On the Origins of Enhanced Reactivity of Five-Membered Cyclic Phosphate Esters. The Relative Contributions of Enthalpic and Entropic Factors

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Abstract: The hydrolysis of five-membered cyclic phosphate and phosphonate esters is about 10^6-fold more rapid than the hydrolysis of related acyclic esters. The origin of the enhanced reactivity of the cyclic esters has been ascribed to enthalpic factors, associated with ground-state strain, and to entropic factors, associated with optimal orbital orientations. The temperature dependence of the rates of alkaline hydrolysis of ethyl and methyl esters of ethylene phosphate and of ethyl and methyl esters of propylphostonate have been determined. The enthalpies of activation for the cyclic esters are much less than those for the corresponding acyclic esters while differences in entropies of activation are dependent on the nature of the alkyl substituent, varying from less than 1 cal mol^{-1} deg^{-1} (eu) for the methyl esters to about 8 eu for the ethyl esters. The data in the present study indicate that the report of an unusually low entropy of activation (17 eu less than the acyclic analogue) for the hydrolysis of the cyclic phosphonate ester, ethyl propylphostonate (Aksnes, G.; Bergesen, K. *Acta Chem. Scand.* **1966** *30*, 2508) is in error and thus cannot support assumptions that the high reactivity of cyclic esters is due primarily to entropic effects. The acceleration of the rate of hydrolysis of cyclic phosphate esters is therefore due to enthalpic factors, consistent with interpretations based on ring strain.

The enzyme-catalyzed hydrolysis of ribonucleic acids occurs by a pathway in which cyclic 2′–3′ phosphate esters form as intermediates.[1] These intermediates are also formed readily under non-enzymic reaction conditions and react with water much more rapidly than do acyclic phosphate esters.[1] The enhanced reactivity of five-membered cyclic esters derived from RNA is also exhibited by simple, unsubstituted cyclic phosphates.[2,3] Thus, the rates of reaction of five-membered cyclic phosphate and phosphonate esters with hydroxide are approximately 10^6 times greater than those of their acyclic analogues.[4]

The source of the enhanced rate of reaction of the cyclic materials was first proposed by Westheimer to be due to differential ring strain in the reactant and the transition state for nucleophilic addition.[5] Calorimetric data indicate that the hydrolysis of the five-membered cyclic phosphate triester, methyl ethylene phosphate, is between 5.5 and 9 kcal/mol more exothermic than that of an acyclic triester.[6-8] The heat evolved in the hydrolysis of the cyclic ester is consistent with the presence of ring strain in the molecule.[6] The rapid hydrolysis of methyl ethylene phosphate leads to both cyclic and acyclic products.[9] Therefore, any acceleration due to relief of ring strain must occur in the transition state for nucleophilic addition to the cyclic phosphate. The structure of this transition state is expected to be similar to that of a pentacoordinated phosphorus derivative (trigonal bipyramid) and should be free of ring strain with the five-membered ring still intact.[4,10] While it is clear that formation and reaction of the five-membered ring in these systems is significant, the role of ring strain has not been directly established.

Kinetic factors leading to acceleration of the hydrolysis of cyclic phosphates should be manifested in differences in enthalpy and entropy of activation for the reactions of cyclic and acyclic phosphates. Ring strain and its relief are enthalpic phenomena and if the acceleration in the cyclic cases is due to relief of ring strain in the transition state of the rate-determining step, then differences between cyclic and acyclic materials should appear in enthalpies of activation. However, a large entropic contribution to the rate acceleration in the cyclic compounds was noted by Aksnes and Bergesen in 1966.[11] These workers reported that the entropy of activation for the alkaline hydrolysis of ethyl propylphostonate is −17 cal mol^{-1} deg^{-1} (eu), whereas the entropy of activation for an acyclic phosphonate ester, diethyl ethylphosphonate, is considerably larger in absolute magnitude, −34 eu.[11] On the basis of the equations of transition-state theory, the difference, 17 eu, is equivalent to a factor of about 10^4 in the observed rate acceleration, leaving enthalpic strain to account for at most a factor of 100.

