

previous darkening at 65°. The infrared spectrum had bands at 3.28(w), 3.36(w), 3.42(w), 3.50(w), 4.88(m), 5.07(vs), 5.98(s), 6.30(vw), 6.38(vw), 6.75(m), 6.98(m), 7.10(vw), 7.28(w), 7.52(vw), 7.67(w), 7.82(w), 8.01(vw), 8.31(w), 8.42(w), 8.65(w), 8.95(w), 9.11(m), 9.31(w), 9.71(w),

10.00(w), 10.30(vw), 10.77(w) and 11.40(w)  $\mu$  in  $\text{CCl}_4$ , and at 13.10(m), 13.40(s), 14.15(s), 14.41(vs) and 14.95(m)  $\mu$  in  $\text{CS}_2$ .

Anal. Calcd. for  $\text{C}_2\text{H}_2\text{O}_4\text{PCo}$ : C, 65.12; H, 5.08. Found: C, 65.41; H, 5.29.

[CONTRIBUTION FROM THE JAMES BRYANT CONANT LABORATORY OF HARVARD UNIVERSITY, CAMBRIDGE 38, MASS.]

## Hydrolysis and Exchange in Esters of Phosphoric Acid<sup>1a,b</sup>

BY PAUL C. HAAKE<sup>1c</sup> AND F. H. WESTHEIMER

RECEIVED SEPTEMBER 9, 1960

The acid-catalyzed hydrolysis of ethylene hydrogen phosphate in water enriched in  $^{18}\text{O}$  is accompanied by exchange of heavy oxygen into the unreacted ester. The ratio of the rate of hydrolysis to the rate of exchange is about 5. This ratio, under somewhat different experimental conditions, is about 20 for dimethyl hydrogen phosphate and about 5 for methyl dihydrogen phosphate. Previous work has shown that ethylene hydrogen phosphate hydrolyzes about  $10^7$  as fast as does dimethyl hydrogen phosphate, and thermochemical studies have suggested that the large difference in rate is largely caused by strain in the cyclic ester. The results of the present work show that the exchange reaction, as well as the hydrolysis, has been strongly accelerated in ethylene hydrogen phosphate. Since strain is not relieved in the product of the exchange reaction (as it is in that of hydrolysis) these findings bear on the mechanism of the exchange reaction of phosphate esters. The argument is advanced that, provided that hydrolysis and exchange occur by parallel mechanisms, the activated complexes for the acid hydrolysis and exchange of these phosphates do not have the usual configuration for  $\text{S}_\text{N}2$  displacement reactions. An activated complex with the geometry of a trigonal bipyramid or of a square pyramid, with reaction at a basal position in either case, is in accord with the experimental evidence. Additional chemistry of exchange and hydrolysis is presented and discussed.

The hydrolysis of five-membered cyclic esters of phosphoric acid is enormously more rapid than that of the corresponding open-chain analogs.<sup>2</sup> In particular, salts of ethylene phosphate<sup>3,4</sup> hydrolyze in alkali about  $10^7$  times as fast as do those of dimethyl phosphate, and ethylene hydrogen phosphate hydrolyzes in acid<sup>5,6</sup> about  $10^7$  times as fast as does dimethyl hydrogen phosphate. Salts of trimethylene phosphate, however, hydrolyze only slightly faster than those of dimethyl phosphate.<sup>7,8</sup>

The effect of structure on the lability of cyclic phosphates thus differs sharply from that for lactones,<sup>9</sup> where the six-membered lactone hydrolyzes most rapidly, and where the maximum rate factor (ca. 6,500) is much smaller than that for the phosphates. The proximate cause of the rapid hydrolysis of the five-membered cyclic phosphate esters has been shown to lie in strain in the ester itself; the heat of hydrolysis of methyl ethylene phosphate exceeds that for dimethyl hydroxyethyl phosphate by about 7–9 kcal./mole.<sup>10</sup>

(1) (a) Presented at the Gordon Conference on Isotopes, New Hampton, New Hampshire, July 6, 1960. (b) Mono- and diesters are named specifically where possible (e.g., dimethyl hydrogen phosphate, or dimethyl phosphate anion); where it is impossible to specify, the general name (e.g., dimethyl phosphate) is used. (c) U. S. Rubber Fellow, 1957–1958; N. S. F. Fellow, 1958–1960.

(2) O. Bailly, *Bull. soc. chim. France*, **31**, 848 (1922); A. Fono, *Archiv. Kemi Mineral Geol.*, **24A**, 34, 15 (1947); R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952); D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 52, 2708 (1952); T. Ukita, N. A. Bates and H. E. Carter, *J. Biol. Chem.*, **216**, 867 (1955).

(3) J. Lecocq, *Compt. rend.*, **242**, 1902 (1956).

(4) J. Kumamoto, J. R. Cox, Jr., and F. H. Westheimer, *J. Am. Chem. Soc.*, **78**, 4858 (1956).

(5) F. H. Westheimer, *Spec. Publ. of Chem. Soc.*, **8**, 1 (1957).

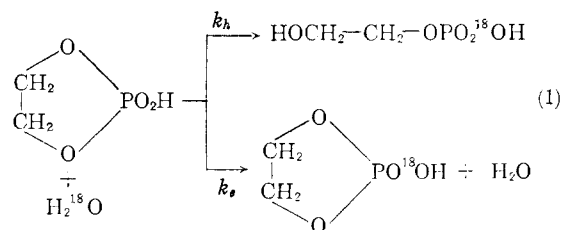
(6) J. R. Cox, Jr., Thesis, Harvard University, 1958.

(7) H. G. Khorana, G. M. Tener, R. S. Wright and J. G. Moffatt, *J. Am. Chem. Soc.*, **79**, 430 (1957); see also E. Cherbuliez, H. Probst and J. Rabinowitz, *Helv. Chim. Acta*, **42**, 1377 (1959).

(8) The approximate rate for the alkaline hydrolysis of salts of ethylene phosphate given in ref. 7 is about ten times that reported in ref. 4. However, in a private communication (4/6/60), Professor Khorana has stated that a reinvestigation of the reaction velocity has yielded figures in agreement with these reported by Kumamoto, *et al.*

(9) R. Huisgen and H. Ott, *Tetrahedron*, **6**, 253 (1959).

In the present work, the position of cleavage of ethylene and dimethyl phosphates in both acid and base has been investigated using  $^{18}\text{O}$  as tracer. The cyclic ester hydrolyzes (within experimental error) exclusively with P–O cleavage, whereas dimethyl phosphate (as anion or acid) hydrolyzes in large part with C–O cleavage. Therefore the rate of hydrolysis of ethylene phosphate anion or of ethylene hydrogen phosphate exceeds the corresponding rate for dimethyl phosphate at the P–O bond by a factor of  $10^8$  or more. Further investigations measured the exchange which accompanies hydrolysis (eq. 1), modeled on Bender's pioneering study of the hydrolysis of esters of carboxylic acids.<sup>11</sup>



Oxygen exchange accompanies the hydrolysis of ethylene phosphate in acid solution, but not in base. The conclusion can reasonably be drawn that the exchange of  $^{18}\text{O}$  into ethylene hydrogen phosphate has been strongly accelerated (as is hydrolysis) relative to the rate for an open-chain phosphate ester. On the basis of these facts, the geometry of the transition state for the acid hydrolysis and exchange of these phosphate esters may be partially delineated.

