

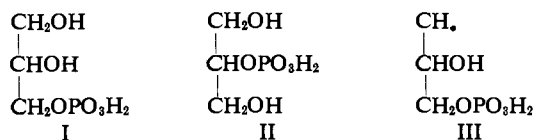
# Solvolysis of Phosphoric Acid Esters. Hydrolysis and Rearrangement Reactions of 1- and 2-Glycero Dihydrogen Phosphates and 1,2-Propanediol 1-Dihydrogen Phosphate

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**Abstract:** The rates of the acid-catalyzed hydrolysis and 1,2 rearrangement reactions of 1- and 2-glycero phosphates and of 1,2-propanediol 1-phosphate were determined at several temperatures, in acid concentrations up to 8 *M* perchloric acid. At constant ionic strength, the rate of hydrolysis of these esters is linearly related to the perchloric acid concentration. The interconversion of 1- and 2-glycero dihydrogen phosphates is strongly acid catalyzed, the rate increasing also with ionic strength. At ionic strength  $\mu = 4$ , the rate is proportional to the perchloric acid concentration. Acid-catalyzed hydrolysis of 1,2-propanediol dihydrogen phosphate, as well as of the glycero phosphates, involves initially rapid rearrangement to the equilibrium mixture of the 1 and 2 isomers. Since this rearrangement occurs with P-O bond fission, acid-catalyzed hydrolysis in  $H_2^{18}O$  produces  $H_3P^{18}O_4$ , with almost complete oxygen exchange. On the basis of the Zucker-Hammett hypothesis, the mechanism of hydrolysis in strongly acid solutions is considered to involve nucleophilic attack of a water molecule on the phosphorus atom of the conjugate acid of the substrate. In weakly acid solutions, these esters have a maximum in rate of hydrolysis at about pH 4, with complete P-O bond fission.

Many naturally occurring phosphate esters, such as phospholipids and sugar phosphates, contain a 2-hydroxy group on the carbon chain. Their hydrolytic reactions often involve isomerization with 1,2 rearrangements, presumably through a cyclic intermediate.<sup>1-4</sup> The formation and hydrolytic breakdown of such five-membered cyclic phosphate esters seem also to occur in the hydrolysis of ribonucleic acid. Rates of hydrolysis and of rearrangement of 1- and 2-glycero phosphates (I and II) in a few acidic media have been



measured,<sup>5</sup> specific rate constants for the hydrolysis of the monoanions were determined, and  $^{18}O$ -tracer experiments were used to determine the points of bond fission during hydrolysis.<sup>6</sup> The synthesis of 1,2-propanediol 1-dihydrogen phosphate (III) has been described,<sup>7</sup> but only isolated data on the rates of its hydrolytic breakdown have been reported.<sup>8</sup> The mechanism of these reactions is not yet clearly understood. Particularly, the possible role of water as a nucleophilic reagent participating in the rate-determining step has not been investigated.

(1) H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, Inc., New York, N. Y., 1961, p 44.

(2) O. Bailly and J. Gaume, *Bull. Soc. Chim. France*, **2**, 354 (1935).

(3) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, **52** (1952); *Ann. Rev. Biochem.*, **24**, 311 (1955); D. M. Brown, G. E. Hall, and H. M. Higson, *J. Chem. Soc.*, 1360 (1958); D. M. Brown and N. K. Hamer, *ibid.*, 406, (1960).

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(5) M. C. Bailly, *Bull. Soc. Chim. France*, **9**, 314, 340, 405 (1942).

(6) P. A. T. Swoboda and E. M. Crook, *Biochem. J.*, **59**, XXIV (1955); P. W. C. Barndard, C. A. Bunton, D. R. Llewellyn, K. G. Oldham, B. L. Silver, and C. A. Vernon, *Chem. Ind. (London)*, 760 (1955).

(7) (a) F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, 382 (1945); (b) T. Ukita, K. Nagasawa, and M. Irie, *Pharm. Bull. (Tokyo)*, **5**, 121 (1957).

(8) (a) O. N. Miller, C. G. Huggins, and K. Arai, *J. Biol. Chem.*, **202**, 263 (1953); (b) O. Lindberg, *Arkiv Kemi Mineral. Geol.*, **23A**, 1 (1946).

The present work attempts to present a more detailed study of the reactions of 2-hydroxy-substituted alkyl dihydrogen phosphates by applying kinetic and tracer methods.

## Experimental Section

**Materials.** 1,2-Propanediol 1-dihydrogen phosphate was prepared<sup>7,8b</sup> by mixing disodium hydrogen phosphate (72 g) in water (500 ml) with propylene oxide (24 g). Water was added until the solution was homogeneous. After 20 days at room temperature, barium hydroxide (100 g) was added to precipitate the unreacted orthophosphate. The precipitate was filtered off, and carbon dioxide was passed through the filtrate to remove the barium ions. The filtrate was evaporated on a steam bath to 300 ml. Addition of ethanol resulted in an oil<sup>7b</sup> containing a mixture of mono- and dialkyl phosphates ( $R_f$  0.4 and 0.74 by ascending paper chromatography<sup>9</sup> in *n*-propyl alcohol-ammonia-water, 6:3:1). The di-propane-1,2-diol phosphate was decomposed by dissolving the mixture in water (200 ml), adding the acid form of Dowex-50-WX12 cation exchanger, and boiling for 1 hr. After filtration, the solution was brought to pH 9 with cyclohexylamine. Addition of acetone precipitated the dicyclohexylamine salt of 1,2-propanediol phosphate, which was recrystallized from aqueous acetone, yielding colorless needles, mp 158°,  $R_f$  0.40 (determined as above). *Anal.* Calcd for  $C_{11}H_{25}O_5N_2P \cdot 0.5H_2O$ : C, 49.60; H, 9.92; N, 7.72; P, 8.55. Found: C, 49.60; H, 10.1; N, 7.02; P, 8.86. The  $^{31}P$  nmr spectrum had the triplet peak required for the 1,2-propanediol 1-phosphate ( $\delta_P = -4.04$  ppm and  $J_{PH} = 6.7$  cps), as well as a small doublet peak (~10% of the total, with  $\delta_P = -5.05$  ppm and  $J_{PH} = 3.0$  cps), which may be due to the 1,2-propanediol 2-phosphate isomer. 1-Glycero disodium phosphate (Fluka) was recrystallized from aqueous ethanol. 2-Glycero disodium phosphate (Sigma Chemical Co.) was used without further purification.