Such a dominant entropic component implicates phenomena related to orientation and restricted motion rather than ring strain. Stereoelectronic effects (of the type presented by the antiperiplanar lone pair hypothesis[12,13]) depend on the proper alignment of occupied nonbonding orbitals on oxygen with respect to σ bonds to phosphorus. It has been proposed that the large entropic factor reported by Aksnes and Bergesen[11] should be an indication of the importance of these effects.[13]

In order to establish the relative importance of entropic and enthalpic factors in accelerating the reactions of cyclic phosphates, we have determined the activation parameters for the hydroxide-catalyzed hydrolysis of a set of related cyclic five-membered phosphonates and phosphates (ethyl propylphostonate, EPP; ethyl ethylene phosphate, EEP; methyl ethylene phosphate, MEP; methyl propylphostonate, MPP) and have compared these with parameters for acyclic phosphates (diethyl ethylphosphonate, DEEP; triethyl phosphate, TEP; trimethyl phosphate, TMP; dimethyl methylphosphonate, DMMP).

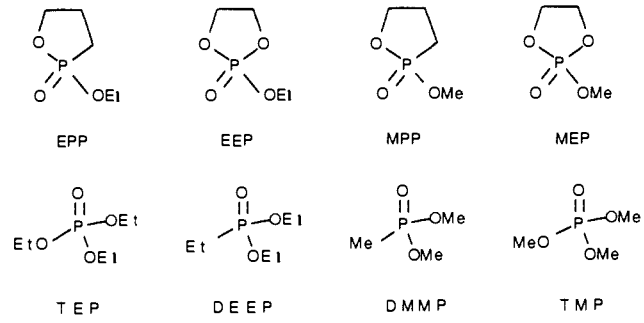

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Table I. Second-Order Rate Constants for the Base-Catalyzed Hydrolysis of Cyclic and Acyclic Phosphonates and Phosphates at 25 °C

compd	k_2, M^{-1} s^{-1}	k_c/k_{ac}[d]
ethyl propylphostonate	21.8	7.8×10^5
diethyl ethylphosphonate	2.80×10^{-5}[a]	
ethyl ethylene phosphate	43.8	5.1×10^6
triethyl phosphate	8.54×10^{-6}[a]	
methyl propylphostonate	41.2	1.8×10^5
dimethyl methylphosphonate	2.24×10^{-3}[b]	
methyl ethylene phosphate	81.4	4.7×10^5
trimethyl phosphate	1.74×10^{-4}[c]	

[a] Calculated from data in ref 11. [b] Calculated from data in Hudson, R. F.; Keay, L. *J. Chem. Soc.* **1956**, 2463. [c] Calculated from data in Barnard, P. W. C.; Bunton, C. A.; Llewellyn, D. R.; Vernon, C. A.; Welch, J. A. *J. Chem. Soc.* **1961**, 2670. [d] Rate constant ratio of cyclic (c) and acyclic (ac) compounds.

Experimental Section

Materials and Methods. All chemicals used in syntheses were purchased from the Aldrich Chemical Co. Solvents were purchased from Caledon Laboratories Ltd. and BDH Chemicals. Phosphorus NMR spectra were recorded on a Varian XL-200 instrument and were subject to decoupling of proton nuclear spins. Chemical shifts are reported in parts per million (δ) relative to external 85% phosphoric acid. Proton NMR spectra were recorded on a Varian Gemini-200 or Varian XL-200 with chemical shifts reported in parts per million relative to internal TMS. Chloroform-*d* was used as a solvent for these spectra.

