statistical probability of 4 to 3. By analogy one would expect that the single hydrogen ion held by the central P atom of triphosphoric acid to be even more acidic. These arguments indicate that the H^+ ion held by the central P atom is the most highly dissociated. The second ion can then ionize from either terminal P atom to yield identical structures. If this is the case K_2 is a real equilibrium constant.

The calculations of K_4 and K_5 were simple since the constants were sufficiently different in magnitude so that the addition of hydrogen ion was stepwise and the degree of dissociation was negligible compared to the concentrations of the triphosphate species involved. Furthermore the effect of hydrolysis was negligible except in the most dilute solutions near a *p*H of 9. The hydrolysis correction consists of the addition or subtraction of $K_w/f[H^+]$ due to the following type of reaction

$$P_3O_{10}{}^{5-} + HOH \rightleftarrows HP_3O_{10}{}^{4-} + OH^-$$

The values of K_4 and K_5 were accordingly calculated by the following general equation for the acid titration.

$$K_n = \frac{[H^+][(a + 1 - n)C_p - K_w/[H^+]f + [H^+]/f]}{[(n - a)C_p + K_w/[H^+]f - [H^+]/f]}$$

where f is the activity coefficient of a univalent ion.

At an ionic strength adjusted to unity with $(CH_3)_4NCl$, the values of pK_4 and pK_5 were found to be 5.83 and 8.81. At an ionic strength of 0.1 the values of pK_4 and pK_5 were found to be 6.00 and 8.73, respectively. Extrapolated to zero ionic strength the values were $pK_4 = 6.26$ and $pK_5 = 8.90$.

Although the experiments were performed using cells with liquid junctions the error due to this cause is believed to be very small since the effect is in part cancelled through the use of standard buffers and hydrochloric acid solutions of known concentrations to standardize the *p*H meter. Experiments are in progress to determine the thermodynamic functions using cells without liquid junction at various temperatures.

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COLUMBUS, OHIO

[CONTRIBUTION FROM THE MALLINCKRODT LABORATORIES OF HARVARD UNIVERSITY]

Barium Ethylene Phosphate

BY JUNJI KUMAMOTO, JAMES R. COX, JR., AND F. H. WESTHEIMER

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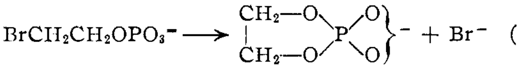
The barium salt of ethylene phosphate has been prepared from barium bromoethyl phosphate. Salts of ethylene phosphate undergo hydrolysis in alkaline solution to salts of hydroxyethyl phosphate; the rate of the reaction is almost ten million times that for the corresponding hydrolysis of salts of dimethyl phosphate.

A series of recent investigations[1] have shown that five-membered cyclic phosphate esters are intermediates in the hydrolysis of ribonucleic acids. These cyclic phosphates rapidly hydrolyze to open-chain monoesters of phosphoric acid.[1,2] However, qualitative data[3] suggest that most diesters of phosphoric acid are relatively resistant to alkaline hydrolysis. The cyclic phosphate esters previously[1,2] examined contained at least one additional hydroxyl group. In order to investigate further the peculiarities of five-membered cyclic phosphate esters, the simplest member of the series, ethylene phosphate (I) has been synthesized according to equation 1, and the rate of its hydrolysis has been compared to that for dimethyl phosphate.


(1)

I

Experimental

Barium bromoethyl phosphate was synthesized by Flexser's modification[4] of Outhouse's procedure.[5] Eleven g. of water was slowly introduced into 110 g. of phosphorus oxychloride. After the initial reaction subsided, the mixture was cooled to 0°, and 100 g. of ethylene bromohydrin was slowly added. The reaction mixture was warmed under vacuum for a half an hour to remove hydrogen chloride. The product (about 100 cc.) was again cooled to 0°, and added in small portions to 100 cc. of cold water in a large porcelain mortar. Between each addition of the phosphate ester mixture, solid $Ba(OH)_2 \cdot 8H_2O$ was added, and ground in the mortar until it reacted; the *p*H was maintained around 8–9 (external indicator). The mixture was filtered, and the filtrate discarded. Barium bromoethyl phosphate was leached from the wet filter cake with successive 100-cc. portions of water. An equal volume of ethanol was added to the aqueous extracts; the desired salt precipitated from the aqueous alcohol. After six extractions, the yield was 30 g., or about 12%. The compound could be recrystallized from water by the addition of alcohol.