**Kinetic Methods.** A. Rates of hydrolysis were determined by thermostating ( $\pm 0.1^\circ$ ) about 0.003 *M* solutions of phosphate esters in sealed tubes. The reaction was interrupted by cooling in ice water, and the orthophosphate produced was measured by the molybdate method.<sup>10</sup> The initial concentration of glycerophosphate was determined by oxidation with periodic acid to glycolaldehyde phosphate,<sup>11</sup> which undergoes rapid acid-catalyzed hydrolysis (in 2.5 *N* sulfuric acid) to orthophosphate,<sup>12</sup> and using

(9) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(10) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 377 (1925).

(11) C. F. Burmaster, *ibid.*, **164**, 233 (1946).

(12) P. Fleury, J. Courtois, and A. Desjoberg, *Bull. Soc. Chim. France*, **15**, 694 (1948).

colorimetric phosphate assay, as above. The errors in rate constants were about  $\pm 3\%$ .

**B. Rates of rearrangement** were measured in about 0.05 *M* solutions of 2-glycero dihydrogen phosphate in volumetric flasks kept in appropriate thermostats ( $\pm 0.05^\circ$ ). Aliquots (0.5 ml) were pipetted into a slight excess of aqueous sodium hydroxide to quench the reaction. The 1-glycero dihydrogen phosphate formed was determined by oxidation with periodic acid (20 min at pH 4) and titrating the excess periodic acid iodometrically with arsenite.<sup>13</sup> Errors in rate constants were  $\pm 5$ –10%.

**C. Bond breakage during hydrolysis** of the phosphate esters was measured by dissolving in  $^{18}\text{O}$ -enriched water. After more than one half-time, the orthophosphate was precipitated by adding barium chloride and bringing the solution with sodium hydroxide to pH 9. The precipitate was washed, dissolved in hydrochloric acid, reprecipitated with sodium hydroxide, dissolved by shaking with the protonated form of Dowex-50WX12 ion exchanger, brought to pH 4 with 3 *N* potassium hydroxide, and precipitated as  $\text{KH}_2\text{PO}_4$  by addition of acetone and cooling overnight in a deep freeze. The potassium dihydrogen phosphate was washed with acetone, dried, at  $60^\circ$  under reduced pressure, and analyzed for  $^{18}\text{O}$  by the method of Boyer, *et al.*<sup>14</sup>

**D. Oxygen exchange** during the rearrangement of 1,2-propanediol dihydrogen phosphate (0.1 *M*) in perchloric acid (1 *M*; containing 10 atm %  $^{18}\text{O}$  in the water) was measured by maintaining the solution for 0.5 hr at  $100^\circ$ . Orthophosphate was removed by precipitation as the barium salt.

The unreacted propanediol phosphate was purified from traces of  $^{18}\text{O}$ -orthophosphate by isotopic dilution—normal orthophosphate was added and precipitated as the barium salt. Barium propanediol phosphate, as well as other salts, were precipitated by addition of acetone. The precipitate was dissolved in normal water (for isotopic dilution of the  $^{18}\text{O}$ -enriched solvent) and reprecipitated in acetone. The solution was brought to pH 4 with hydrochloric acid and was hydrolyzed by maintaining for 18 hr at  $100^\circ$  in a sealed tube (about one half-time). The resulting orthophosphate was isolated and analyzed for  $^{18}\text{O}$  as above.

## Results

Hydrolytic decomposition may be due to reactions of the various charge states of the substrate, the conjugate acid, the neutral molecule, and the monoanion. Rate constants associated with these reactions will be described by  $k_A$ ,  $k_N$ , and  $k_M$ . Specific rate constants due to 1- or 2-glycero dihydrogen phosphate ( $\alpha$ - or  $\beta$ -glycero dihydrogen phosphate), will be marked with the additional subscript  $\alpha$  or  $\beta$ . The rates of the rearrangement reaction of 1- and 2-glycero dihydrogen phosphate will be described by  $k_\alpha$  and  $k_\beta$ .

**A. Acid-Catalyzed Hydrolysis of Glycero Dihydrogen Phosphates.** The rates of the acid-catalyzed hydrolysis of 1- and of 2-glycero phosphate are equal, within experimental error, as shown in Table I. Thus, their hydrolyses must involve rapid, reversible equilibration.

Table I. Comparison of Rates of Acid-Catalyzed Hydrolysis of 1- and 2-Glycero Phosphates at  $100^\circ$

| $[\text{HClO}_4]$ ,<br><i>M</i> | $10^6 k_\alpha^a$ ,<br>$\text{sec}^{-1}$ | $10^6 k_\beta^b$ ,<br>$\text{sec}^{-1}$ |
|---------------------------------|--|---|
| 0.80                            | 3.2                                      | 3.2                                     |
| 1.60                            | 4.3                                      | 4.3                                     |
| 3.30                            | 5.8                                      | 6.0                                     |

<sup>a</sup> 1-Glycero dihydrogen phosphate. <sup>b</sup> 2-Glycero dihydrogen phosphate.

The dependence of the rate of hydrolysis on acid concentration was measured in aqueous perchloric

(13) F. O. Treadwell, "Analytical Chemistry," Vol. II, 9th ed, John Wiley and Sons, Inc., New York, N. Y., 1958, p 602.

(14) P. D. Boyer, D. J. Graves, C. H. Suelter, and M. E. Dempsey, *Anal. Chem.*, **33**, 1906 (1961).

acid, either without or with control of ionic strength. Results are presented in Table II and show that the rate increases linearly with the acid concentration and that added sodium perchlorate has little effect on the rate of hydrolysis. Assuming the reaction in strongly acid media to be due both to a first-order hydrolysis of the neutral substrate and to a second-order, acid-catalyzed reaction, we get  $k_{\text{obsd}} = k_N + k_A[\text{HClO}_4]$ . From the slope and intercept of the plots of  $k_{\text{obsd}}$  against  $[\text{HClO}_4]$ , a least-square fit gave the values shown in Table III for the rate of reaction of the neutral species and for the acid-catalyzed reaction.