### Experimental

**Materials.**—Barium ethylene phosphate, prepared by Cox,<sup>4,6</sup> was recrystallized from ethanol-water. The sample was bromide-free, and the structure previously assigned to

(10) J. R. Cox, Jr., R. E. Wall and F. H. Westheimer, *Chemistry & Industry*, 929 (1959).

(11) M. L. Bender, *J. Am. Chem. Soc.*, **73**, 1626 (1951).

this salt<sup>4</sup> was confirmed by its nuclear magnetic resonance spectrum in D<sub>2</sub>O as solvent. The spectrum (determined with a Varian Associates model V4300B nuclear magnetic resonance spectrometer equipped with a "Superstabilizer" and operated at 40.01 Mc.) consisted of two equal bands at +17 and +27 c.p.s., relative to water.<sup>12</sup> These are assigned to the equivalent methylene groups of the ethylene phosphate anion, as split by the <sup>31</sup>P atom. Barium hydroxyethyl phosphate was isolated as the hydrolysis product of barium ethylene phosphate<sup>4</sup> and was also synthesized (a) from ethylene oxide and sodium dihydrogen phosphate,<sup>13</sup> and (b) from chloroethanol and disodium hydrogen phosphate by a procedure modeled on that of Plimmer and Burch.<sup>14</sup> The material in all cases was recrystallized to constant infrared spectrum. Barium dimethyl phosphate was isolated from Eastman methyl phosphoric acid. This latter is a mixture of monomethyl dihydrogen phosphate and dimethyl hydrogen phosphate. The mixed acids (48 g.) dissolved in 800 ml. of 50% methanol were neutralized with a 4% excess of solid barium hydroxide, and the barium methyl phosphate removed by filtration. The excess barium hydroxide was neutralized with carbon dioxide, and barium carbonate removed by filtration. The residual solution of barium dimethyl phosphate was evaporated to dryness under vacuum in a rotary evaporator, and the residue recrystallized three times from ethanol-water. The final infrared spectrum was identical to that of barium dimethyl phosphate prepared by the hydrolysis of trimethyl phosphate. The infrared spectrum of barium monomethyl phosphate was identical to that of a sample prepared by Butcher<sup>15</sup> by Outhouse's procedure.<sup>16</sup>

Alkaline phosphatase was obtained from the Sigma Chemical Co. and from Nutritional Biochemicals Co. Water enriched with <sup>18</sup>O was obtained from the Stuart Oxygen Co. and from the Weizmann Institute; the water containing 30% <sup>18</sup>O was a gift of Dr. David Samuel of that laboratory. All other chemicals were of reagent grade.

**Methods.** Drying.—Prior to analysis for <sup>18</sup>O, the phosphates were carefully dried. Potassium dihydrogen phosphate was dried overnight at 110° (0.1 mm.); the salts of the organic phosphates were dried at 150° (0.03 mm.) for 12 hours. On subsequent determination of their equivalent weights, they were found to be anhydrous. The analysis was conducted by passing a solution of each salt through a column<sup>17</sup> of Dowex 50-H<sup>+</sup> followed by titration of the eluate with standard base.

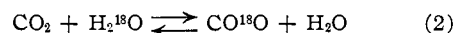
**Hydrolysis of 2-Hydroxyethyl Phosphate.**—Barium 2-hydroxyethyl phosphate (60–100 mg.) was converted into inorganic phosphate and ethylene glycol for <sup>18</sup>O analysis by hydrolysis using alkaline phosphatase<sup>18</sup> (5–10 mg.) as catalyst. The solution of these components in a buffer consisting of 0.1 M sodium barbital and 0.008 M magnesium bromide at pH 8.9 was kept at 37° for 24 hours. Then the precipitate of barium phosphate which had formed was dissolved by the addition of 0.2 ml. of 8.6 M hydrobromic acid. Barbital crystallized and was removed by filtration. An aliquot (0.1 ml.) was removed for phosphate analysis,<sup>19</sup> and to the rest was added 1 ml. of saturated barium hydroxide solution and enough sodium hydroxide solution to raise the pH to 12. The precipitated barium phosphate was isolated by centrifugation, and the supernatant (solution A) set aside. The precipitate was dissolved in 1 ml. of 0.5 M hydrobromic acid, and the resulting cloudy liquid clarified by centrifugation, and then passed through 2 g. of Dowex 50-H<sup>+</sup> and washed through the column with 3 ml. of water. The eluate was taken to pH 4.4 with 5 M potassium hydroxide solution, and evaporated to 0.5 ml. in a rotary evaporator. Addition of 4 ml. of absolute ethanol precipitated potassium dihydrogen phosphate, which was washed with alcohol and

dried, or, in some experiments, redissolved, reprecipitated, washed and dried.<sup>20</sup>

In three instances, 4.1 g. of sodium hydroxide was added to solution A, and the alkaline mixture shaken for half an hour with 20 ml. of carbon tetrachloride and 5.6 g. of benzoyl chloride. The carbon tetrachloride layer was separated, the solution further extracted with carbon tetrachloride, and the organic layer washed with water. From the carbon tetrachloride, glycol dibenzoate (m.p. 70.5–71.5°) was isolated.

**Analysis for <sup>18</sup>O.**—In all experiments, carbon dioxide was analyzed for <sup>18</sup>O with a Consolidated Engineering Corp. model 21-103C mass spectrometer. A complete spectrum was always run to make sure that impurities which might affect the analysis were absent. Tank carbon dioxide was analyzed before and after each determination; with two exceptions, all these controls, determined over a period of 6 months, gave values between 0.204 and 0.206 atom % <sup>18</sup>O.

Water was obtained from potassium dihydrogen phosphate and analyzed either by the method of Cohn<sup>20</sup> or by converting it to carbon dioxide with mercuric cyanide and mercuric chloride by the method of Anbar and Guttmann.<sup>21</sup> The methods of calculation appropriate to these two analytical methods differ slightly but significantly because the equilibrium constant for the reaction



is not precisely 2.000. The required equations have been presented by Dostrovsky and Klein.<sup>22</sup> Eight samples of water were analyzed by both techniques; the data yielded a value of  $K_{\text{eq}}$  of 2.09, in reasonable agreement with theory and the earlier experiments. Potassium dihydrogen phosphate was analyzed by the method of Williams and Hager,<sup>23</sup> glycol dibenzoate by the method of Rittenberg and Ponticorvo<sup>24</sup> and all other compounds (salts of phosphate esters) by a slight modification of the method of Anbar and Guttmann.<sup>21</sup> Typically, 5–10 mg. of phosphate and 20–40 mg. of a 1:1 mixture of mercuric chloride and mercuric cyanide were placed in a break-seal tube, evacuated, sealed and heated for an hour at 400–440°. The tube was placed in a larger one containing a drop of quinoline and attached to a vacuum line. After the seal was broken, the hydrogen chloride generated during the heating was trapped by the quinoline<sup>24</sup> and the carbon dioxide distilled on the vacuum line into a trap at –135°, and from this trap to one at –195°. Before the trap at –195° was put in place, a small amount of gas, consisting largely of nitrogen and carbon monoxide, was pumped from the –135° trap and discarded. In the analysis of monomethyl and dimethyl phosphates, the carbon dioxide initially contained a large amount of methyl chloride as impurity. However, when the non-condensable gases were removed prior to distillation of the carbon dioxide, the product collected in the –195° trap was almost pure. Some trial and error was required to fix the time (less than a second in our apparatus) necessary for the elimination of the low boiling components of the mixture; subsequently, carbon dioxide of high purity could conveniently be obtained.