Anal. Calcd. for $C_2H_4BrPO_4Ba \cdot H_2O$: C, 6.70; H, 1.68; Br, 22.31. Found: C, 7.14; H, 1.70; Br, 22.66.

This salt (the monohydrate) had previously been obtained by another method.[8]

Barium Ethylene Phosphate.—Barium bromoethyl phosphate (6.5 g.) was dissolved in 100 cc. of water, and warmed to 75° for 15 minutes. During this interval, the *p*H was maintained at 7.0 with barium hydroxide added by means of

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(1) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952); D. Lipkin, P. T. Talbert and M. Cohn, THIS JOURNAL, **76**, 2871 (1954); *cf.* A. Fono, *Archiv. Kemi. Mineral. Geol.*, **24A**, 34, 19 (1947); D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 52 (1952).

(2) D. M. Brown, D. I. Magrath and A. R. Todd, *ibid.*, 2708 (1952); T. Ukita, N. A. Bates and H. E. Carter, *J. Biol. Chem.*, **216**, 867 (1955).

(3) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, Chapter 9.

(4) (a) L. A. Flexser and W. G. Farkas, *Chem. Eng. News*, **29**, 3947 (1951); (b) W. W. Butcher and F. H. Westheimer, THIS JOURNAL, **77**, 2420 (1955).

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

(6) F. Zetzsche and M. Nachmann, *Helv. Chim. Acta*, **8**, 943 (1925).

a Beckman model K automatic titrator; the *p*H was then raised to 8.5, and the mixture filtered. The filtrate was then rapidly evaporated to dryness at 75° and 20 mm. pressure. The residue was dissolved in 10 cc. of water and 10 cc. of alcohol added. The crystals of barium hydroxyethyl phosphate which formed during the next half hour were removed by filtration and discarded, and the filtrate diluted to 250 cc. with absolute ethanol. About 3 g. of barium ethylene phosphate crystallized from the solution on standing; it was repeatedly recrystallized by dissolving 3 g. in 20 cc. of 50% ethanol, adding 60 cc. of absolute ethanol and seeding. This procedure separates the barium ethylene phosphate from barium bromide, which is more soluble in aqueous ethanol; the best samples gave no test for halogen with acidified silver nitrate solution. The crystals, under 100 × magnification, generally appeared as flat hexagonal or diamond-shaped plates; occasionally needles were obtained. The analysis of the salt for phosphorus proved difficult; consistent results were obtained by William Sascheck (University of Chicago).

Anal. Calcd. for $(C_2H_4PO_4)_2Ba$: C, 12.52; H, 2.10; P, 16.16; Ba, 35.84; mol. wt., 383.4. Found: C, 12.55; H, 2.24; P, 15.52; 15.60; Ba, 35.50, 35.93; mol. wt. (f.p. method in water) 415, 430, 423; mol. wt., cor., 364, 377, 372. (The corrected molecular weights were obtained by taking into account deviations from ideal solution behavior as exemplified by $BaCl_2 \cdot 2H_2O$.)

Cyclohexylammonium Ethylene Phosphate.—A solution of 2.0 g. of barium ethylene phosphate was added to a solution of 1.30 g. of cyclohexylammonium sulfate in 20 cc. of water. The barium sulfate was removed by centrifugation and an equal volume of ethanol added to the clear solution. The mixture was again filtered, and 200 cc. of acetone added to precipitate residual cyclohexylammonium sulfate. The new filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 20 cc. of *n*-propyl alcohol and the salt crystallized by the addition of ether. The total yield (two crops) was 1.65 g. of fine needles, m.p. 168°. Titration of a 0.1-g. sample showed no inflection in the region of *p*H 7.