Table II. Rates of Hydrolysis of Glycero Dihydrogen Phosphates in Aqueous Perchloric Acid without Control of Ionic Strength (Column A), and with Constant Ionic Strength by Added Sodium Perchlorate<sup>a</sup>

| Temp,<br>$^\circ\text{C}$ | $[\text{HClO}_4]$ ,<br><i>M</i> | First-order rate constants,<br>$10^6 k$ , $\text{sec}^{-1}$ |     |      |
|---------------------------|---------------------------------|---|-----|------|
|                           |                                 | A   | B   | C    |
| 100.0                     | 1.00 $\pm$ 0.02                 | 3.1   | 2.7 | 2.9  |
|                           | 2.00                            | 4.3   | 4.1 | 4.9  |
|                           | 3.00                            | 6.1   | 5.7 | 6.4  |
|                           | 4.00                            | 8.0   |     | 8.0  |
|                           | 5.00                            | 10.0  |     | 9.5  |
|                           | 6.00                            | 11.8  |     | 11.0 |
|                           | 7.00                            | 13.8  |     |      |
|                           | 8.00                            | 16.0  |     |      |
|                           | 9.00                            | 18.2  |     |      |
| 85.5                      | 2.00                            | 1.00  |     |      |
|                           | 4.00                            | 2.10  |     |      |
|                           | 6.00                            | 3.18  |     |      |
| 72.3                      | 2.00                            | 0.30  |     |      |
|                           | 4.00                            | 0.62  |     |      |
|                           | 6.00                            | 1.01  |     |      |

<sup>a</sup>  $\mu = 4$  (column B) and  $\mu = 6$  (column C). In runs A, 1-glycero phosphate was used; in runs B and C, 2-glycero phosphate.

Table III

| Temp,<br>$^\circ\text{C}$ | $10^6 k_N$ ,<br>$\text{sec}^{-1}$ | $10^6 k_A$ ,<br>1. mole <sup>-1</sup><br>$\text{sec}^{-1}$ |
|---------------------------|-----------------------------------|--|
| 100.0                     | 0.6 $\pm$ 0.2                     | 1.91 $\pm$ 0.04  |
| 85.5                      |                                   | 0.55 $\pm$ 0.01  |
| 72.3                      |                                   | 0.14 $\pm$ 0.01  |

**B. Acid-Catalyzed Rearrangement and Hydrolysis of 1,2-Propanediol 1-Dihydrogen Phosphate.** 1,2-Propanediol 1-dihydrogen phosphate in perchloric acid solution (1 *M*) was found to undergo rapid oxygen exchange with the solvent. At  $100^\circ$ , the isotopic equilibration was complete in 30 min, and resulted in exchange of *three* oxygen atoms, as shown in Table IV.

Table IV

| Min | Atom % excess $^{18}\text{O}$ |                        | No. of O<br>atoms<br>exchanged |
|-----|-------------------------------|------------------------|--------------------------------|
|     | In water                      | In ortho-<br>phosphate |                                |
| 30  | 10.1 $\pm$ 0.1                | 7.8                    | 3.1 $\pm$ 0.1                  |
| 180 | 11.9 $\pm$ 0.1                | 8.4                    | 2.9 $\pm$ 0.1                  |

In these experiments, unhydrolyzed propanediol phosphate was recovered after the time stated and analyzed for  $^{18}\text{O}$  enrichment by hydrolysis at pH 4 in

normal water, which occurs with P-O bond fission only. Assay of  $^{18}\text{O}$  on the resulting orthophosphate gave the result in column three. About 75% of the complete exchange had occurred, or three out of four oxygen atoms in orthophosphate had exchanged. In analogy to the glycerol phosphates, this rapid oxygen exchange in propanediol phosphate is probably due to a  $1 \rightleftharpoons 2$  rearrangement of the phosphoric ester group.

The hydrolysis of 1,2-propanediol phosphate is strongly acid catalyzed. Observed first-order rate constants for the hydrolysis in aqueous perchloric acid are presented in Table V. The present results for the

**Table V.** Rates of Hydrolysis of 1,2-Propanediol 1-Phosphate in Strongly Acid Media without Control of Ionic Strength (Column A), and with Constant Ionic Strength by Added Sodium Perchlorate<sup>a</sup>

| Temp, °C | [HClO <sub>4</sub> ], M | 10% <i>k</i> <sub>obsd</sub> , sec <sup>-1</sup> |      |      |       |
|----------|-------------------------|--|------|------|-------|
|          |                         | A  | B    | C    | D     |
| 100.0    | 0.50                    | 2.3  |      |      |       |
|          | 1.00                    | 3.0  | 6.1  | 13.2 | 29.0  |
|          | 2.00                    | 4.78   | 9.7  | 24.5 | 67.0  |
|          | 3.00                    | 8.9  | 14.0 | 35.5 | 110   |
|          | 4.00                    | 17.2   |      | 47.6 | 140   |
|          | 4.78                    | 29.0   |      |      |       |
|          | 5.00                    |  |      | 63.7 | 250   |
|          | 6.00                    | 68.4   |      |      | 288   |
|          | 7.00                    | 123  |      |      |       |
|          | 8.00                    | 449  |      |      |       |
| 87.7     | 10.00                   | 3500   |      |      |       |
|          | 1.00                    |  | 0.79 |      |       |
|          | 2.00                    |  | 1.68 | 3.59 | 9.88  |
|          | 3.00                    |  | 2.20 |      |       |
|          | 4.00                    | 3.12   |      | 8.40 | 18.60 |
|          | 6.00                    | 13.5   |      |      | 47.80 |
| 72.0     | 8.00                    | 87.0   |      |      |       |
|          | 1.00                    |  | 0.18 |      |       |
|          | 2.00                    |  | 0.30 | 0.69 | 1.68  |
|          | 3.00                    |  | 0.43 |      |       |
|          | 4.00                    | 0.61   |      | 1.58 | 3.42  |
|          | 6.00                    | 2.38   |      | 2.38 | 8.85  |
| 8.00     | 22.1                    |  |      |      |       |

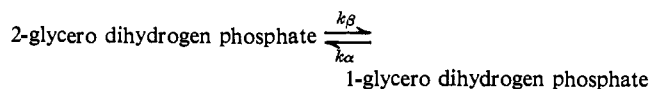
<sup>a</sup>  $\mu = 4$  (column B),  $\mu = 6$  (column C), and  $\mu = 8$  (column D).

rate in aqueous perchloric acid are fairly similar to those calculated from previous reports on the yields of hydrolysis at 100° in 1 N hydrochloric acid ( $2.2 \times 10^{-6} \text{ sec}^{-1}$ ), and in 5 N sulfuric acid ( $17 \times 10^{-6} \text{ sec}^{-1}$ ).<sup>8</sup>

The plot of  $k_{\text{obsd}}$  against perchloric acid concentration is linear only if the ionic strength is kept constant. A least-square fit of the plot  $k_{\text{obsd}} = k_{\text{N}} + k_{\text{A}}[\text{HClO}_4]$  results in  $k_{\text{N}} = (2.35 + 0.38) \times 10^{-6} \text{ sec}^{-1}$ ,  $k_{\text{A}} = (3.70 \pm 0.14) \times 10^{-6} \text{ l. mole}^{-1} \text{ sec}^{-1}$ , and  $k_{\text{N}} = (2.37 \pm 0.57) \times 10^{-6} \text{ sec}^{-1}$ ,  $k_{\text{A}} = (11.08 \pm 0.15) \times 10^{-6} \text{ l. mole}^{-1} \text{ sec}^{-1}$  at 100° and ionic strengths  $\mu = 4$  and 6, respectively. Thus, only the acid-catalyzed reaction is affected by a change in ionic strength. In contrast, in the hydrolysis of the glycerol dihydrogen phosphates, neither the neutral nor the acid-catalyzed reactions are subject to an ionic strength effect. Both the neutral and the acid-catalyzed reactions of propanediol phosphate are faster than those of the glycerol phosphates.