**Hydrolyses.**—The hydrolysis of barium ethylene phosphate in the presence of either potassium or barium hydroxide in H<sub>2</sub><sup>18</sup>O goes nearly to completion in 2–10 hours at room temperature. In either case, barium hydroxyethyl phosphate was isolated from the concentrated aqueous solution by the addition of ethanol. In several experiments, a solution of barium ethylene phosphate was added to an equivalent solution of sodium or potassium sulfate, and the barium sulfate which formed was removed by centrifugation. Solid sodium or potassium hydroxide was added for the hydrolysis; subsequently, barium hydroxide was added, and the barium salt of the product isolated as before, and identified by its infrared spectrum. For acid hydrolysis, barium ethylene phosphate in H<sub>2</sub><sup>18</sup>O was added to aqueous perchloric acid, and the hydrolysis allowed to go to comple-

(12) J. D. Roberts, "Nuclear Magnetic Resonance Spectroscopy," McGraw-Hill Book Co., Inc., New York, N. Y., 1959.

(13) F. R. Atherton, H. T. Openshaw and A. R. Todd, *J. Chem. Soc.*, 382 (1945).

(14) R. H. A. Plimmer and W. J. N. Burch, *ibid.*, 279 (1929).

(15) W. W. Butcher, Dissertation, University of Chicago, 1953.

(16) E. L. Outhouse, *Biochem. J.*, **31**, 1459 (1937).

(17) E. Lederer and M. Lederer, "Chromatography," 2nd ed. Elsevier Press, New York, N. Y., 1957.

(18) G. Schmidt and S. J. Thianhauser, *J. Biol. Chem.*, **149**, 369 (1943).

(19) E. J. King, *Biochem. J.*, **26**, 292 (1932).

(20) M. Cohn, "Methods in Enzymol.," (S. Colowick and N. Kaplan, Eds.) Academic Press, New York, N. Y. 1957, Vol. 4, p. 905.

(21) M. Anbar and S. Guttmann, *Int. J. Appl. Rad. and Isotopes*, **4**, 233 (1959).

(22) I. Dostrovsky and F. S. Klein, *Anal. Chem.*, **24**, 414 (1952).

(23) F. R. Williams and L. P. Hager, *Science*, **128**, 1434 (1958).

(24) D. Rittenberg and L. Ponticorvo, *Int. J. Appl. Rad. and Isotopes*, **1**, 208 (1956).

tion at room temperature. The solution was then evaporated in vacuum, and the water saved for  $^{18}\text{O}$  analysis. The remainder of the solution was brought to pH 11 with barium hydroxide, the solution evaporated to a sirup, diluted to 2 cc. with water and then to 12 cc. with absolute ethanol. The barium hydroxyethyl phosphate which precipitated was centrifuged, washed with alcohol, and dried. Experiments without barium ion were conducted by a procedure paralleling that described for basic solutions.

For partial acid hydrolysis, barium ethylene phosphate in 20 cc. of aqueous perchloric acid (from water enriched in  $\text{H}_2^{18}\text{O}$ ) was allowed to react for 3-4 minutes at room temperature, and then the reaction stopped by the rapid addition of 5.0 ml. of 0.46 *N* barium hydroxide solution. This brought the pH to 6.8-8.6. The pH was then adjusted with barium hydroxide to 9.6, and the solution evaporated below room temperature in vacuum to 2 ml. From the analysis of the distillate for  $^{18}\text{O}$  and the known volumes of all the solutions added, the initial isotopic composition of the aqueous acid was calculated. The solution was centrifuged, and 10 ml. of absolute ethanol added to the supernatant. The precipitated barium hydroxyethyl phosphate was isolated by centrifugation, washed with ethanol, and dried. In two experiments, it was redissolved in water and reprecipitated with ethanol.

The supernatant from the precipitation of barium hydroxyethyl phosphate was diluted with 12 ml. of acetone; barium ethylene phosphate slowly crystallized from the initially clear solution. The solid was separated by centrifugation, washed with absolute ethanol and ether, and dried. In two experiments it was recrystallized from ethanol-water-acetone.

The purity of barium ethylene phosphate was determined by infrared analysis. Barium hydroxyethyl phosphate has a strong band at 10.25  $\mu$ , which is clearly separated from the band at 9.95  $\mu$  in barium ethylene phosphate (spectra in KBr disks). Impurity of the order of 2% of barium hydroxyethyl phosphate in barium ethylene phosphate can be detected by infrared spectra.

The hydrolysis of dimethyl phosphate ion in alkaline solution was conducted at 125° in a Teflon tube.<sup>6</sup> After hydrolysis, the solution was concentrated to 2 ml., and barium perchlorate and 4 ml. of ethanol added to precipitate barium methyl phosphate. This salt, isolated by centrifugation, was redissolved and reprecipitated twice. Barium dimethyl phosphate was isolated by evaporating the supernatant from the precipitation of barium methyl phosphate to a sirup and adding absolute ethanol and acetone. It was redissolved and reprecipitated after the addition of unenriched barium methyl phosphate to dilute any labeled impurity. Experiments with sodium dimethyl phosphate paralleled those for sodium ethylene phosphate. The experiments in acid were conducted with solutions of sodium dimethyl phosphate plus aqueous perchloric acid in sealed Pyrex tubes at 100°. Solid potassium hydroxide was added to neutrality, and the solution was freeze-dried to recover the enriched water without bumping. The residue was dissolved in 5 ml. of water and potassium perchlorate separated by centrifugation. The supernatant was passed through a column of Dowex-50-H<sup>+</sup>, and the eluate brought to pH 10 with barium hydroxide. Barium phosphate was removed by centrifugation, and the volume reduced under vacuum to 6 ml. Addition of an equal volume of ethanol precipitated a solid which was separated by centrifugation from the supernatant B. The solid, barium methyl phosphate, was dissolved in water, along with unenriched barium hydrogen phosphate; the former was then reisolated. In two experiments it was purified by solution in water and reprecipitation with ethanol. The supernatant B from the isolation of barium methyl phosphate was evaporated to dryness in vacuum at 20-25° and dissolved in 0.8 ml. of water. Addition of 10 ml. of acetone produced a gel which, after much scratching, crystallized to needles of barium dimethyl phosphate. The crystals were washed with ethanol and ether, and dried.

Barium methyl phosphate was sealed in an ampoule with 11.6 *M* perchloric acid enriched in  $^{18}\text{O}$ , and heated at 75 ± 2° for 102 hours. The solution was brought to pH 3 with potassium hydroxide, and potassium perchlorate removed by centrifugation; the supernatant was made basic (pH 11) with saturated barium hydroxide solution, and barium phosphate removed by centrifugation. The latter was converted to potassium dihydrogen phosphate

by procedures already outlined, and the barium methyl phosphate in the supernatant was precipitated with ethanol and purified by procedures described above.