Anal. Calcd. for $C_8H_{18}PO_4N$: C, 43.05; H, 8.13; N, 6.28; P, 13.88. Found: C, 43.32, 42.91; H, 8.13, 8.04; N, 6.55, 6.24; P, 13.55, 14.19.

Cyclohexylammonium ethylene phosphate was reconverted to the barium salt by adding 0.21 g. of barium bromide in 10 cc. of 95% ethanol to 0.3 g. of cyclohexylammonium ethylene phosphate dissolved in the same solvent. The gelatinous mass which first formed turned powdery on standing, gave only a faint test for halogen, and was identified as the barium salt by its infrared spectrum in a KI window.

Product.—A solution of 102 mg. of barium ethylene phosphate and 83 mg. of barium hydroxide octahydrate in 5 cc. of water was heated for an hour on the steam-bath. The solution was filtered hot, and diluted with an equal volume of alcohol; the resulting precipitate was digested to facilitate filtration. The product (141 mg., or 96% yield) was recrystallized from water, and identified as barium hydroxyethyl phosphate by a comparison of its infrared spectrum with that of an authentic sample.[7,4(b)]

Solutions of Potassium Ethylene Phosphate.—Most of the kinetic experiments were carried out with solutions of the potassium salt of ethylene phosphate. A weighed amount of the barium salt, dissolved in water, was treated with a solution of the calculated quantity of potassium sulfate. Barium sulfate was removed by centrifugation, and the precipitate washed with water and again spun down. The combined supernatant liquids were transferred to a volumetric flask, and diluted to volume. The solid potassium salt proved too hygroscopic for convenient handling or for analysis.

Trimethyl phosphate was prepared by the method of Becher.[8] It boiled at 92.5° (16 mm.).

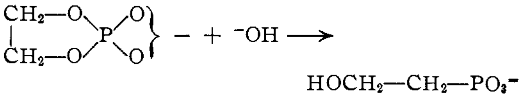
Barium dimethyl phosphate was prepared by the partial hydrolysis of the triester[9] with barium hydroxide.

Cyclohexylammonium dimethyl phosphate was prepared by treating a solution of the barium salt with a solution of cyclohexylammonium sulfate. The salt could be recovered from the aqueous solution after removing the precipitated barium sulfate. On recrystallization from a propyl alcohol-ligroin mixture, it melted at 78°.

Anal. Calcd. for $C_8H_{20}PO_4N$: C, 42.67; H, 8.96; P, 13.75; N, 6.23. Found: C, 42.73; H, 8.98; P, 13.72; N, 6.13.

Solutions of potassium dimethyl phosphate were prepared from the barium salt by a method analogous to that used for the corresponding salt of ethylene phosphate. The solid could be recrystallized from methanol, but proved too hygroscopic for analysis.

Kinetic Method.—The hydrolysis of barium ethylene phosphate in alkaline solution was followed by a titration procedure. The reaction consumes alkali; however, in most of the experiments here reported, the concentration of alkali was much higher than that of the ester and so changed very little during any experiment. The salt of hydroxyethyl phosphate, however, can be titrated by taking advantage of the fact that primary esters of phosphoric acid have their second ionization constants[10] around 10^{-7}.


(2)

A solution of potassium ethylene phosphate, in aqueous alkali, was therefore thermostated, and aliquots removed at known intervals; these aliquots were quenched by adding enough acid to bring the *p*H to about 10. The solutions were then titrated with a Beckman model K automatic titrator, and the amount of alkali consumed between *p*H 9.5 and *p*H 5.5 was taken as equivalent to the hydroxyethyl phosphate formed.