**C. Rearrangement of Glycerol Dihydrogen Phosphates.** 1,2-Intramolecular migration of the phosphoric acid group, as was shown by Bailly,<sup>5</sup> occurs only in solutions more acidic than pH 3. With higher acid concentrations, the rate of rearrangement increases

more rapidly than the rate of hydrolysis. The rate of rearrangement is extremely sensitive to the ionic strength of the solution (see Table VI). The equilibrium constant for the rearrangement



was found to be independent both of the concentration of perchloric acid and of the temperature, within the range -3 to 100° and 2 to 8 M perchloric acid (see Table VII). The equilibrium mixture contained  $0.865 \pm 0.006$  equiv of 1-glycerol phosphate and  $0.135 \pm 0.006$  equiv of 2-glycerol phosphate. Therefore the equilibrium constant is  $k_{\beta}/k_{\alpha} = 0.865/0.135 = 6.41$ , which is in good agreement with the ratio 6.70 reported by Verkade, *et al.*,<sup>15</sup> and Bailly<sup>5</sup> for boiling solutions.

**Table VI.** Rates of Rearrangement of Glycerol Dihydrogen Phosphates, in the Absence and Presence of Sodium Perchlorate, as a Function of Perchloric Acid Concentration

| Temp, °C | [HClO <sub>4</sub> ], M | [NaClO <sub>4</sub> ], M | 10% <i>k</i> <sub>obsd</sub> , sec <sup>-1</sup> | 10% <i>k</i> <sub>α</sub> , sec <sup>-1</sup> | 10% <i>k</i> <sub>β</sub> , sec <sup>-1</sup> |
|----------|-------------------------|--------------------------|--|---|---|
| 21.8     | 3                       |                          | 49   | 7   | 42  |
|          | 4                       |                          | 75   | 10  | 65  |
|          | 5                       |                          | 120  | 16  | 104   |
|          | 6                       |                          | 200  | 27  | 173   |
|          | 8                       |                          | 420  | 57  | 363   |
|          | 1                       | 3                        | 21   | 3   | 18  |
|          | 2                       | 2                        | 32   | 4   | 28  |
|          | 3                       | 1                        | 50   | 7   | 43  |
|          | 1                       | 5                        | 32   | 4   | 28  |
|          | 5                       | 1                        | 130  | 18  | 112   |
| 31.8     | 1                       |                          | 22   | 3   | 19  |
|          | 2                       |                          | 45   | 6   | 39  |
|          | 3                       |                          | 120  | 16  | 104   |
|          | 4                       |                          | 190  | 26  | 164   |
|          | 5                       |                          | 376  | 51  | 325   |
|          | 6                       |                          | 548  | 74  | 474   |
|          | 7                       |                          | 780  | 105   | 675   |
|          | 8                       |                          | 1080   | 145   | 935   |
|          | 1                       | 3                        | 64   | 9   | 55  |
|          | 2                       | 2                        | 117  | 16  | 101   |
| 45.3     | 1                       | 5                        | 103  | 14  | 89  |
|          | 2                       | 4                        | 145  | 20  | 125   |
|          | 4                       | 2                        | 320  | 43  | 277   |
|          | 2                       | 6                        | 259  | 35  | 225   |
|          | 5                       | 3                        | 430  | 58  | 372   |
|          | 6                       | 2                        | 595  | 80  | 515   |
|          | 2                       |                          | 337  | 45  | 292   |
|          | 4                       |                          | 815  | 110   | 705   |
|          | 6                       |                          | 2900   | 400   | 2500  |
|          | 8                       |                          | 5000   | 680   | 4320  |
|          | 1                       | 3                        | 220  | 30  | 190   |
|          | 2                       | 2                        | 445  | 60  | 385   |
|          | 3                       | 1                        | 710  | 95  | 615   |
|          | 1                       | 5                        | 323  | 43  | 280   |
|          | 3                       | 3                        | 890  | 120   | 770   |
|          | 4                       | 2                        | 1280   | 170   | 1110  |
|          | 2                       | 6                        | 755  | 102   | 653   |
|          | 6                       | 2                        | 2500   | 340   | 2160  |

From the observed first-order rate constants  $k_{\text{obsd}}$  for appearance of glycerol 1-dihydrogen phosphate (measured by the periodic acid method) in acidic solutions of glycerol 2-dihydrogen phosphate, together with the

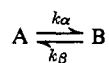
(15) P. E. Verkade, J. C. Stoppelenburg, and W. D. Cohen, *Rec. Trav. Chim.*, **59**, 886 (1940).

**Table VII.** Equilibrium Ratio of Glycero 1-Dihydrogen Phosphate [ $A_e$ ] to Total Glycero Dihydrogen Phosphates [ $A_e + B_e$ ] at Several Temperatures and Perchloric Acid Concentrations

| Temp, °C | [HClO <sub>4</sub> ], M | [A <sub>e</sub> ]/[A <sub>e</sub> + B <sub>e</sub> ] |
|----------|-------------------------|--|
| -3.0     | 4                       | 0.873  |
|          | 6                       | 0.867  |
|          | 8                       | 0.859  |
| 21.8     | 4                       | 0.865  |
|          | 6                       | 0.867  |
|          | 8                       | 0.860  |
| 31.8     | 3                       | 0.855  |
|          | 4                       | 0.866  |
|          | 5                       | 0.871  |
|          | 6                       | 0.857  |
| 45.3     | 7                       | 0.876  |
|          | 2                       | 0.870  |
|          | 4                       | 0.864  |
|          | 6                       | 0.856  |
| 100.0    | 8                       | 0.858  |
|          | 2                       | 0.860  |
|          | 5                       | 0.857  |
|          | 8                       | 0.859  |

measured equilibrium constant for the rearrangement,  $K = [A_e]/[B_e]$  (where [ $A_e$ ] and [ $B_e$ ] are the equilibrium concentrations of 1- and 2-glycerophosphate), it was possible to derive the specific constant  $k_\alpha$  and  $k_\beta$  for the rates of migration of glycero 1- and 2-dihydrogen phosphates.