## Results

**Ethylene Phosphate. Positions of Bond Cleavage.**—The hydrolysis of ethylene phosphate anion or of ethylene hydrogen phosphate in  $\text{H}_2^{18}\text{O}$  must yield hydroxyethyl phosphate with at least one atom of  $^{18}\text{O}$  per molecule. Subsequent hydrolysis with alkaline phosphatase in normal water yields ethylene glycol and inorganic phosphate. The former, isolated as the dibenzoate, proved unenriched even when the hydrolysis was conducted in water containing almost 7%  $^{18}\text{O}$ . Since an enrichment of no more than 0.004 atom %  $^{18}\text{O}$  might have passed undetected because of experimental error, hydrolysis by P-O cleavage must occur at least 300 times faster than hydrolysis by C-O cleavage. When the phosphate fragment was analyzed, it was found to contain about 0.9 atom of oxygen introduced from the solvent. This value, although in approximate agreement with the value of 1.00 expected from the analysis of the glycol, is low; similar slightly low values have been obtained in the alkaline hydrolysis of ribonucleic acid.<sup>25</sup> The cause of the discrepancy is not known. However, control experiments on the enzymatic hydrolysis of barium hydroxyethyl phosphate in water enriched in  $^{18}\text{O}$  showed no exchange of  $^{18}\text{O}$  into the substrate, and approximately one atom of  $^{18}\text{O}$  in the phosphate produced.<sup>26</sup> Therefore the reaction of salts of hydroxyethyl phosphate promoted by alkaline phosphatase occurs exclusively with P-O cleavage.<sup>27</sup> The deviation from unity in the atoms of oxygen introduced into inorganic phosphate following the hydrolysis of ethylene and hydroxyethyl phosphates is not caused by loss of the heavy isotope during the enzymatic reaction, but rather by some other source of error. The data are presented in Table I.

TABLE I

COMPLETE ALKALINE HYDROLYSIS OF ETHYLENE PHOSPHATE ION IN  $\text{H}_2^{18}\text{O}$  AT ROOM TEMPERATURE

Base, <i>N</i>	Base	Ethyl- ene phos- phate anion, <i>N</i>	Time, (hr.)	—Atom % $^{18}\text{O}$ —			<i>Q</i> <sup>d</sup>
				Water	Glycol	Phos- phate	
0.50	KOH	0.098 <sup>a</sup>	15	1.69	0.198		0.00
.50	NaOH	.100 <sup>b</sup>	18	6.70	0.197		.00
.14	NaOH	.068 <sup>c</sup>	2.3	1.50		0.486	.88
.23	NaOH	.054 <sup>c</sup>	10	0.886		.348	.87
.48	KOH	.106 <sup>a</sup>	20	1.63		.521	.90
.40	Ba(OH) <sub>2</sub>	.106 <sup>c</sup>	2.5	1.58		.529	.96
.23	NaOH	.052	2.8	0.198		.198	

<sup>a</sup> Potassium ethylene phosphate. <sup>b</sup> Sodium ethylene phosphate. <sup>c</sup> Barium ethylene phosphate. <sup>d</sup> Atoms of  $^{18}\text{O}$  introduced per molecule of product.

In parallel experiments, the acid hydrolysis of ethylene hydrogen phosphate to hydroxyethyl phosphate was followed by enzymatic cleavage of the latter. No  $^{18}\text{O}$  was introduced into the glycol, but more than one atom of  $^{18}\text{O}$  per molecule was

(25) D. Lipkin, P. T. Talbert and M. Cohn, *J. Am. Chem. Soc.*, **76**: 2871 (1954).

(26) Paul C. Haake, Thesis, Harvard University, 1960.

(27) Cf. S. S. Stein and D. E. Koshland, *Arch. Biochem. Biophys.*, **39**: 229 (1952).

found in the phosphate (Table II). Control experiments showed that the excess  $^{18}\text{O}$  above that calculated for one atom per molecule was not incorporated by acid-catalyzed exchange of oxygen from water, under these experimental conditions, into hydroxyethyl dihydrogen phosphate, nor (as pointed out above) does appreciable exchange accompany the enzymic hydrolysis of hydroxyethyl phosphate. (The enzymic process is carried out in ordinary water, and if exchange occurred it would of course reduce, not enhance, the  $^{18}\text{O}$  content of the phosphate.) The additional  $^{18}\text{O}$  is introduced (as will be shown below) by the exchange which accompanies the hydrolysis of ethylene hydrogen phosphate.

TABLE II

COMPLETE ACID HYDROLYSIS OF ETHYLENE HYDROGEN PHOSPHATE IN  $\text{H}_2^{18}\text{O}$  AT ROOM TEMPERATURE

$\text{HClO}_4$ , N	Ethylene hydrogen phosphate, N	Time, min.	Atom % $^{18}\text{O}$			Q
			Water	Glycol	Phos- phate	
0.10	0.080 <sup>a</sup>	10	6.77	0.199		0.00
.10	.081 <sup>a</sup>	120	1.52		0.571	1.13
.10	.079 <sup>a</sup>	120	1.16		.467	1.12
.10	.078 <sup>a</sup>	260	1.57		.596	1.16
.10	.077 <sup>b</sup>	130	1.57		.598	1.17
.57	.073 <sup>a</sup>	90	1.44		.554	1.14
.10	.071	4	0.198		.198	

<sup>a</sup> Barium salt introduced. <sup>b</sup> Potassium salt introduced.

**Ethylene Phosphate. Exchange Accompanying Hydrolysis.**—Under alkaline conditions, the hydrolysis of ethylene phosphate anion is not accompanied by detectable exchange; recovered barium ethylene phosphate shows no enrichment in  $^{18}\text{O}$ . By contrast, the hydrolysis catalyzed by acid is accompanied by exchange of  $^{18}\text{O}$  into the recovered barium ethylene phosphate. The data are shown in Table III.

TABLE III

EXCHANGE OF  $^{18}\text{O}$  INTO BARIUM ETHYLENE PHOSPHATE DURING PARTIAL HYDROLYSIS IN ACID AT ROOM TEMPERATURE

$\text{HClO}_4$ , N	Barium ethylene phos- phate, N	Hy- drolysis, %	Com- pound isolated <sup>a</sup>	Times re- pptd. <sup>b</sup>	Atom % $^{18}\text{O}$		Q
					Water	Phos- phate	
0.10	0.078	37	BaEP	0	1.63	0.234	0.10
			BaHEP	0		.504	.86
.10	.073	43	BaEP	1	1.58	.236	.11
			BaHEP	2		.548	1.01
.10	.074	46	BaEP	1	1.57	.237	0.11
			BaHEP	1		.529	0.96

<sup>a</sup> BaEP = barium ethylene phosphate; BaHEP = barium hydroxyethyl phosphate. <sup>b</sup> Reprecipitated.

**Dimethyl Phosphate. Position of Bond Cleavage.**—The fraction of cleavage of the phosphorus-oxygen bond during the alkaline hydrolysis of dimethyl phosphate in water enriched in  $^{18}\text{O}$  was determined by isolation of barium methyl phosphate and direct conversion of this compound to carbon dioxide. The results are shown in Table IV.

Barium ion, which catalyzes the hydrolysis of ethylene phosphate,<sup>4</sup> here increases the rate of P-O relative to that of C-O cleavage.