Ethyl Propylphostonate (EPP). The material was prepared by a procedure similar to that reported by Eberhard and Westheimer.[14] Thus, 35 g of diethyl 3-bromopropylphosphonate (0.14 mol, prepared by the method of Helferich and Curtius[15]) was heated at 160–170 °C under nitrogen for 2 h. The resulting liquid was vacuum distilled (Hickman still), yielding the colorless liquid product with a small amount of unreacted starting material. Purified product was obtained by distillation through a vacuum jacketed vigreux column (bp 58–60 °C, 0.05 torr, lit.[14] bp 74 °C, 0.6 torr). Yield: 13.4 g (66%). ^{1}H NMR: δ 1.29–1.41 (t, 3 H), 1.75–1.95 (m, 2 H), 2.10–2.45 (m, 2 H), 4.13–4.35 (m, 4 H). ^{31}P NMR: δ 49.9.

Methyl Propylphostonate (MPP). This was prepared by the method described for ethyl propylphostonate using dimethyl 3-bromopropylphosphonate (18 g, 0.09 mol). Purification required multiple distillations. Yield: 6 g (50%). ^{1}H NMR: δ 1.55–2.70 (m, 4 H), 3.68–3.93 (d, 3 H), 4.00–4.70 (m, 2 H). ^{31}P NMR: δ 51.2. Bp: 50–53 °C, (0.03–0.04 torr).

Ethyl Ethylene Phosphate (EEP). This was prepared according the procedure of Boisdon et al.[16]

Methyl Ethylene Phosphate (MEP). This was prepared according to reported procedures.[17,18]

Kinetic Measurements. Rates of hydrolysis were determined by using a pH-Stat (Radiometer pH meter 27, TTT80 titrator, autoburet 13, GK-202B combination electrode) with data collected with use of a Commodore 8032 microcomputer. The hydrolysis reaction consumes 1 equiv of base per equivalent of ester hydrolyzed so that the addition of base to maintain the measured pH is an accurate measure of the extent of reaction. Reactions were conducted in a jacketed flask under argon with temperature maintained within ±0.1 °C with a Neslab RTE-80 circulating water bath. Potassium chloride solution (7 mL, 0.1 M) was sitrred and equilibrated in the reaction vessel with the apparatus set to maintain the pH through the addition of a 0.1 M potassium hydroxide solution from the autoburet. The reactant (volume 0.5–1.0 μL) was added with a calibrated syringe. All runs were performed in triplicate. NMR analysis of reaction solutions of all materials under the conditions in this study revealed that only ring-opened materials are produced: there were no signals for the alcohols which would be produced by exocyclic cleavage. (It is known that at pH 10, MEP undergoes exclusively ring opening and this was the basis for the choice of conditions.[17])

Data Analysis. Calculations of the best fit of primary kinetic data to the integrated first-order rate law utilized the Swain "Kinetic Overrelaxation" algorithm on a microcomputer.[19] This gave correlation coefficients of at least 0.99999 over at least four half-lives and standard errors (2 standard deviations) of less than 2%. The concentration of hydroxide at each temperature was derived from pH measurements, the temperature-dependent dissociation constant of water,[20] and the activity coefficient of hydroxide ion:[21] $[OH^-] = (K_w/\gamma)(1/a_h)$. This equation was used to calculate second-order rate constants (k_2) from $k_2 = k_{obs}/[OH^-]$. Activation and statistical parameters were calculated by fitting our data to the Eyring equation ($k_2 = (KT/h)e^{-G^\ddagger/RT}$ and $\Delta G^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger$, where T is Kelvin temperature and k_2 is the second order rate constant) with use of a nonlinear regression analysis with the program "GraFit" (Erithacus Software Ltd. with Microsoft "Windows" and an IBM AT compatible microcomputer).

