cooling. In all cases the concentration of methylacetoin ethyl phosphate was at least 1/50 the concentration of base (pseudo-first-order conditions). The hydrolysis was followed by measuring the ratio of the integrated signal due to the α-methyl protons of methylacetoin phosphate and *gem*-dimethyl protons of methylacetoin to the combined integrated signals due to the α-methyl protons of methylacetoin ethyl phosphate (δ 1.4), the α-methyl protons of methylacetoin phosphate, and the *gem*-dimethyl protons of methylacetoin. All runs were performed in duplicate to 2.5–3 half-lives. Rate constants were obtained by fitting the data on an MS-DOS computer to the integrated first-order rate equation by nonlinear regression with the program GRAFIT (Erithacus Software Ltd. with Microsoft Windows). Excellent fit to the first-order rate equation was obtained in all cases ($r > 0.99$). The deviation of two measurements from their average was about 5%. Partial rates for the two modes of breakdown were obtained from product ratios, which were checked for constancy throughout the hydrolysis.

Labeling Experiments. Incorporation of ^{18}O into phosphate products was measured using ^{31}P NMR. Thus, 7.5 mg of methylacetoin ethyl phosphate was added to a 5-mm NMR tube containing 500 μL of either 4 M or 0.1 M NaOD (50% $H_2{}^{18}O$) and incubated at 55 °C (under argon). Once the reaction was complete, 0.25 mL of deuterium oxide was added to the tube, and a spectrum was recorded. Incorporation of ^{18}O isotope into methylacetoin was measured using chemical ionization GC-MS (ClGCMS). Thus, 7.5 mg of methylacetoin ethyl phosphate was added to an NMR tube containing 0.5 mL of either a 4.0 M or 0.1 M NaOH solution (approximately 50% $H_2{}^{18}O$) and incubated at 55 °C. Once the reaction was complete, the sample was transferred to a small test tube, and 1 mL of dichloromethane was added. The mixture was subjected to vortex mixing for 2 min. After a 3-min delay, the organic layer was transferred to a 5-mL round bottom flask and concentrated to about $^1/_5$ volume by rotary evaporation. The mass spectrum of the residue was recorded.

Results and Discussion

The reaction of methylacetoin ethyl phosphate (**1**) in alkaline solution produces two sets of products (Scheme I). The first set, ethanol and methylacetoin phosphate (**2**), arises from cleavage of the P–O bond of the phosphate ester (see below). The second set of products, ethyl phosphate and methylacetoin, is the result of C–O cleavage of the phosphate ester with methylacetoin (**3**) as the leaving group. The relative amounts of the two sets of products vary linearly with the concentration of sodium deuteroxide (Figure 1, Table I) and also with the concentration of sodium chloride (Figure 1, Table I).

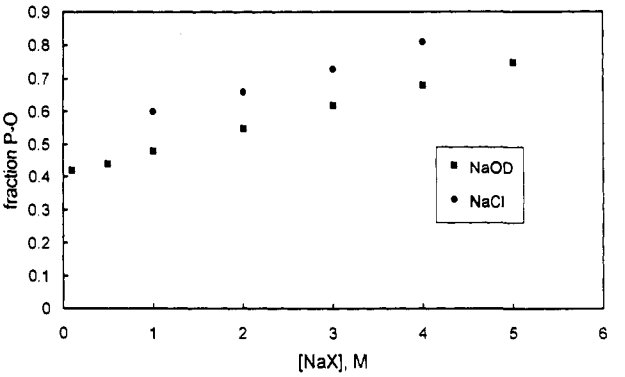
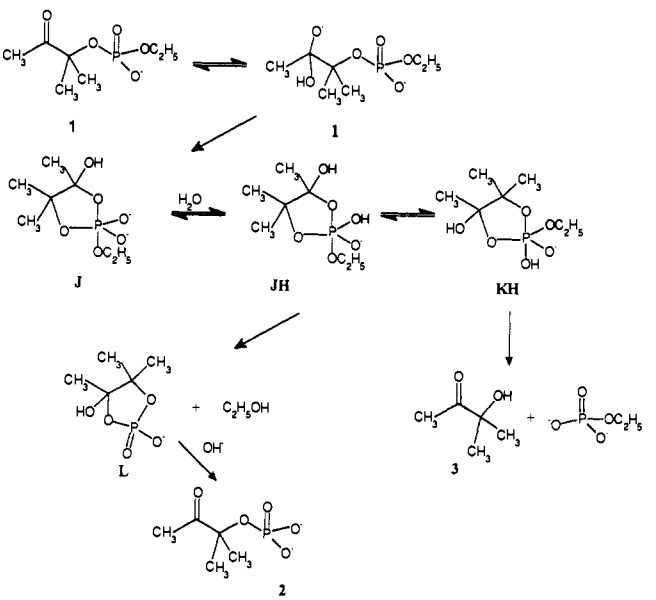


Figure 1. Effect of sodium deuteroxide concentration and sodium chloride concentration on product distribution (% ethyl cleavage product set) with respect to total product sets.