TABLE IV  
ALKALINE HYDROLYSIS OF DIMETHYL PHOSPHATE ION IN  $\text{H}_2^{18}\text{O}$  AT 125°

Base, N	Di- methyl phos- phate anion, N	MBa <sup>++</sup>	Time, hr.	Hy- drolysis, %	Atom % $^{18}\text{O}$ Water	% Methyl phos- phate	Q
2.01 <sup>a</sup>	0.22 <sup>b</sup>	0.00	166	ca. 100	1.58	0.240	0.11
1.00 <sup>c</sup>	.50 <sup>d</sup>	.25	27	27	1.24	.299	.38
1.98 <sup>e</sup>	.44 <sup>d</sup>	.22	20	62	1.55	.302	.30

<sup>a</sup> Potassium hydroxide. <sup>b</sup> Potassium dimethyl phosphate. <sup>c</sup> Sodium hydroxide. <sup>d</sup> Barium dimethyl phosphate.

**Dimethyl Phosphate. Exchange Accompanying Hydrolysis.**—The exchange which accompanies the acid hydrolysis of dimethyl hydrogen phosphate and the fractions of P-O cleavage are recorded in Table V; they will later be compared with those of Bunton, *et al.*<sup>28</sup>

TABLE V

EXCHANGE OF  $^{18}\text{O}$  INTO BARIUM DIMETHYL PHOSPHATE DURING PARTIAL HYDROLYSIS IN ACID AT 100 ± 2°

$\text{HClO}_4$ , N	Time, hr.	Hy- drolysis, %	Compound isolated <sup>a</sup>	Atom % $^{18}\text{O}$		Q
				Water	Phos- phate	
5.2 <sup>b</sup>	6	61	Dimethyl	29.4	0.236	0.0047
5.1 <sup>c</sup>	94 <sup>e</sup>	50	Methyl	22.1	.754	.10
			Dimethyl		.220	.0033
4.8 <sup>b</sup>	6	43	Methyl	1.25	.231	.11
4.7 <sup>b</sup>	0.9	8	Methyl	1.26	.233	.12
4.7 <sup>c</sup>	0.9	8	Methyl	1.35	.236	.12
2.3 <sup>b,f</sup>	24	61	Methyl	1.53	.260	.17
0.50 <sup>a,g</sup>	63	60	Methyl	16.6	1.49	.31
			Dimethyl		0.256	.013
0.50 <sup>a,d,g</sup>	62	42	Methyl	15.1	1.23	.28
			Dimethyl		0.252	.013

<sup>a</sup> Phosphates isolated and analyzed as barium salts. <sup>b</sup> Barium dimethyl phosphate. <sup>c</sup> Sodium dimethyl phosphate. <sup>d</sup> Ionic strength increased to 5.0 with  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$ . <sup>e</sup> 75° rather than 100°. <sup>f</sup> Dimethyl phosphate 0.25 M; all other experiments at 0.5 M. <sup>g</sup> pH 1.0 at 100°.

**Methyl Dihydrogen Phosphate.**—The extents of P-O bond cleavage and exchange for methyl dihydrogen phosphate have been published by Bunton, *et al.*<sup>29</sup> The data in Table VI are in qualitative agreement with their findings, but show more P-O cleavage and less exchange. Whether the differences result from a systematic error in the analysis of the phosphates (in our work) or of methanol (in theirs) or from some other cause has not yet been established.

TABLE VI

THE AMOUNT OF EXCHANGE AND P-O CLEAVAGE DURING THE ACID HYDROLYSIS OF METHYL DIHYDROGEN PHOSPHATE

$\text{HClO}_4$ , N	Barium methyl phos- phate, M	Temp., °C.	Hy- droly- sis, %	Phosphate analyzed	Atom % $^{18}\text{O}$		Q
					Water	Phos- phate	
2.4	0.20	100	46	$\text{KH}_2\text{PO}_4$	1.17	0.350	0.63
				Methyl <sup>a</sup>		.214	.05
5.0	.46	75	4	$\text{KH}_2\text{PO}_4$	1.25	.335	.52
5.0	.34	75	32	$\text{KH}_2\text{PO}_4$	1.25	.398	.76
				Methyl <sup>a</sup>		.213	.04

<sup>a</sup> Barium methyl phosphate.

(28) C. A. Bunton, M. M. Mhala, K. G. Oldham and C. A. Vernon *J. Chem. Soc.*, 3293 (1960).

(29) C. A. Bunton, D. R. Llewellyn, K. G. Oldham and C. A. Vernon, *ibid.*, 3574 (1958).

**Ratio of Rates of Hydrolysis and Exchange.**—From the data of Tables I–VI and eq. 3 (see appendix) the ratio of rates of hydrolysis to exchange can be calculated

$$k_h/k_e = \frac{\ln(D/D_0)}{2 \ln(1 - D'/2D)} \quad (3)$$

where  $k_h$  is the rate constant for hydrolysis,  $k_e$  that for exchange,  $D$  the total concentration of the dialkyl phosphate,  $D'$  the concentration of the enriched ester, and  $D_0 = D$  for zero time. The rate constant<sup>6</sup> for the hydrolysis of ethylene hydrogen phosphate in 0.10 *M* perchloric acid at 30° is  $2.06 \times 10^{-3}$  sec.<sup>-1</sup>, so (from Table VII) the rate constant for exchange is  $4 \times 10^{-4}$  sec.<sup>-1</sup>. Ideally, values of  $D'$  and  $D$  should be available at many concentrations rather than only at one, and the results in Table VII are calculated from the data of Table IV on the assumption that the exchange reaction is first order in the ester.

TABLE VII  
RATIO OF THE RATE CONSTANTS FOR HYDROLYSIS AND EXCHANGE OF ETHYLENE HYDROGEN PHOSPHATE IN ACID SOLUTION

$D'/D$	$D/D_0$	$k_h/k_e$
0.100	0.63	4.5
.110	.57	5.0
.114	.54	5.3
	Av.	4.9

Table IV gives both the <sup>18</sup>O content of recovered barium ethylene phosphate and the <sup>18</sup>O content of the barium hydroxyethyl phosphate obtained as product during partial acid hydrolysis. Similarly, Table II gives the <sup>18</sup>O content of barium phosphate isolated after complete acid and enzymatic hydrolysis of ethylene phosphate. One atom of <sup>18</sup>O per molecule should be incorporated from hydrolysis, and additional <sup>18</sup>O because of exchange into ethylene phosphate. The amount of <sup>18</sup>O expected in hydroxyethyl phosphate can be calculated from the  $k_h/k_e$  ratio of Table VII and eq. 4 (see Appendix).

$$M'/M = 3 - 4k_h/(2k_h + k_e) - \frac{2k_h D'}{(2k_h + k_e)(D_0 - D)} \quad (4)$$

Here  $M'$  is the concentration of enriched monoester,  $M$  is the total concentration of monoester, and the other symbols have already been defined. Application of eq. 4 to the experimental data<sup>26</sup> shows that the amount of <sup>18</sup>O found in the phosphate is slightly less than that calculated. The data in acid solution are thus consistent with those for completely hydrolysis in base, where somewhat less <sup>18</sup>O was incorporated into the phosphate than was predicted from the results on glycol dibenzoate.