Table II. Second-Order Rate Constants (M^{-1} s^{-1}) for Alkaline Hydrolysis of Cyclic Phosphonates and Phosphates, Using Data from Pseudo-First-Order Conditions at pH 10

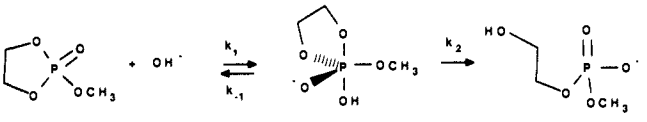
	k_2			
T, °C	EPP	EEP	MPP	MEP
5		16.9	15.0	27.9
10		21.6	19.7	38.0
15	13.2	27.2	24.1	50.4
20	16.5	37.5	31.4	63.1
25	21.8	43.8	41.2	81.4
30	28.3	53.8	52.7	98.2
35	34.1	71.0	65.8	129
40	41.7	84.6		

Table III. Calculated[a] Enthalpies and Entropies of Activation for Alkaline Hydrolysis of Phosphates and Phosphonate

compd	$\Delta H^\ddagger$, kcal/mol	$\Delta S^\ddagger$, eu	$\Delta\Delta S^\ddagger$,[e] eu
ethyl propylphostonate	7.6	−27	−8
diethyl ethylphosphonate	13.4[b]	−35[b]	
ethyl ethylene phosphate	7.4	−26	−8
triethyl phosphate	14.1[b]	−34[b]	
methyl propylphostonate	7.9	−24	0
dimethyl methylphosphonate	14.0[c]	−24[c]	
methyl ethylene phosphate	7.8	−23	0
trimethyl phosphate	15.6[d]	−23[d]	

[a] Values determined from literature data were calculated in the same manner as our data as described in the Experimental Section. [b] Calculated from data in ref 11. [c] Calculated from data in Hudson, R. F.; Keay, L. *J. Chem. Soc.* **1956**, 2463. [d] Calculated from data in Barnard, P. W. C.; Bunton, C. A.; Llewellyn, D. R.; Vernon, C. A.; Welch, J. A. *J. Chem. Soc.* **1961**, 2670. [e] Difference in entropy of activation between cyclic (c) and acyclic (ac) compounds.

Scheme I



Results and Discussion

Second-order rate constants for the alkaline hydrolysis of cyclic phosphates and phosphonates and for related acyclic materials are presented in Table I. The variation of rate constants with temperature is given in Table II. These data were plotted as described in the Experimental Section to give entropies and enthalpies of activation. Table III contains activation parameters for the hydrolysis of compounds in this study. The calculated errors for compounds in our study (based on nonlinear regression analysis) is ±1 eu for the entropies of activation and ±0.2 kcal/mol for the enthalpies of activation. We also calculated activation parameters for comparison compounds on the basis of reported rate data (uncertainties for those data are unknown).

A stepwise mechanism for the reaction of methyl ethylene phosphate with hydroxide is shown in Scheme I. The formation of a pentacoordinate intermediate is consistent with the observed change in product distribution with pH.[17,18]

The simplified mechanism is a two-step process in which formation of the intermediate, decomposition of the intermediate,

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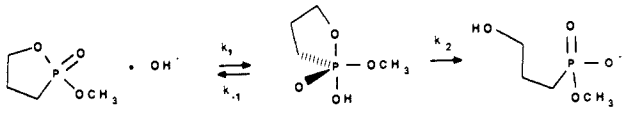
Scheme II



or a combination is rate-determining. In this case, the alkoxy group in the ring is the leaving group. The pK_A of ethylene glycol monomethyl ether is 14.8[22] and the pK_A of water is 15.7.[20] Since the leaving group associated with k_{-1} is a stronger base than the group associated with k_2, it is likely that the first step is rate-determining. The same reasoning applies to the reaction of ethyl ethylene phosphate with hydroxide. For the phosphonate esters, the alkoxide derived from the chain of the ring is a poorer leaving group than in the phosphates (pK_A for ethanol = 16)[22] and is probably a poorer leaving group than hydroxide (Scheme II). Therefore, the second step in the mechanism shown in Scheme II should be rate-determining for the cyclic phosphonate esters, methyl propylphostonate and ethyl propylphostonate.