Scheme II



Rapid oxygen exchange occurs into carbonyl groups of ketones via a base-catalyzed hydration–dehydration mechanism.[11-13] We have estimated the rate constant for the base-catalyzed exchange of solvent oxygen into the carbonyl group of pinacolone to be about 10 M^{-1} s^{-1}.[8] If we assume that the rate constant for base-catalyzed exchange of solvent oxygen into methylacetoin ethyl phosphate is similar to that for pinacolone, then complete exchange of solvent oxygen into the carbonyl group of methylacetoin ethyl phosphate will occur before cyclization of the hydrate to the intermediate can take place.

There are two mechanisms by which methylacetoin ethyl phosphate can react with hydroxide to produce methylacetoin and diethyl phosphate rapidly. In the first, the conjugate base of the hydrate **I** attacks the adjacent phosphate moiety to form intermediate **J** (Scheme II). This may either expel ethoxide (to give a cyclic phosphate ester which reacts with hydroxide to give **2**), or it may pseudorotate to form the isomeric phosphorane **KH** which then breaks down to form methylacetoin and ethyl phosphate.

The mechanism in Scheme III also can produce methylacetoin and ethyl phosphate via attack of the conjugate base of the hydrate

(10) Pregosin, S.; Kuntz, R. W. *31-P and 13-C NMR of Transiton Metal Phosphine Complexes*; Springer: Berlin, 1979; p 14.

(11) Greenzaid, P.; Luz, Z.; Samuel, D. *J. Am. Chem. Soc.* **1967**, *89*, 749.
(12) Greenzaid, P.; Luz, Z.; Samuel, D. *Trans. Faraday Soc.* **1968**, *64*, 2780.
(13) Greenzaid, P.; Luz, Z.; Samuel, D. *Trans. Faraday Soc.* **1968**, *64*, 2787.

Scheme III

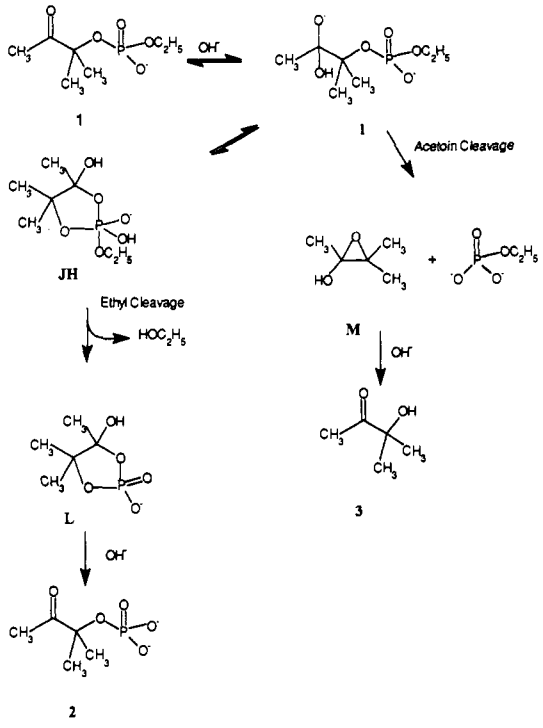


Table II. Incorporation of Solvent Oxygen into the Products Resulting from the Basic Hydrolysis of Methylacetoin Ethyl Phosphate

product	[base],[a] M	equiv solvent oxygen[b]
methylacetoin[c]	4.0	2
	0.1	2
ethyl phosphate[d]	4.0	0
	0.1	0
methylacetoin phosphate[e]	4.0	1
	0.1	1