For the reversible first-order reactions



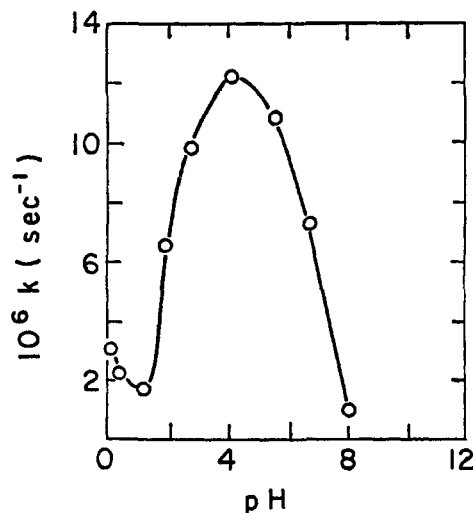
it can be shown,<sup>16a</sup> that if [ $B_0$ ], [ $B_t$ ], and [ $B_e$ ] denote the concentrations of glycero 2-dihydrogen phosphate at times zero,  $t$ , and equilibrium, the rate of approach to equilibrium is a first-order reaction, with an observed first-order rate constant, which is the sum of rate constants for the forward and reverse reactions  $\ln \frac{([B_0] - [B_e])}{([B_t] - [B_e])} = (k_\alpha + k_\beta)t = k_{\text{obsd}}t$ .

Thus, a plot of  $\ln \frac{([B] - [B_e])}{([B_0] - [B_e])}$  against  $t$  gives the slope  $k_{\text{obsd}}$ . Using Guggenheim's method<sup>16b</sup> of determining the rate constant without requiring knowledge of the equilibrium concentration [ $B_e$ ], plots were made of  $\ln \frac{([B_t] - [B_{t+\Delta t}])}{([B_0] - [B_{0+\Delta t}])}$  against  $t$ , where [ $B_t$ ] and [ $B_{t+\Delta t}$ ] are the concentrations of glycero 2-phosphate at time  $t$ , and after a constant interval  $\Delta t$ . The slopes of these plots are  $k_{\text{obsd}} = (k_\alpha + k_\beta)$ . The specific rate constants  $k_\alpha$  and  $k_\beta$  were found from the following equations, using the measured equilibrium constant  $[A_e]/[B_e]$ ,  $k_\alpha[A_e] = k_\beta[B_e]$ . Hence,  $k_\alpha = k_{\text{obsd}}[B_e]/([A_e] + [B_e])$  and  $k_\beta = k_{\text{obsd}}[A_e]/([A_e] + [B_e])$ .

Results, for the rearrangement of glycero 2-dihydrogen phosphate in aqueous perchloric acid, without and with control of ionic strength, are included in Table VI. In contrast to the hydrolysis, the rate of rearrangement of glycero dihydrogen phosphate is not linearly related to the perchloric acid concentration. The rate of rearrangement in D<sub>2</sub>O is larger than in water. At 45.3° in 0.96 M perchloric acid,  $k_{D_2O}/k_{H_2O} = 1.23 \pm 0.09$ .

**D. Reactions of the Monoanions of 1- and 2-Glycero Hydrogen Phosphates.** In moderately acid solutions, above pH 2, the rate of rearrangement of the

(16) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961: (a) p 186, (b) p 49.



**Figure 1.** Rates of hydrolysis of 1,2-propanediol 1-dihydrogen phosphate at 100° as a function of pH.

glycerophosphates is negligible in comparison with the rates of hydrolysis. In this region, the rates of hydrolysis of the two isomers differ. In the present work, constants observed at 100° in solutions of pH 4.3 are  $k_{M\alpha} = 1.44 \times 10^{-5} \text{ sec}^{-1}$  and  $k_{M\beta} = 3.22 \times 10^{-5} \text{ sec}^{-1}$  for 1- and 2-glycero phosphates. In these solutions, essentially all the substrate is in form of the monoanion, and therefore these values are also the specific rate constants for the unimolecular hydrolysis of the monoanions of the two isomers. Previously reported values for these specific rate constants are<sup>5</sup>  $k_{M\alpha} = 1.35 \times 10^{-5} \text{ sec}^{-1}$  and  $k_{M\beta} = 2.77 \times 10^{-5} \text{ sec}^{-1}$ .

**E. Reactions of the Monoanion of 1,2-Propanediol 1-Dihydrogen Phosphate.** As with the glycero phosphates and other simple alkyl phosphates, the reaction above pH 1 is mainly due to hydrolysis of the monoanion. The rate has a rather marked maximum at about pH 4 (see Table VIII and Figure 1), at which the

**Table VIII.** Dependence of Rate of Hydrolysis of 1,2-Propanediol Dihydrogen Phosphate on pH at 100°

| pH   | Buffer solution                                  | $10^6 k_{\text{obsd}}$ , $\text{sec}^{-1}$ |
|------|--|--|
| 1.10 | 0.1 M HClO <sub>4</sub>                          | 1.7  |
| 1.80 | 0.264 N HCl + 0.047 M KCl                        | 6.6  |
| 2.65 | KHP <sup>a</sup> + 0.039 N HCl                   | 9.8  |
| 4.25 | KHP <sup>a</sup>                                 | 12.2                                       |
| 5.50 | KHP <sup>a</sup> + 0.024 N NaOH                  | 10.8                                       |
| 6.70 | KHP <sup>a</sup> + 0.046 N NaOH                  | 7.2  |
| 7.90 | 0.082 M sodium diethylbarbiturate + 0.0177 N HCl | 1.01                                       |

<sup>a</sup> KHP = potassium hydrogen phthalate, 0.05 M.

concentration of the monoanion is largest. In alkaline solutions, the rate of hydrolysis is negligible; thus, there was no measurable production of orthophosphate after 4 days in 2 N sodium hydroxide at 100°. Assuming the maximal rate at pH 4 to be due only to decomposition of the monoanion, we get  $k_M = 1.22 \times 10^{-5} \text{ sec}^{-1}$ , which is similar to the value obtained above for the rate of hydrolysis of the monoanion of 1-glycero phosphate.