In both the phosphates and phosphonates, application of the Hammond postulate suggests that the transition state for the rate-determining step resembles the pentacoordinated intermediate. Since the leaving groups are strongly basic, there is a sufficient kinetic barrier to their expulsion to permit the intermediate to have a lifetime sufficient to avoid a concerted mechanism.[10]

The magnitudes of the rate constants for the reactions of methyl ethylene phosphate and methyl propylphostonate with hydroxide are similar (Table I). Since the two reactions are likely to have different rate-determining steps (due to the differences in basicities of the leaving group in the ring), it is probable that the differences in barriers associated with k_1 and k_2 are small in comparison to the thermodynamic barrier to formation of the intermediate. Because the intermediate generated by addition of hydroxide to the phosphonate esters (Scheme II) is expected to lack differential acceleration due to a stereoelectronic component (due to the lack of an oxygen atom in the ring in the equatorial plane of the intermediate[12,13]), these results argue against significant assistance in either case from stereoelectronic factors.

In general, the major difference between the activation parameters for cyclic and acyclic esters is observed for the enthalpy of activation while the entropy of activation is related to the alkyl substituent. This makes the choice of a reference material critical, since the hydrolysis of triethyl phosphate has a considerably higher entropy of activation than does that of trimethyl phosphate (Table III). Two ethyl groups together are bulkier than the five-membered ring of the cyclic compound. Therefore, part of the difference in activation entropies between cyclic esters containing one ethyl group and acyclic esters containing two or three ethyl groups is due to the requirements of the alkyl substituents.

The difference in the entropies of activation of methyl ethylene phosphate and trimethyl phosphate is less than 1 eu. However, the difference in enthalpies of activation of methyl ethylene phosphate and trimethyl phosphate is over 7 kcal/mol, about the same as the amount of excess heat released in the overall hydrolysis reaction.[6-8] The difference in enthalpies of activation for ethyl ethylene phosphate and triethyl phosphate is about 7 kcal/mol, and the difference in entropies of activation is 8 eu. As we have noted, part of this difference is due to the more crowded situation caused by three ethyl groups compared to one ethyl group and an ethylene bridge. Thus, the cyclic phosphate's enhanced reactivity arises from differences in enthalpies of activation, consistent with the effect of ring strain.

The case of the phosphonate esters is similar. The methyl esters, methyl propylphostonate, and dimethyl methylphosphonate differ by about 6 kcal/mol in enthalpy of activation and the entropies of activation are within 1 eu. The ethyl esters, ethyl propylphostonate, and diethyl ethylphosphonate differ by 8 eu in entropies of activation and differ in enthalpy of activation by 6 kcal/mol. The entropy of activation for ethyl propylphostonate is −27 eu (Aksnes and Bergesen report −17 eu[11]) and the enthalpy of activation is 7.6 kcal/mol (Aksnes and Bergesen report 11.1 kcal/mol[11]). Aksnes and Bergesen based their reported parameters on a plot of only two points, both measured at low temperatures, without reported uncertainties.[11] Experimental details are not reported so we could not check their experiments for reproducibility. Extrapolations of our data to the temperatures of those measurements suggest that the rate constants are both in error by at least a factor of 2.

Conclusions

Differences in the entropy of activation between cyclic and acyclic phosphates and phosphonates are not the source of the differences in rate as has previously been suggested.[13] We conclude that ring strain, as originally proposed by Westheimer,[23] rather than orbital orientation, is by far the major factor in enhancing the reactivity of cyclic phosphates and phosphonates. Sinnott's proposal that stereoelectronic effects can play no more than a small role in enhancing the reactivity of cyclic esters appears to be correct.[24]

Acknowledgment. We thank Professor F. H. Westheimer for providing us with data from the thesis of F. Covitz (Harvard University, 1963) for comparison with our own for the activation parameters for the hydrolysis of methyl ethylene phosphate and for helpful comments regarding the sources of useful data for these calculations. We are also grateful for support from the Natural Sciences and Engineering Research Council of Canada through an operating grant (R.K.) and a postgraduate fellowship (S.D.T.).

Supplementary Material Available: Table of enthalpy and entropy values (weighted) and graph of K_2 vs T for EPP at pH 10 (2 pages). Ordering information is given on any current masthead page.

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