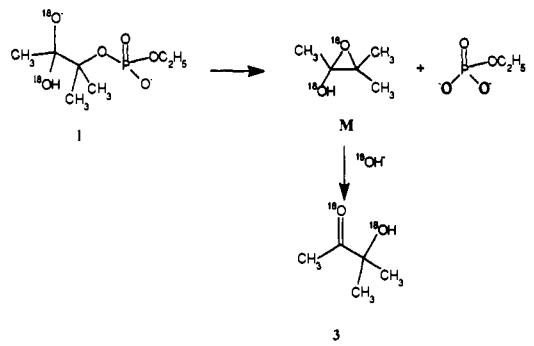
[a] NaOH was used for reactions analyzed by mass spectroscopy. NaOD was used for reactions analyzed by ^{31}P NMR. [b] Comparison of the total fraction of oxygen isotope (^{18}O) in products and isotope fraction in NaOD or NaOH solution. [c] Isotope incorporation determined by mass spectroscopy. Uncertainty is ±0.02 equiv of solvent oxygen (i.e. 2 ± 0.02 equiv of solvent oxygen incorporated) based upon the standard deviation from two determinations. [d] Isotope incorporation determined by ^{31}P NMR. [e] Isotope incorporation into phosphate moiety only by ^{31}P NMR. Uncertainty is ±0.03 equiv of solvent oxygen based upon the standard deviation from three determinations.

on the adjacent α-carbon, expelling ethyl phosphate. This results in formation of the oxirane which rapidly reacts with hydroxide to form methylacetoin (**3**).

The pattern of ^{18}O incorporation into the products shown in Table II (from $H_2{}^{18}O$ solvent) distinguishes the two mechanisms. Since isotopic exchange occurs into the carbonyl group of the substrate much faster than the hydrate cyclizes to form the phosphorane intermediate, the mechanism in Scheme II will lead to incorporation of one oxygen atom derived from the solvent into both methylacetoin and ethyl phosphate. However, we observe that where methylacetoin and ethyl phosphates are the products, ^{18}O is incorporated only into methylacetoin (in both the carbonyl and hydroxyl groups). The lack of ^{18}O incorporation into the phosphate product is the result which rules out this path.

The alternative mechanism in Scheme III is consistent with the observed labeling pattern. Attack of the conjugate base of the labeled hydrate on the α-carbon will result in the expulsion of unlabeled ethyl phosphate. Whether the resulting oxirane intermediate breaks down via elimination or by nucleophilic attack of hydroxide, label will be incorporated into both the carbonyl and hydroxyl oxygens of the product, methylacetoin, as shown in Scheme IV. Thus, methylacetoin ethyl phosphate hydrolyzes

Scheme IV



in base via competing P–O and C–O cleavage in the two sets of products.

The mechanism in Scheme III, involving an intermediate oxirane, was first proposed as a possibility for reactions of β-carbonyl phosphate triesters in analogy to the reaction of methylacetoin diethyl phosphate with Grignard reagents,[9] but the mechanism was ruled out by solvent labeling patterns.[8] Recently, Corrie and Trentham[14] proposed a similar mechanism in the reactions of triesters of benzoin phosphate on the basis of product analysis. The involvement of oxirane intermediates is observed for reactions of certain hydroxyalkyl phosphate esters.[6,15,16] Our current results show that oxirane formation is also a significant pathway in the hydrolysis of a phosphodiester with an adjacent carbonyl group.

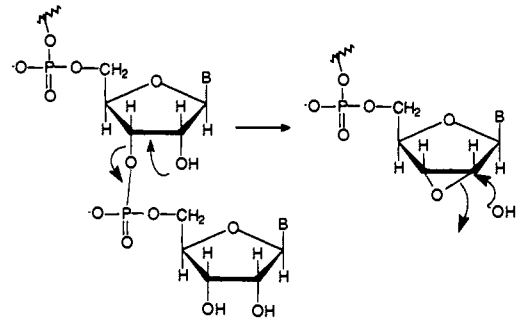
On the basis of literature values for rate constants and activation energies,[17] the observed first-order rate constant for the hydrolysis of dimethyl phosphate in 1.0 M hydroxide solution at 55 °C should be about 3×10^{-9} s^{-1}. Ten percent of the products of hydrolysis come from a pathway involving P–O cleavage, giving a net rate constant for those routes of 3.3×10^{-10} s^{-1}. In 1.0 M NaOD the hydrolysis of methylacetoin ethyl phosphate involves about 50% P–O cleavage. Therefore, the rate constant for P–O cleavage under these conditions is about 1.4×10^{-5} s^{-1} and the rate enhancement for P–O cleavage in methylacetoin ethyl phosphate is at least 2×10^{-5}. In alkaline solution, triethyl phosphate hydrolyzes in base about 10 times faster than trimethyl phosphate. If a similar rate difference exists between dimethyl and diethyl phosphate, the rate enhancement is 10^6.