The hydrolysis of ethylene hydrogen phosphate has been shown<sup>5,6</sup> to be controlled by a kinetic equation of the form

$$v = k(\text{EHP})(\text{H}^+) \quad (5)$$

and the hydrolysis occurs exclusively with P–O cleavage (Table II). However, the hydrolysis of dimethyl phosphate obeys an equation<sup>28</sup> of the form

$$v = k(\text{DMHP}) + k'(\text{DMHP})(\text{H}^+) \quad (6)$$

where DMHP = dimethyl hydrogen phosphate,

and even in moderately concentrated acid solutions, the first term cannot be neglected. In their careful work, Bunton, *et al.*,<sup>28</sup> have evaluated  $k$  and  $k'$  as a function of ionic strength up to 5 *M*, but in such concentrated solutions the ionic strength principle does not hold accurately, and specific ion effects may be quite important. In these circumstances, the determination of the percentages of the reaction which proceed by way of the undissociated acid and by way of the acid-catalyzed reaction is subject to large errors.

The kinetic equation of Bunton, *et al.*,<sup>28</sup> predicts that, at pH 1.24, substantially all of the reaction proceeds by way of the undissociated acid, whereas in 5 *M* perchloric acid about 33% of the reaction proceeds through the undissociated acid and 67% by way of the acid-catalyzed reaction. The fourth from last line of Table V shows that the hydrolysis of the undissociated acid proceeds with 31% P–O cleavage; a calculation from the rest of the data (Table VIII) shows that the uncatalyzed reaction will account for all the observed P–O cleavage. Furthermore, the exchange into dimethyl hydrogen phosphate (Table IX) can be accounted for exclusively as exchange of the undissociated acid. This conclusion is not in agreement with that which Bunton, *et al.*,<sup>28</sup> drew from their data. The percentage of P–O cleavage in the undissociated acid was here found to be 31%, whereas Bunton, *et al.*, found 21%. The difference is not large considering both the complexity of the experiments and the diversity of methods; the English workers analyzed the methanol whereas we analyzed the barium methyl phosphate produced in the hydrolysis. However, calculation for the reaction controlled by  $k'$  led them to the conclusion that 11% proceeds by P–O cleavage. Since the percentage of P–O cleavage *via* the acid-catalyzed reaction depends critically on small differences between larger numbers, only considerable additional careful work can resolve the question, which is here left open.

TABLE VIII  
PREDICTED AMOUNTS OF EXCHANGE AND P–O CLEAVAGE THROUGH UNDISSOCIATED DIMETHYL HYDROGEN PHOSPHATE

$\text{HClO}_4$ , <i>N</i>	Hydrolysis through DMHP, %	Starting material			Product	
		$\frac{Q}{\text{calcd.}}$	$\frac{Q}{\text{found}}$	Reacn., %	$\frac{Q}{\text{calcd.}}$	$\frac{Q}{\text{found}}$
5.2	33	0.0044	0.0047	61	0.10	
5.1	34	0.0033	0.0033	50	.11	0.10
4.8	36				.11	.11
4.7	37				.12	.12
2.3	61				.19	.17
0.5	100					.31

TABLE IX  
RATIO OF THE RATE CONSTANT FOR HYDROLYSIS TO THAT FOR EXCHANGE OF DIMETHYL HYDROGEN PHOSPHATE

$\text{HClO}_4$ , <i>N</i>	$E'/E$	Hy- drolysis, %	$k_h/k_e$	% P–O cleav- age	$k_{P-O}/k_e$
5.2	0.0047	61	205	10	21
5.1	.0033	50	216	10	22
0.5	.013	60	71	31	22
0.5 <sup>a</sup>	.013	42	42	28	12

<sup>a</sup> Ionic strength of 5.0, made up with sodium perchlorate.

The rather large change in  $k_{P-O}/k_e$  at 0.5 *M* acid in the presence of sodium perchlorate underlines the difficulties of a complete analysis of the situation at high ionic strengths.

Calculations from the data of Table VI and eq. 4 show that the  $k_h/k_{P-O}$  ratio for the acid hydrolysis of methyl dihydrogen phosphate is 5–6.

### Discussion

**Relative Rates of Hydrolysis of Dimethyl and Ethylene Phosphates.**—The ratios previously reported<sup>4–8</sup> between the rate constants for the hydrolysis of ethylene phosphate and those of dimethyl phosphate did not take into account the differences in the point of bond cleavage between the two compounds. The results here reported show that, in alkaline solution, only about 10% of the hydrolysis in dimethyl phosphate occurs with P–O cleavage and that the ratio of rates for P–O cleavage is therefore tenfold greater than the published factor of  $10^7$ . The comparison for acid solutions, however, is more complex. The hydrolysis of ethylene hydrogen phosphate occurs predominantly if not exclusively by way of the acid-catalyzed reaction. Although the hydrolysis of dimethyl hydrogen phosphate occurs by both an acid-catalyzed reaction and by solvolysis of the undissociated acid (see eq. 6), only the latter reaction is accompanied by much P–O cleavage. According to the data of Bunton, *et al.*,<sup>28</sup> 10% of  $k'$ , eq. 6, refers to breaking the phosphorus to oxygen bond; the data here reported suggest that the extent of this cleavage is smaller; 3% seems a plausible upper limit. The relative rates of P–O cleavage in alkaline solution at 25° for ethylene and dimethyl phosphates therefore approximate  $10^8$ , and the ratio in acid solution at 100° exceeds  $10^8$ . These values are somewhat uncertain because of the needed extrapolations from widely different temperatures. The rate for the alkaline hydrolysis<sup>4</sup> of dimethyl phosphate was observed at 125 and 115°, and that for ethylene phosphate at 25°; in acid solution<sup>5,6</sup> the rate of hydrolysis of dimethyl phosphate was measured at 100°, and that for ethylene phosphate at 0° and 30°. Despite the errors inherent in these extrapolations, the rate factors are obviously enormous.

**The Geometry of the Activated Complex in the Acid Hydrolysis of Esters of Phosphoric Acid.**—Ethylene hydrogen phosphate exchanges oxygen atoms with the solvent during acid hydrolysis; the ratio of the rate constants for hydrolysis and exchange is about 5 (Table VII). The rate of hydrolysis at the P–O bond exceeds that for dimethyl hydrogen phosphate by more than  $10^8$ . Previously, the argument has been advanced that the enhanced rate of hydrolysis of ethylene hydrogen phosphate relative to that of dimethyl hydrogen phosphate has resulted from the relief of strain in the hydrolytic reaction.<sup>10</sup> Now it appears that the exchange of oxygen atoms between the solvent and ethylene hydrogen phosphate is also rapid, although, in the "final" product (recovered barium ethylene phosphate) the strain is still present. This finding requires that the strain be relieved in the transition states for both hydrolysis and exchange.

The geometries of three possible activated complexes for hydrolysis and exchange are illustrated in pairs in formulas I–VI of Fig. 1. In conformity with eq. 5, each formula represents ethylene hydrogen phosphate plus a proton plus a water molecule, with the phosphorus atom at the center of the diagram. The positive charge is divided between the positions marked (+); the O–P–O angle of the ethylene phosphate ring is specially marked. In the trigonal bipyramids and in the square pyramids, the basal plane is indicated by dotted lines.