Table IX. Positions of Bond Breakage during Hydrolysis at 100° in <sup>18</sup>O-Enriched Water

| Phosphate (ester), <i>M</i> | Solvent                      | Time, hr | 10 <sup>6</sup> <i>k</i> , sec <sup>-1</sup> | <i>a</i> (water, atom % excess <sup>18</sup> O) | <i>b</i> (PO <sub>4</sub> <sup>3-</sup> , atom % excess <sup>18</sup> O) | 100 <i>b/a</i> |
|-----------------------------|------------------------------|----------|--|---|--|----------------|
| Propanediol-1 (0.3)         | 4 <i>M</i> HClO <sub>4</sub> | 22       | 17.2   | 16.9  | 15.8   | 94 ± 4         |
| Propanediol-1 (0.3)         | pH 4                         | 17       | 12.2   | 26.7  | 6.7  | 25 ± 2         |
| 2-Glycero (1.0)             | 4 <i>M</i> HClO <sub>4</sub> | 48       | 6.8  | 16.0  | 16.3   | 102 ± 4        |
| 2-Glycero (0.5)             | pH 4.3                       | 6        | 2.70   | 32.2  | 0.75   | 27 ± 2         |

**F. Positions of Bond Breakage during Hydrolysis.** Hydrolysis of the two isomeric glycerol phosphates and 1,2-propanediol 1-phosphate may occur with alkyl-oxygen or with phosphorus-oxygen bond breakage. Each charge state of the substrate, the conjugate acid, the neutral molecule, and the monoanion, may react by a different process. Using <sup>18</sup>O-enriched water as solvent, the extent of P-O bond breakage is determined by the <sup>18</sup>O content of the orthophosphate produced by the hydrolysis. Three reaction processes are possible: (a) carbon-oxygen bond breakage; in this case the orthophosphate is unlabeled (no excess <sup>18</sup>O); (b) irreversible phosphorus-oxygen bond breakage; the orthophosphate is labeled with one <sup>18</sup>O atom (<sup>18</sup>O excess 25% of that of the water, and therefore 100% P-O bond fission); (c) rapid, reversible phosphorus-oxygen bond breakage, through intermediate formation of a cyclic phosphate ester, ring opening by nucleophilic attack of water on the phosphorus atom, followed by hydrolysis as in a or b, above; in this case the orthophosphate becomes equilibrated with the water (<sup>18</sup>O excess 75-100% of the water).

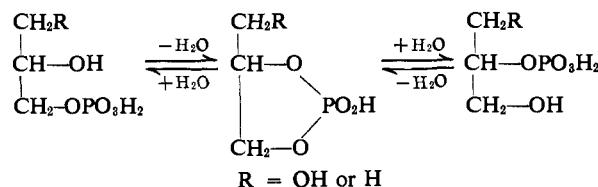
As shown in Table IX, in strongly acid solutions, the hydrolysis of both the propanediol and the glycerol phosphates occurs with formation of orthophosphate which is essentially in isotopic equilibrium with the solvent water. The ratio 100*b/a* (last column of the table) is close to 100%, and the mechanism c is in operation. Thus the hydrolysis of propanediol dihydrogen phosphate, as of the glycerol phosphates, involves only P-O bond fission; this agrees with the observation that glycerol isolated after hydrolysis of glycerophosphates in H<sub>2</sub><sup>18</sup>O did not become isotopically enriched.<sup>6</sup>

In weakly acid solutions, at about pH 4, in which the predominant species is the monoanion, the hydrolysis produces orthophosphate containing just 25% of the <sup>18</sup>O excess of the solvent and therefore the reaction involves only P-O bond fission, by mechanism b (above).

## Discussion

In the acid-catalyzed reaction, both the two isomeric glycerol phosphates and 1,2-propanediol 1-phosphate undergo hydrolysis with almost complete oxygen exchange in the phosphate group. Following the pioneering discovery of Chargaff that the equilibration between 1- and 2-glycerol phosphates in acid solution is intramolecular,<sup>17</sup> this has been explained by a mechanism involving a cyclic phosphate ester.<sup>6</sup> Each step of ring closure and ring opening occurs only with P-O bond fission; these steps are more rapid than hydrolysis. The actual concentration of the cyclic inter-

mediate must be very small. For the glycerol phosphates, the equilibrium mixture contains 86.5% of 1-glycerol and 13.5% of 2-glycerol phosphate. The preferred abundance for the 1 isomer may be only partly due to the twice as large probability to be bound to the 1-hydroxy group. The statistical distribution should be 66.6 and 33.3% for the 1- and 2-glycerol phosphates. The additional preference for the primary ester bond may be due to steric hindrance.



The larger rate of acid-catalyzed rearrangement in D<sub>2</sub>O indicates that in the fast reversible proton transfer preceding the rate-determining step, the deuterated substrate is more stable than the protonated substrate.

The acid-catalyzed hydrolysis of propanediol 1-hydrogen phosphate is similar to that of the glycerol phosphates in involving rapid reversible formation of a cyclic intermediate, as shown by the complete incorporation of oxygen from water into the orthophosphate. Separation of the observed rate constants (*k*<sub>obsd</sub>) into the specific rate constants (*k*<sub>N</sub> and *k*<sub>A</sub>) for the unimolecular and bimolecular rate constants of hydrolysis of the neutral species and the acid-catalyzed reaction reveals that *k*<sub>N</sub> is not affected by changes in ionic strength with either substrate. On the other hand *k*<sub>A</sub> is sensitive to changes in ionic strength for propanediol phosphate, but is insensitive to ionic strength for the glycerol phosphates. The marked effect of ionic strength on the acid-catalyzed hydrolysis of propanediol phosphate may possibly be due to a transition state, which has a larger charge separation than the corresponding transition state for the glycerol phosphates.

While the rate of hydrolysis of the glycerol phosphate is proportional only to the stoichiometric perchloric acid concentration (Figure 2), for propanediol phosphate it is possible also to obtain a linear relationship of log (*k*<sub>obsd</sub> - *k*<sub>N</sub>) against the Hammett acidity function *H*<sub>0</sub>, but with a slope of 0.65. On the basis of the Zucher-Hammett hypothesis,<sup>18</sup> it may therefore be concluded that the hydrolysis of the glycerol phosphates definitely involves a water molecule in the rate-determining step, and is thus a bimolecular reaction, while the hydrolysis of propanediol 1-phosphate is a borderline case between unimolecular and bimolecular reactions.

