the detailed phase diagram, and the kinds of materials which can be dissolved.

K. D. Lawson, T. J. Flautt
The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45239

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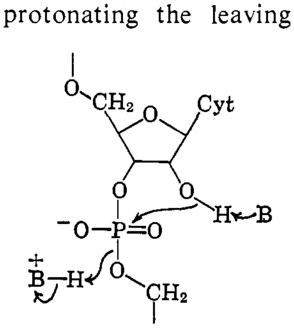
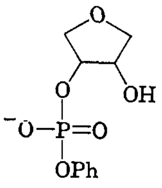
Models of Ribonuclease Action. I. General Species Catalysis in the Hydrolysis of a Nucleotide Diester Analog[1]

Sir:

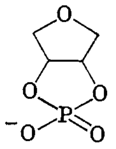
The first step in the action of the enzyme ribonuclease-A on a ribonucleic acid or dinucleoside phosphate substrate is believed to be the formation of the nucleoside 2′,3′-cyclic phosphate,[2] and a considerable amount of evidence implicates the imidazole groups of histidine-12 and histidine-119 in the catalytic process.[3] In one of the currently discussed mechanisms for this reaction,[3,4] one imidazole acts as a general base toward the 2′-hydroxyl group while the other acts as a general acid, protonating the leaving group (I). However, there has been to date no demonstration of the involvement of imidazole or of any other nitrogenous base in the nonenzymic hydrolysis of a nucleic acid or nucleic acid analog.[5] We now report that hydrolysis of the phenyl ester of *cis*-tetrahydrofuran-3,4-diol monophosphate (II), a model of a dinucleoside phosphate, shows general species catalysis by imidazole and by morpholine and involves initial formation of the cyclic phosphate III.



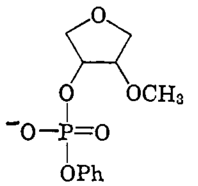
I



II



III



IV

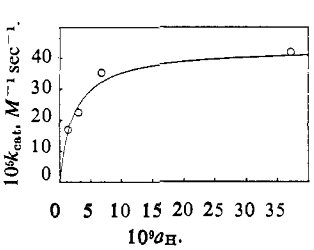


Figure 1. The dependence of k_{cat} on a_H (morpholine buffers; 50°; ionic strength 0.1). The rate and equilibrium constants given in the text give rise to the solid line; the experimental points are denoted by circles.

The phenyl ester II[6] was prepared by the reaction of phenyl phosphate with *cis*-tetrahydrofuran-3,4-diol using trichloroacetonitrile as the condensing agent.[7] The comparison ester IV was prepared by the reaction of diphenyl phosphorochloridate[8] with *cis*-4-methoxy-3-tetrahydrofuranol, followed by the removal of one phenyl group. Analyses for C, H, and P for all new compounds and for the cyclic phosphate III[9] were within 0.2% of theory. The rate of reaction of II was measured in a Cary Model 15 spectrophotometer by following the rate of formation of phenol. All of the experiments were carried out at 50° at an ionic strength of 0.1 (maintained with potassium chloride); the pH values of the buffer solutions were measured before and after each run, and the ultraviolet spectra of the hydrolysis products were the same as those of a mixture of phenol with the appropriate buffer.

The rate of production of phenol from II was found to be subject to general species catalysis,[10] but for morpholine the standard method[11] of dividing the catalytic effect between that due to general base and that due to general acid was inappropriate; our present data show that the catalysis changed from apparently general acid at high buffer ratio (base:base hydrochloride) to apparently general base at low buffer ratio. This is shown in Figure 1 and is reasonably well fit by an equation of the form

$$k_{cat} = \frac{(43.3 \times 10^{-5})a_H}{a_H + (2.26 \times 10^{-9})}$$

where the constants were obtained by a linear least-squares analysis of a plot of the experimental values of $1/k_{cat}$ *vs.* $1/a_H$.

The cyclic phosphate is the sole initial product, as was shown by paper chromatography and by isolation of the cyclic phosphate (infrared spectrum of the lithium salt) from the reaction mixture after ten half-lives (morpholine buffer, 1:1, 0.1 M free base).[12] It is unlikely that the reaction involves nucleophilic attack on phosphorus by the general base followed by ring closure: (1) nitrogen bases are not particularly efficient

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(1) We thank the National Institutes of Health for support of this research under Grant No. GM 13335.

(2) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952).

(3) For a review of this subject see J. P. Hummel and G. Kalnitsky, *Ann. Rev. Biochem.*, **33**, 15 (1964). A symposium "Ribonuclease—Recent Advances" was held in Buffalo on May 31 and June 1, 1967.