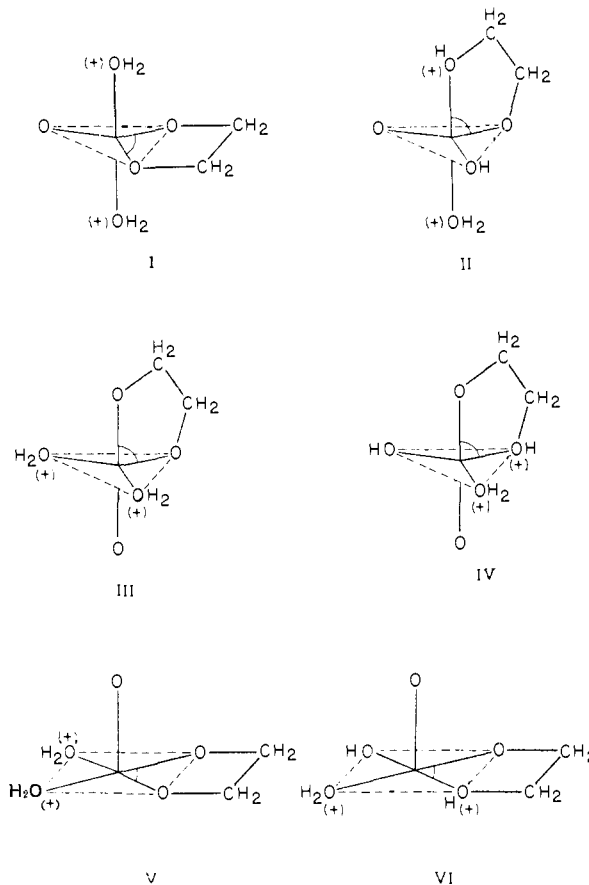


Fig. 1.—Possible geometric arrangements of the activated complexes for the acid-catalyzed hydrolysis and exchange of ethylene hydrogen phosphate.

Formulas I and II illustrate the geometries to be expected in a transition state for an  $S_N2$  displacement reaction with water as the nucleophile. In I the O–P–O angle of the ring has been expanded to 120°, and in II this angle has been contracted to 90° from the normal, near-tetrahedral size presumably present in ethylene hydrogen phosphate. It is difficult to see how both the expansion and the contraction of the tetrahedral angle could relieve the strain present in ethylene hydrogen phosphate. Formulas I and II therefore represent an improbable geometry for the transition states.

Formulas III and IV represent trigonal bipyramids in which the entering and leaving groups are at basal positions. Both III and IV have O–P–O angles in the ring of 90°, and would therefore be expected to have comparable energies relative to starting material. Exchange and hydrolysis could

therefore proceed at comparable rates through III and IV, and these transition states are consistent with the experimental evidence here presented. Formulas V and VI have the geometry of a square pyramid with entering and leaving groups in basal positions and O-P-O angles in the ring of 90°, so they also represent plausible transition states for hydrolysis and exchange. This entire argument is based on the assumption that here, as with carboxylic esters,<sup>11</sup> hydrolysis and exchange occur by similar pathways and that the small ratio of  $k_h/k_e$  is not fortuitous. Calculations of angle strain for this new model cannot be performed at present. Presumably the rehybridization of the orbitals would make an O-P-O angle of 90° one of the normal ones for a phosphorus atom in a trigonal bipyramid, but the appropriate P-O bond length, and P-O-C bond angle are unknown. Various plausible assumptions suggest, however, that a reduction of the O-P-O bond angle could reduce the strain in the ring. Until the nature of the strain in ethylene phosphate is better understood, further speculation seems premature.

The above reasoning would be more conclusive if the rates of the hydrolysis and exchange of ethylene hydrogen phosphate could be directly compared to those for open-chain alkyl phosphates. However, within the experimental error of these investigations, the exchange and P-O cleavage of dimethyl phosphate was found to occur by way of the uncatalyzed solvolysis of the undissociated acid. For this latter reaction, the ratio of  $k_{P-O}/k_{ex}$  is about 20. The hydrolysis of methyl dihydrogen phosphate<sup>29</sup> shows a ratio of  $k_{P-O}/k_{ex}$  of about 5 or 6, but it has not yet been established whether this reaction is an acid catalyzed or an uncatalyzed reaction of the undissociated acid (or is an average value for both reactions). Although these data are therefore not directly comparable to those for the acid-catalyzed hydrolysis and exchange of ethylene hydrogen phosphate, the assumption that the rates of acid-catalyzed hydrolysis and exchange are similar for the three compounds appears reasonable. Furthermore, if the inherent rate of exchange in the acid-catalyzed reaction were very great compared to that for hydrolysis, some exchange by way of the acid-catalyzed reaction presumably would have been detected with dimethyl hydrogen phosphate.

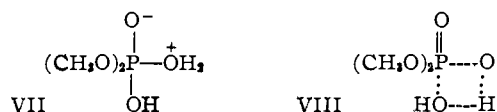
Incidentally, the stereochemical consequences of reaction *via* III and IV differ from those *via* V and VI; III may lead to inversion, whereas V leads to retention of configuration. Pertinent data for phosphates in acid solution are, however, lacking.<sup>30</sup> It should perhaps be noted that the trigonal bipyramid with reaction at the basal positions could, in principle, be the correct geometry for some reaction for which the SN2 mechanism has been assumed; inversion accompanying substitution does not distinguish unambiguously between these geometries. Further, the square pyramid provides a suitable geometry for the SNi reaction with retention of configuration. The geometrical configura-

(30) The stereochemistry of the hydrolysis of phosphonates in alkalis probably accompanied by inversion; see M. Green and R. Hudson, *Proc. Chem. Soc.*, 227 (1959); J. Michalski and A. Ratajczak, *Chemistry & Industry*, 1241 (1960).

tions suggested here may also provide an explanation for some of the rates observed by Sommer and his collaborators<sup>31</sup> for the interaction of water with substituted derivatives of silane. The "hydrolysis" of a silane presumably occurs by the addition of the oxygen atom of water to silicon to form a pentacovalent intermediate. A study of models reveals that the reaction with 1-silabicyclo[2,2,1]-heptane cannot occur by an SN2 process, since the rings constrain the incoming water molecule and departing hydrogen molecule to adjacent positions. A model can be constructed, however, for a distorted trigonal bipyramid with incoming and leaving groups at basal positions, and similarly a model can be made for a distorted square pyramid. The change in geometry of the central atom when it becomes pentacovalent might then provide relief of strain in the solvolysis of the silabicycloheptane and so explain the enhanced rate observed.<sup>31</sup> The new geometrical arrangements may even prove general for reactions of second row elements.

**Intermediates or Transition States**—On the basis of the information at hand, III, IV, V or VI might be either an intermediate or transition state. Furthermore, an intermediate (*e.g.*, III) could add water to an apex and lose it from a basal position (or *vice versa*) without violating microscopic reversibility, provided that the reactions forming III from protonated ethylene phosphate were freely reversible at both basal and apical positions.

The exchange of <sup>18</sup>O from water into dimethyl hydrogen phosphate by an uncatalyzed process suggests an intermediate of the form



This intermediate could then transfer a proton internally or through the solvent from the  $-\text{OH}_2^+$  group to the  $-\text{OH}$  group; reversal of the formation of VII would account for exchange. Although the concerted mechanism through a transition state, VIII, has not been excluded, the latter mechanism requires the simultaneous formation of two bonds and cleavage of two others, and therefore appears less probable than the formation of a real intermediate, VII.