(18) L. Zucker and L. P. Hammett, *J. Am. Chem. Soc.*, **61**, 2791 (1939).

(17) E. Chargaff, *J. Biol. Chem.*, **144**, 455 (1942).

Table X. Slopes for Rates of Hydrolysis of Alkyl Dihydrogen Phosphates Plotted by Various Acidity Functions

| R in ROPO <sub>3</sub> H <sub>2</sub>     | Log<br>( $k_{\text{obsd}} - k_N$ )<br>vs. log [H <sup>+</sup> ]            | Log<br>( $k_{\text{obsd}} - k_N$ )<br>vs. $H_0$ | Log<br>( $k_{\text{obsd}} - k_N$ )/[H <sup>+</sup> ]<br>vs. log $a_{\text{H}_2\text{O}}$<br>(slope = $w^*$ ) | Log<br>( $k_{\text{obsd}} - k_N$ ) + $H_0$<br>vs. log $a_{\text{H}_2\text{O}}$<br>(slope = $w$ ) | Suggested<br>mechanism |
|---|--|---|--|--|------------------------|
| Methyl <sup>c</sup> (hydrolysis)          | 1.0  | Not linear                                      | -0.08 ± 0.05<br>(D) <sup>a</sup>   | +4.0 ± 0.31  | Bimolecular            |
| Ethyl <sup>d</sup> (hydrolysis)           | At constant $\mu$<br>1.05 ± 0.05   | Not linear                                      | -0.88  | Not linear   | Bimolecular            |
| Isopropyl <sup>d</sup> (hydrolysis)       | Not linear   | 1.30 ± 0.04                                     | -5   | 0.2 ± 0.1  | Unimolecular           |
| <i>t</i> -Butyl <sup>e</sup> (hydrolysis) | 2  | 1.0   | -5 (I) <sup>b</sup>  | 0.1 ± 0.1  | Unimolecular           |
| Propane-1,2-diol<br>1- (hydrolysis)       | Not linear (at<br>$\mu = 6$ , a linear<br>plot, with slope<br>1.02 ± 0.03) | 0.65 ± 0.03                                     | Not linear -3 (I) <sup>b</sup>   | Not linear 2 (D) <sup>a</sup>  | Borderline             |
| 1- and 2-glycero<br>(hydrolysis)          | 1.04 ± 0.01  | Not linear                                      | -0.2 ± 0.1   | Not linear   | Bimolecular            |
| 1- and 2-glycero<br>(rearrangement)       | 3  | Not linear                                      | Not linear   | 3.0 ± 0.5  | Borderline             |

<sup>a</sup> D = steadily decreasing absolute slope. <sup>b</sup> I = steadily increasing absolute slope. <sup>c</sup> See ref 20. <sup>d</sup> See ref 21. <sup>e</sup> See ref 22.

Another approach for testing the acid-catalyzed hydrolysis, as proposed by Bunnett,<sup>19</sup> involves plots of  $\log(k_{\text{obsd}} - k_N)/[\text{H}^+]$  or of  $\log(k_{\text{obsd}} - k_N) + H_0$  vs.  $\log a_{\text{H}_2\text{O}}$ , where  $k_{\text{obsd}}$  and  $k_N$  are the rates for the observed and the neutral reactions,  $[\text{H}^+]$  is the stoichiometric concentration of acid, and  $a_{\text{H}_2\text{O}}$  is the activity of the water in the solution.

For substitution reactions on carbon atoms, the empirical criteria of Bunnett for assigning reaction mechanism on the basis of the values of  $w$  and  $w^*$  seem satisfactory. However, for the examples of alkyl dihydrogen phosphates, for which kinetic data were then available, no rational explanation was possible.<sup>19</sup> The additional results presented in the present work enable a separate empirical classification of reaction mechanisms for alkyl dihydrogen phosphates.

Results for several of these plots are summarized in Table X, which includes comparison with results on other alkyl dihydrogen phosphates. The plot of  $\log(k_{\text{obsd}} - k_N)/[\text{H}^+]$  against  $\log a_{\text{H}_2\text{O}}$  for the hydrolysis of the glycerol dihydrogen phosphates in acid solution is linear, with a slope  $w^* = -0.2 \pm 0.1$ . This put the hydrolysis of this substance in the same mechanistic group as methyl dihydrogen phosphate ( $w^* = -0.08 \pm 0.05$ ).<sup>20</sup> For the hydrolysis of propanediol dihydrogen phosphate, neither the plot of  $\log(k_{\text{obsd}} - k_N)/[\text{H}^+]$  nor the plot of  $\log(k_{\text{obsd}} - k_N) + H_0$  against  $\log a_{\text{H}_2\text{O}}$  are completely linear. In the 3 to 6 *M* HClO<sub>4</sub> range the slope of the plot of  $\log(k_{\text{obsd}} - k_N)/[\text{H}^+]$  is however fairly linear, with  $w^* = -3$ , which is intermediate between that for methyl dihydrogen phosphate and isopropyl<sup>21</sup> and *t*-butyl<sup>22</sup> dihydrogen phosphate (both with  $w^* = -5$ ). For the rearrangement of the glycerol phosphates, a linear plot of  $\log(k_{\text{obsd}} - k_N) + H_0$  against  $\log a_{\text{H}_2\text{O}}$  was obtained, with a slope  $w = 3.0 \pm 0.5$ . This is less than the value  $w = +4.0 \pm 0.31$  reported for methyl dihydrogen phosphate<sup>19,20</sup> which undergoes bimolecular acid hydrolysis, but much

more than the value  $w = 0.1-0.2$  calculated for the unimolecular hydrolysis of isopropyl<sup>21</sup> and *t*-butyl<sup>22</sup> dihydrogen phosphate. Therefore, it seems necessary to place the rearrangement reaction of the glycerol phosphates into a borderline category—between the two limiting mechanisms of uni- and bimolecular hydrolysis—but more nearly similar to a bimolecular reaction, with a water molecule participating in the transition state.

