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ACETYL ETHYLENE PHOSPHATE. EXTERNAL STRAIN ACTIVATION

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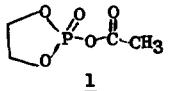
Our interest in factors controlling the site of reactivity of mixed anhydrides in biological systems prompted us to investigate the effect of strain external to the anhydride linkage on the reactivity of acyl phosphates. This mode corresponds to models proposed for metal ion catalysis in enzymic phosphate transfer reactions[2,3]. While strained carboxylic-phosphoric anhydrides which incorporate the linkage into a five-membered alkyl ring have been proposed as reactive intermediates[4,5] and have recently been synthesized,[6] no compound of this class has been available which might react to cleave the mixed anhydride while maintaining intact the strained ring. Rapid reaction at phosphorus with external cleavage would require either rapid isomerization of a pentacovalent intermediate or a relatively unfavorable transition state.[7] This behavior would be in contrast to the reaction of acyclic neutral acyl phosphates which undergo preferable reaction at the carboxyl moiety.[8]

We prepared acetyl ethylene phosphate (1) by the oxidation of the corresponding phosphite[9] in methylene chloride at 0° with ozone.[10] The oxidation proceeds via an ozone adduct which decomposes slowly at room temperature to give 1 (precipitated by the addition of ether in a dry



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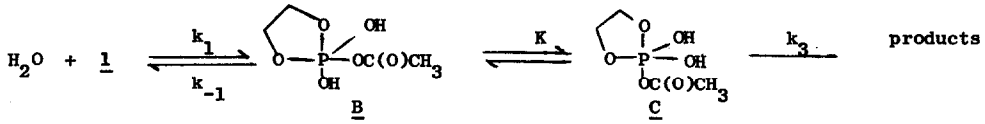
atmosphere: needles, mp 50-51°) and oxygen. The extremely hygroscopic compound shows appropriate spectroscopic features: ir (CH_2Cl_2): P=O, 1280 cm^{-1}; C=O 1785 cm^{-1}. nmr (CD_2Cl_2): δ 2.25, d, 3H, J_{P-H} = 1.5 Hz; δ 4.6, d, 4H, J_{P-H} = 11 Hz.

Acetyl ethylene phosphate is extremely reactive toward water. At 5°, pH stat methods were inadequate to follow the reaction which was complete within (at most) 15 seconds and a rate constant of 8 min^{-1} or less. The reaction is therefore at least 20 times more rapid than that

of the corresponding acyclic compound, acetyl dimethyl phosphate.[8] This ratio serves only as a lower limit but firmly establishes that the enhanced carboxyl activity has been exceeded. The products of the hydrolysis were determined to be acetic acid and ethylene phosphate by running the reaction in deuterium oxide and comparing the spectrum thus obtained with genuine samples of possible reaction products. The rate of reaction of acyl phosphates undergoing hydrolysis via reaction at the carboxyl center (leading to C-O cleavage of the anhydride) is proportional to the pK_a of the conjugate acid of the leaving group.[8] Therefore, rapid reaction indicates either that ethylene phosphoric acid is a much stronger acid than dimethyl phosphoric acid or that hydrolysis proceeds by attack of water at the phosphoryl center, rather than the carboxyl center. Sodium ethylene phosphate was prepared by treatment of methyl ethylene phosphate[11] with sodium iodide in acetone. The pK, determined by partial titration at 25° is 1.29 + .05, equal to that of dimethyl phosphate.[12] Therefore, the reactivity of acetyl ethylene phosphate is due to enhanced reactivity at phosphorus. Since reaction occurs without ring opening (the possibility of a ring opening followed by reclosure is ruled out by the slow rate of ring opening, compared to the rate in question, of other neutral cyclic phosphates),[11] it is likely that an initially formed pentacovalent intermediate isomerizes in accord with routes proposed for similar cyclic phosphates[7] so that acetate may be expelled. The low pK of acetic acid makes the possibility that rapid decomposition of the intermediate can occur without catalysis likely. It is expected that the neutral intermediate will isomerize rapidly since it contains ligands of high and relatively equal electronegativity.[7] A mechanism that has been formulated in accord with these considerations is presented in Scheme 1. Proton transfer steps are not included.