(4) D. Findlay, D. G. Herries, A. P. Mathias, B. R. Rabin, and C. A. Ross, *Nature*, **190**, 781 (1961); A. P. Mathias, A. Deavin, and B. R. Rabin in "Structure and Activity of Enzymes," Symposium No. 1 of the Federation of European Biochemical Societies, London, 1964, Academic Press, London, 1964, pp 19–30; J. E. Erman and G. G. Hammes, *J. Am. Chem. Soc.*, **88**, 5614 (1966), and earlier papers. See also H. G. Gassen and H. Witzel, *European J. Biochem.*, **1**, 36 (1967), and earlier papers.

(5) Hydrolysis of the cyclic triester, methyl ethylene phosphate, is subject to general base catalysis (F. Covitz and F. H. Westheimer, *J. Am. Chem. Soc.*, **85**, 1773 (1963)) and could be considered to be a model for the second step of this reaction.

(6) The only aryl esters of a similar *cis*-1,2-glycol phosphate that have been previously reported are the 2′- and 3′-uridine α-naphthyl phosphates (M. Z. Kowalczewska, H. Sierakowska, and D. Shugar, *Acta. Biochim. Polon.*, **13**, 237 (1966)); these were not obtained in analytically pure form.

(7) F. Cramer, W. Rittersdorf, and W. Böhm, *Ann.*, **654**, 180 (1962).

(8) D. M. Brown, *Advan. Org. Chem.*, 75 (1963).

(9) P. Carré, *Ann. Chim. Phys.*, [8] **5**, 394 (1905).

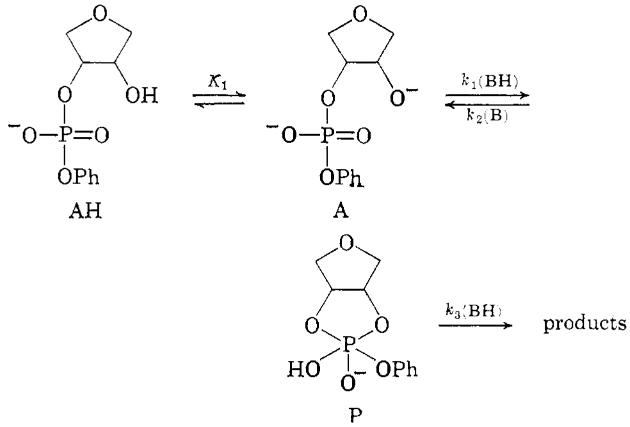
(10) The production of phenol from II is of course also subject to specific acid and base catalysis. The latter may for simplicity be considered to be a concerted reaction in the example given here.

(11) R. P. Bell and E. C. Baughan, *J. Chem. Soc.*, 1947 (1937).

(12) The general species catalyzed process accounts for one-third of the rate in this buffer.

nucleophiles with respect to phosphorus;[13] (2) the *cis*-2-methoxy ester IV was completely unchanged under conditions that represented 70 half-lives for ester II; (3) the sterically hindered 2-methylimidazole also showed general species catalysis of the hydrolysis of II.

The many mechanisms that could fit our present data all involve some form of intermediate that can either return to starting material or go on to product; the partitioning is unsymmetrically catalyzed. One mechanism is presented here as an example only, and should not be taken literally. In this mechanism there is no necessity for the intermediate species to be pentacoordinate, though a phosphorane with finite lifetime is implicit in recent theories[14] concerned with the hydrolysis of cyclic phosphates and has been invoked as part of the mechanism of hydrolysis of ribonucleic acid by ribonuclease.[3,4] An alternative cyclic triester is considered by us to be an unlikely candidate.



AH

A

P

In the pH range 7–9, A << AH, and the assumption of a steady-state concentration for P gives $k_{\text{cat}} = k_1k_3K_1a_{\text{H}}/(K_ak_2 + k_3a_{\text{H}})K_a$, where K_a is the acid dissociation constant of the general acid, $k_{\text{cat}} = (k_{\text{obsd}} - k_{\text{hyd}})/$(free base), and k_{hyd} is the rate constant for hydrolysis at zero buffer concentration at that pH. If the value 13 is given to pK_1,[15] then for morpholine $k_1 = 29$ M^{-1} sec^{-1} and $k_2/k_3 = 0.33$.

The phenyl ester of *cis*-cyclopentane-1,2-diol monophosphate has also been synthesized and interestingly shows a hydroxide ion catalyzed rate of hydrolysis 80 times less than that of II, while the specific acid catalyzed rates are almost identical at pH 1.2.

Future research is directed toward achieving a more complete description of the mechanism of this reaction, its possible significance with respect to the action of ribonuclease, and the synthesis of polyfunctional molecules that may more efficiently catalyze the decomposition of II and related esters.