β-Hydroxyl vs β-Carbonyl Group. Because of its relevance to ribonuclease action, the effect of a neighboring hydroxyl group on the hydrolysis of phosphodiesters has been studied in considerable detail. Brown and Usher examined the reactivity patterns and determined the rates of base-catalyzed hydrolysis of a variety of β-hydroxyalkyl phosphate diesters.[15,16] The hydrolysis of these compounds proceeds by two mechanisms similar to those presented above for methylacetoin ethyl phosphate. The first is via a cyclic phosphorane intermediate, and the second is via an oxirane intermediate. Based upon the rates of hydrolysis of analogous compounds as determined by Brown and Usher, in 1.0 M NaOH solution at 55 °C the rate constant for hydrolysis of the hydroxy ester is about 4×10^{-4} s^{-1}. This is within an order of magnitude of the rate constant for hydrolysis of methylacetoin ethyl phosphate via P–O cleavage in 1.0 M NaOD.

Dianionic and Monoanionic Intermediates. We have recently shown that the triester analogue of **1**, methylacetoin diethyl phosphate, hydrolyzes in alkaline solution via a stepwise mechanism involving phosphorane intermediates. We have also depicted the reaction of **1** leading to ethyl cleavage occurring via a monoanionic trigonal bipyramidal intermediate (**JH** in Scheme III). **JH** is similar to the first intermediate that is formed during the hydrolysis of the phosphotriester, except that an equatorial ethoxy

(14) Corrie, J. E. T.; Trentham, D. R. *J. Chem. Soc., Perkin Trans. 1*, in press.
(15) Brown, D. M.; Usher, D. A. *J. Chem. Soc.* **1965**, 6547.
(16) Brown, D. M.; Usher, D. A. *J. Chem. Soc.* **1965**, 6558.
(17) Kumamoto, J.; Cox, J. R.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 4858.

Scheme V



group is replaced by a hydroxy group. Since pseudorotation of the triester occurs in 0.1 M NaOD,[8] we expected products resulting from pseudorotation of **JH**. However, our ^{18}O studies show that there are no products resulting from pseudorotation nor is there any exchange of label back into the phosphate moiety of the starting material during the course of the reaction. If the intermediate exists as the dianion **J**, pseudorotation would be inhibited since it would require placing an oxyanion in the stereoelectronically unfavorable apical position.[18] Alternatively, the in-line decomposition might occur directly with the intermediate having a lifetime too short for pseudorotation. In the extreme, the intermediate has no lifetime and is a transition state.[19]

Origins of Product Distribution. As the concentration of hydroxide is increased, the relative amount of phosphorane product increases with a slope of 0.07, which is too small to be associated with a kinetic term in hydroxide. We examined the change in product distribution as a function of ionic strength by reacting **1** in 1.0 M NaOD in the presence of added sodium chloride. The relative amount of ethyl cleavage product also increases linearly with added sodium chloride (Figure 1) in parallel to the effect of sodium deuteroxide. This suggests that the change in product distribution with increasing base concentration may be due to the effects of changes in sodium ion concentration or ionic strength on equilibria between intermediates. We note that the initial carbonyl hydrate intermediate should be present predominantly as the conjugate base **I** since carbonyl hydrates have relatively low pK_as.[11-13]

We have shown that the hydrolysis of phosphodiesters can be accelerated by nucleophiles generated from adjacent carbonyl groups by pathways involving intermediates generated by attack at carbon or phosphorus, fitting patterns observed for hydroxyl participation in related systems. The pathway involving formation of the phosphate ester of the carbonyl hydrate appears to be a good model for the formation of a phosphate ester from the tetrahedral adduct which would form from addition of biotin to bicarbonate.[7,8] In addition, the pathway in which the phosphate ester leaves as the result of intramolecular attack at carbon may be relevant for considering cases of unusual cleavage patterns of RNA in the reactions catalyzed by RNAzymes. These reactions do not follow the mechanistic patterns of protein-derived ribonucleases, although many similarities do exist.[20,21] A pathway in which the ribosyl 2′ hydroxyl attacks the 3′ carbon would lead to formation of the oxirane and strand scission by hydroxide attack at the 2′ or 3′ carbon. The unstable oxirane would react to form the terminal ribose group, effectively giving cleavage at the 3′ site (Scheme V). Oxygen labeling studies would test such a mechanism.

Acknowledgment. Support from an Operating Grant (to R.K.) and a fellowship (to S.D.T.) from The Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged. We thank Dr. J. E. T. Corrie for a preprint of ref 14.

(18) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.
(19) Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1990**, *112*, 5872.
(20) Cech, T. R. *Science (Washington, D.C.)* **1987**, *236*, 1532.
(21) Westheimer, F. H. *Nature (London)* **1986**, *319*, 534.