Scheme 1



The corresponding rate equation is: $v = k_{obsd}(1) = k_3(C)$; assuming B and C are steady state intermediates, then: $C = \frac{k_1(1)}{\frac{k_{-1}}{K} + k_3}$; it is reasonable to assume that the value of K will be

approximately unity[7] and the uncatalyzed expulsion of acetate should be much more rapid than expulsion of water, so that $k_3 \gg k_{-1}$. Therefore: $k_{obsd} = \frac{k_1 k_3}{k_3} = k_1$. Thus the rate of cleavage of the strained acyl phosphate external to the ring depends on the rate of hydration of the starting material which is markedly accelerated by the strain relieved in proceeding toward the transition state.[7]

Methanolysis experiments were conducted with materials maintained at -35° in an nmr tube. Reaction rate is too rapid to measure under these conditions. The exclusive products of reaction are methyl ethylene phosphate and acetic acid (determined by in situ comparison with the spectral properties of added genuine samples of possible products). This confirms that the preferred mode of attack upon this molecule by hydroxylic nucleophiles under these conditions is at phosphorus. An initial ring-opening reaction would give the unstrained neutral acyl phosphate which would necessarily undergo methanolysis at the carboxyl center[8] and would lead to the production of methyl acetate.

We conclude that strained acyl phosphates can react rapidly to cleave the anhydride linkage between phosphorus and oxygen, even when this occurs without ring opening. The enzymatic phosphorylation reactions involving acetyl phosphate can be catalyzed by binding of the substrate to metal ions in a manner that simulates the ethylene bridge, promoting the phosphorylating ability of the compound. Since metal ion- catalyzed hydrolysis of acetyl phosphate has recently been shown to involve C-O cleavage in most cases in the absence of enzyme,[13] catalysis by enzymes involving metal ions[3] must proceed by a mechanism different from the reaction in the absence of enzyme. The strain inducement mechanism[2,3] appears to be an attractive one.

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REFERENCES

1. Fellow of the Alfred P. Sloan Foundation. Address correspondence to Department of Chemistry University of Toronto, Toronto, Canada M5S 1A1.
2. F.J. Farrell, W.A. Kjellstrom and T.G. Spiro, Science, 164, 320 (1969).
3. A.S. Mildvan, The Enzymes, 2, 466 (1970).
4. V.M. Clark and A.J. Kirby, J. Amer. Chem. Soc., 85, 3705 (1963).
5. S.J. Benkovic and K.J. Schray, J. Amer. Chem. Soc., 91, 5653 (1969).

6. F. Ramirez, S. Glaser, P. Stern, P.D. Gillespie, and I. Ugi, Ang. Chem. Int. (Eng.), 12, 65 (1973).

7. F.H. Westheimer, Accounts Chem. Res., 1, 70 (1968). F. Ramirez, ibid., 168 (1968).

8. J.L. Dever and J.J. Hodan, U.S. Pat. 3,482002 (1970); Chem. Abstr., 72, 19051k (1970).

9. W.S. Knowles and Q.E. Thompson, Chem. Ind. (London), 121 (1959).

10. R. Kluger and P. Wasserstein, Biochemistry, 11, 1544 (1972).

11. R. Kluger, F. Covitz, E. Dennis, L.D. Williams, and R.H. Westheimer, J. Amer. Chem. Soc., 91, 6066 (1969).

12. W.D. Kumler and J.J. Eiler, J. Amer. Chem. Soc., 65, 2355 (1943).

13. J.P. Klinman and D. Samuel, Biochemistry, 10, 2126 (1971).