The alkaline hydrolysis of the ethylene phosphate anion is not accompanied by oxygen exchange. This fact, however, does not help in the elucidation of mechanism. In contrast to the intermediate in the saponification of carboxylic esters, a postulated intermediate formed from the ethylene phosphate anion and hydroxide ion is doubly charged, and might well lose either alkoxide ion or the incoming hydroxide ion more rapidly than it undergoes the proton shift necessary to exchange.

**Origin of the Strain.**—The origin of the strain in cyclic phosphates is still undetermined. However, the X-ray crystallographic data on dibenzylphosphoric acid<sup>32</sup> suggest that the P-O bonds in the

(31) L. H. Sommer, O. F. Bennett, P. G. Campbell and D. R. Weyenberg, *J. Am. Chem. Soc.*, 79, 3295 (1957); L. H. Sommer and C. L. Frye, *ibid.*, 81, 1013 (1959).

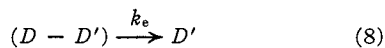
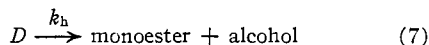
(32) J. D. Dunitz and J. S. Rollett, *Acta Cryst.*, 9, 327 (1956).



ester are shorter by 0.2 Å. than can be calculated from bond radii. Since then the P-O bonds are essentially double, the force constants for bending the O-P-O angle, and possibly the P-O-C angles, must be large. This double bond character might directly account for the strain, since even small angular distortions will require much energy. Alternatively, it might provide the stabilizing influence on geometry to tend to force the two P-O-C bonds at a sharp dihedral angle to one another, and thus<sup>4</sup> provide the strain. These hypotheses are under investigation.

### Appendix

Equation 3 may be derived as follows



where  $D$  and  $D'$  have already been defined, and  $D''$  is double labeled diester. Since in these experiments,  $D''$  is very small, almost all the enrichment

is in  $D'$ , and

$$dD'/dt = k_e(D - D') + k_e D'/2 - k_h D', \text{ where } D = D_0 e^{-k_h t} \quad (10)$$

Substitution and integration yields eq. 3.

When the extent of the reaction is small

$$k_h/k_e = (D/D') \ln (D_0/D) \quad (11)$$

If  $M$  is the total concentration of monoester, and  $M'$  is the concentration of enriched monoester, then

$$M = D_0(1 - e^{-k_h t}) \quad (12)$$

$$(D - D') \xrightarrow{k_h} M' \quad (13)$$

$$D' \xrightarrow{k_h} 2M' \quad (14)$$

$$dM'/dt = k_h(D + D') = k_h D_0 e^{-k_h t} (3 - 2e^{-k_h t/2}) \quad (15)$$

After integration

$$M'/M = 3 - 4k_h/(2k_h + k_e) - 2k_h D'/(2k_h + k_e)(D_0 - D) \quad (4)$$

At complete reaction

$$M'/M = (2k_h + 3k_e)/(2k_h + k_e) \quad (16)$$

**Acknowledgments.**—The authors express their gratitude to the National Science Foundation for the support of this work.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NOTRE DAME, NOTRE DAME, IND.]

## The Alkaline Decomposition of Organic Disulfides. I. Some Dithiodicarboxylic Acids<sup>1</sup>

BY JAMES P. DANEHY AND JOHN A. KREUZ<sup>2</sup>

RECEIVED SEPTEMBER 9, 1960

The decompositions of three dithiodicarboxylic acids, in aqueous alkaline solution at 35.2° in an atmosphere of nitrogen, were followed as a function of time at several constant pH values by quantitative measurement of residual disulfide, mercaptan and hydrogen sulfide, the sum of which accounted for 93 to 100% of the original sulfur. The rate of decomposition of dithiodiacetic acid (DTDA) increases rapidly over the pH range of 11.9 to 12.4. A detailed mechanism is proposed which accounts for the quantitative data. The decomposition of dithiodipropionic acid (HOOCCH<sub>2</sub>CH<sub>2</sub>SSCH<sub>2</sub>CH<sub>2</sub>COOH, DTDP) as anticipated, is much slower than that of DTDA and may proceed by a different mechanism since no hydrogen sulfide is produced. The decomposition of 2,2'-dimethyldithiodiacetic acid (HOOCCH(CH<sub>3</sub>)SSCH(CH<sub>3</sub>)COOH, DMDT) proceeds at rates intermediate between those found for DTDA and for DTDP, by a mechanism analogous to that for DTDA.

### Introduction

More than a century ago Mulder<sup>3</sup> observed that relatively mild alkaline treatment sufficed to remove sulfur (though not all of it, as he reports) from several proteins. Eventually it was established that the sulfur was lost at the expense of the disulfide linkage of the cystinyl residues of the protein. Many studies on the alkaline decomposition of disulfide linkages in proteins, of cystinyl peptides, and of simpler aliphatic disulfides have been reviewed in some detail by Schöberl and Wagner<sup>4</sup> and more recently Parker and Kharasch<sup>5</sup> have set the problem in the larger context.

Briefly, four different mechanistic interpretations have been offered to account for the alkaline cleavage of aliphatic disulfides, the fourth of which is a

more modern and more inclusive version of the first.

Schöberl<sup>6</sup> proposed the direct hydrolytic cleavage of the disulfide bond to furnish mercaptide ion and sulfenic acid, with the further decomposition of the latter providing the products actually obtained. He has consistently interpreted the results of his numerous studies on model compounds<sup>7</sup> on the basis of this assumption, though direct evidence for aliphatic sulfenic acids is lacking.



Tarbell and Harnish<sup>8</sup> suggested an alternative elimination mechanism, initiated by the ionization of a hydrogen from a carbon β to a sulfur atom.

(6) A. Schöberl, *Ann.*, **507**, 111 (1933); A. Schöberl, E. Berninger and F. Harren, *Ber.*, **67B**, 1545 (1934).

(7) A. Schöberl and H. Eck, *Naturwissenschaften*, **23**, 391 (1935); A. Schöberl, *Ber.*, **69B**, 1955 (1936); A. Schöberl and H. Eck, *Ann.*, **522**, 97 (1936); A. Schöberl, *Ber.*, **70B**, 1186 (1937); A. Schöberl and T. Hornung, *Ann.*, **534**, 210 (1938); A. Schöberl and P. Rambacher, *ibid.*, **538**, 84 (1939); A. Schöberl and P. Rambacher, *Biochem. Z.*, **306**, 269 (1940); A. Schöberl, P. Rambacher and A. Wagner, *ibid.*, **317**, 171 (1944).

(8) D. S. Tarbell and D. P. Harnish, *Chem. Revs.*, **49**, 11 (1951).

(1) Presented at the 136th Meeting of the American Chemical Society, Atlantic City, N. J., September 15, 1959.

(2) Toni-Gillette Fellow, 1956-1959.

(3) G. J. Mulder, *Ann.*, **28**, 73 (1838).

(4) A. Schöberl and A. Wagner, Houben-Weyl's "Methoden der organischen Chemie," 4th edition, Thieme, Stuttgart; Vol. 9, 1955, p. 75; Vol. 11 (2), 1958, p. 436.

(5) A. J. Parker and N. Kharasch, *Chem. Revs.*, **59**, 583 (1959).