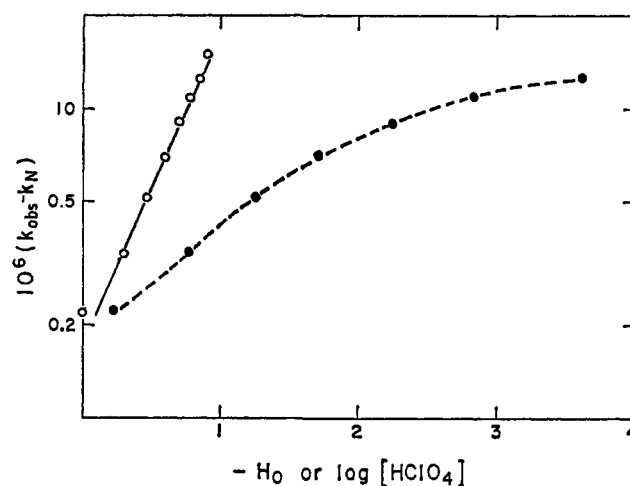


Figure 2. Plot of  $\log(k_{\text{obsd}} - k_N)$  against  $H_0$  (broken line) and against  $\log [\text{HClO}_4]$  (full line) for the hydrolysis of 2-glycero dihydrogen phosphate at 100°, without control of ionic strength.

Further information on the extent of involvement of water molecules in the rate-determining step of hydrolysis may sometimes be obtained by a consideration of the entropy of activation. Reactions with more negative entropy of activation may be understood to have a larger increase in the number of bound water molecules in the transition state. Energies and entropies of activation for the acid-catalyzed reactions of these phosphate esters in 4 *M* perchloric acid at 100° are given in Table XI.

- (19) J. F. Bunnett, *J. Am. Chem. Soc.*, **83**, 4956, 4968, 4978 (1961).  
 (20) C. A. Bunton, D. R. Llewellyn, K. G. Oldham, and A. Vernon, *J. Chem. Soc.*, 3574 (1958).  
 (21) M. Halmann and L. Kugel, to be published.  
 (22) A. Lapidot, D. Samuel, M. Weiss-Brodsky, *J. Chem. Soc.*, 637 (1964).

Table XI

| Ester       | Reaction            | $E_a$ ,<br>kcal mole <sup>-1</sup> | $\Delta S^\ddagger$ , eu |
|-------------|---------------------|------------------------------------|--------------------------|
| Glycero     | Hydrolysis          | $23.4 \pm 0.4$                     | $-21.5$                  |
| Glycero     | Rearrangement (1→2) | $19.1 \pm 0.9$                     | $-18.8$                  |
| Glycero     | Rearrangement (2→1) | $18.4 \pm 0.9$                     | $-17.4$                  |
| Propanediol | Hydrolysis          | $30.1 \pm 0.4$                     | $-2.3$                   |

The acid-catalyzed hydrolysis of glycero dihydrogen phosphate is seen to involve the most negative entropy of activation; this result seems to support the above conclusion based on the Zucker-Hammett hypothesis that a water molecule participates in the rate-determining step of hydrolysis. A fairly similar conclusion can be derived for the mechanism of the rearrangement reaction, which also has a rather negative entropy of activation. On the other hand, the hydrolysis of propanediol phosphate has an entropy of activation of only  $-2.3$  and is thus intermediate in behavior between a uni- and a bimolecular reaction. For the hydrolysis of isopropyl<sup>21</sup> and of *t*-butyl<sup>22</sup> dihydrogen

phosphates, which undergo acid-catalyzed hydrolysis by a completely unimolecular mechanism, entropies of activation of  $+8.2$  and  $+7.2$  eu were found.

The <sup>18</sup>O-tracer experiments showed that the acid-catalyzed rearrangements and hydrolysis of the glycero dihydrogen phosphates and of propanediol dihydrogen phosphate occur only with P-O bond breakage. This result is similar to that recently reported for the hydrolysis of some phosphonates, cyclic esters of hydrogen propylphosphonic acid, which are also cleaved only with P-O bond fission.<sup>23</sup>

The predominance of P-O bond fission during ring formation, ring opening, and hydrolysis of 2-hydroxy-substituted phosphate esters explains the retention of configuration in reactions of such asymmetric phosphates.

**Acknowledgment.** This investigation was supported by Research Grant GM-05842 from the U. S. Public Health Service.

(23) A. Eberhard and F. H. Westheimer, *J. Am. Chem. Soc.*, **87**, 253 (1965).

## Reactions of Methyl Chloromethylphosphinate with Nucleophiles<sup>1</sup>

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*Contribution from the Department of Chemistry, California State College at Los Angeles, Los Angeles, California 90032. Received March 18, 1966*

**Abstract:** A wide spectrum of reactivity is shown by methyl chloromethylphosphinate. Thus, it reacts with methoxide ion, methylamine, and dimethylamine to produce derivatives of methylphosphonic acid. An addition occurs with ketene diethyl acetal in the presence of tertiary amine. With thiophenoxide a displacement of chloride occurs. Reaction with Grignard reagents yields tertiary phosphine oxides; studies with deuterated compounds indicate that two mechanisms—an elimination-addition sequence and a hydride shift—may be important in this process.

A number of reactions in which a compound containing the group CXPH (where X is a halogen) is converted by reaction with an appropriate nucleophile into a compound of the type CHPB (where B is an alkoxy, amino, or hydroxyl group) have been described.<sup>2,3</sup>

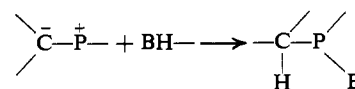
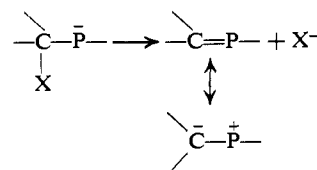
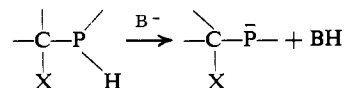
It was suggested<sup>2</sup> that polyfluoroalkylphosphines react with nucleophiles by an elimination-addition process in which an intermediate, highly reactive, phosphalkene is generated. The suggested mechanism is summarized in the following equations.

One reaction of this type has been described for compounds containing pentavalent phosphorus.<sup>3</sup> This reaction is the slow conversion of chloromethylphosphinic acid into methylphosphonic acid by aqueous

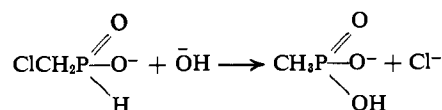
(1) This investigation was supported by Research Grant No. CA-07182 from the National Cancer Institute, Public Health Service; it was presented, in part, at the Western Regional Meeting of the American Chemical Society, Los Angeles, Calif., Nov 1965.

(2) H. Goldwhite, R. N. Haszeldine, and D. G. Rowsell, *J. Chem. Soc.*, 6875 (1965).

(3) E. Uhing, K. Rattenbury, and A. D. F. Toy, *J. Am. Chem. Soc.*, **83**, 2299 (1961).



sodium hydroxide solution. Some hydroxymethyl-



phosphinic acid is also produced. It has recently been