[13] J. R. Cox, Jr., and O. B. Ramsay, *Chem. Rev.*, **64**, 317 (1964).

[14] E. A. Dennis and F. H. Westheimer, *J. Am. Chem. Soc.*, **88**, 3431, 3432 (1966); see also D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 52 (1952); F. Ramirez, *Pure Appl. Chem.*, **9**, 337 (1964).

[15] This figure is used just for illustration, and may well be out by more than one p*K* unit. See P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **82**, 795 (1960). The pK_a value used for morpholine (50°) was 8.2.

[16] Supported by National Institutes of Health Traineeship Grant No. 2T01 GM00834-06.

D. G. Oakenfull,[16] David I. Richardson, Jr., D. A. Usher
Baker Laboratory of Chemistry
Cornell University, Ithaca, New York 14850

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Alkyldiazonium Cations. I. Direct Observation of the 2,2,2-Trifluoroethyldiazonium Ion

Sir:

We wish to report the first direct observation of a stable alkyldiazonium ion in which nitrogen is attached to sp^3 carbon. The ^{1}H and ^{19}F spectra of trifluoromethyldiazomethane in FSO_3H at −60° provide this evidence. Under these conditions the 2,2,2-trifluoroethyldiazonium ion is stable for at least 1 hr.

The alkyldiazonium ion has been postulated as a common intermediate species in the acid-catalyzed decomposition of diazo compounds and the nitrous acid deamination of aliphatic primary amines.[1] Evidence for the RN_2^+ intermediate has come from both rate data and product analysis studies. With bridgehead primary amines, both Curtin and Scherer have succeeded in trapping alkyldiazonium cations using the nitrosyl chloride route to produce RN_2^+.[2] However, vinyldiazonium cations could not be trapped by coupling with β-naphthol.[3]

In addition, a number of stable aliphatic diazonium ions have been reported recently.[4] However, each of these involves extensive stabilization through charge delocalization and nitrogen attached to an sp^2-hybridized carbon. These diazonium ions have been prepared by protonation of the corresponding diazo compounds. In each instance protonation was at a basic atom other than the α-carbon atom.

The great acid stability of diazo compounds, where loss of N_2 in the conjugate acid leads to unstable carbonium ions, is well known. Rate studies of the acid-catalyzed solvolyses of ethyl diazoacetate[5] and α-diazo sulfones[6] have demonstrated a protonation–deprotonation equilibrium preceding the rate determining loss of molecular nitrogen. The alkyldiazonium cation is clearly implicated as an important reaction intermediate. However, despite many attempts to obtain direct evidence for the alkyldiazonium ion in simple aliphatic systems, none had been found previously.

Slow addition of a solution of trifluoromethyldiazomethane[7] in chloroform-*d* to FSO_3H at −78° with good stirring gave a solution of the diazonium ion in the lower acid layer. Both ^{1}H and ^{19}F spectra were run at −60° on the FSO_3H solution. In each case absorptions corresponding to the presence of both the diazonium ion and the final reaction product, 2,2,2-trifluoroethyl fluorosulfate, were observed. Warming the solution to approximately −20° led to the vigorous evolution of a gas. Vapor density measurements showed that the evolved gas was nitrogen.

[1] H. Zollinger, "Azo and Diazo Chemistry," Interscience Publishers, Inc., New York, N. Y., 1961, pp 102–108, 120–136.

[2] (a) D. Y. Curtin, B. H. Klanderman, and D. F. Tavares, *J. Org. Chem.*, 27, 2709 (1962); (b) D. Y. Curtin and B. H. Klanderman, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept 1963, Abstracts, p 2Q; (c) K. V. Scherer, Jr., and R. S. Lunt, III, *J. Am. Chem. Soc.*, **88**, 2860 (1966).

[3] D. Y. Curtin, J. A. Kampmeier, and B. R. O'Connor, *ibid.*, **87**, 863 (1965).

[4] (a) K. Bott, *Angew. Chem. Intern. Ed. Engl.*, **3**, 804 (1964); (b) K. Bott, *Tetrahedron*, **22**, 1251 (1966); (c) H. Reimlinger, *Angew. Chem. Intern. Ed. Engl.*, **2**, 482 (1963).

[5] J. D. Roberts, C. M. Regan, and I. Allen, *J. Am. Chem. Soc.*, **74**, 3679 (1952).

[6] (a) B. Zwanenburg, J. B. F. N. Engberts, and J. Strating, *Tetrahedron Letters*, 547 (1964); (b) B. Zwanenburg and J. B. F. N. Engberts, *Rec. Trav. Chim.*, **84**, 165 (1965).

[7] H. Gilman and R. G. Jones, *J. Am. Chem. Soc.*, **65**, 1458 (1943).