Kinetic Method for Dimethyl Phosphate.—Dimethyl phosphate was generally formed *in situ* by the hydrolysis of trimethyl phosphate; the hydrolysis of the triester is so fast that it could be completed overnight around 25°. At this temperature, the second step of the hydrolysis (equation 4) is so slow that it can be neglected.

$$(CH_3O)_3PO + {}^-OH \longrightarrow CH_3OH + (CH_3O)_2PO_2^- \quad (3)$$

$$(CH_3O)_2PO_2^- + {}^-OH \longrightarrow CH_3OH + CH_3OPO_3^= \quad (4)$$

The hydrolysis of dimethyl phosphate could be carried out only in strong alkali at high temperatures (115 and 125°); glass containers could not, therefore, be used. Separate samples (eight for each kinetic "run") were therefore placed in Teflon tubes which were closed with tapered Teflon plugs; the tubes were supported by brass jackets to provide mechanical strength and tight closure. Individual tubes were withdrawn, quenched by cooling, to 25°, and the contents titrated for monomethyl phosphate by a method analogous to that used for the hydrolysis of ethylene phosphate. Here, however, an end-point at *p*H 5 (rather than 5.5) was chosen.

Kinetic Method for Bromoethyl Phosphate.—The kinetics of the solvolysis of bromoethyl phosphate was conducted in a thermostated cell maintained at constant *p*H by the automatic titrator. At stated intervals, samples were withdrawn and titrated for bromide ion by the Volhard method. The amounts of alkali which had been introduced by the titrator were noted at the same times that the samples were removed for bromide analysis.

Results

Solvolysis of Barium Bromoethylphosphate.—Crude kinetic experiments on the solvolysis of barium bromoethyl phosphate showed that the rate at which bromide ion is liberated at *p*H 7 exceeds the rate at which alkali is consumed by a factor of about 100. At 50°, the half-time for the liberation of bromide ion from a 0.013 *M* solution was about 43 minutes; the half-time from a 0.026 *M* solution was about 45 minutes. Around *p*H 5 (where the ester is protonated) the rate of solvolysis is very much less.

Alkaline Hydrolysis of Salts of Ethylene Phosphate.—The rate constants for the hydrolysis of ethylene phosphate are presented in Table I.

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

[8] P. Becher, THIS JOURNAL, **74**, 2923 (1952).

[9] W. Lossen and A. Kohler, *Ann.*, **262**, 209 (1891).

[10] W. D. Kumler and J. J. Eiler, THIS JOURNAL, **65**, 2355 (1943).

TABLE I

HYDROLYSIS OF SALTS OF ETHYLENEPHOSPHATE AT 25°

KOH, mole/l.	Potassium ethylene phosphate, mole/l.	$BaCl_2$, mole/l.	μ^a	10^4k, sec.$^{-1}$ (mole/l.)$^{-1}$
0.14	0.016	...	0.67	4.39
.14	.032	...	.67	4.74
.133	.032	...	.67	4.86
.14	.064	...	.65	4.89
.63	.032	...	.66	4.54
.14	.043	...	.18	3.65
.13	.032	0.167	.67	39.2
.133	.032	.083	.67	27.5

[a] Maintained with potassium *p*-toluenesulfonate.

The second order rate constant is valid over a four-fold range in the concentration of alkali and of the ethylene phosphate anion. The positive salt effect is expected for a reaction between two negative ions.

Alkaline Hydrolysis of Potassium Dimethyl Phosphate.—The rate constants for the hydrolysis of dimethyl phosphate are presented in Table II.

TABLE II

HYDROLYSIS OF POTASSIUM DIMETHYL PHOSPHATE

Potassium dimethyl phosphate, mole/l.	KOH, mole/l.	μ	T, °C.	10^6k, sec.$^{-1}$ (mole/l.)$^{-1}$
0.0368	1.392	1.43	124.9	6.1
.0583[a]	1.106	1.16	124.9	6.9
.358[a]	0.806	1.16	124.9	5.3
.806[a]	.358	1.16	124.9	4.4
.358[a]	.806	1.16	115.3	2.2

[a] Prepared by hydrolysis of trimethyl phosphate *in situ*; see Experimental.

The rate constants here determined vary somewhat with composition of the solutions, even at constant ionic strength. However, the individual experiments led to good second order rate constants. Besides, the ionic strength principle seldom holds in such concentrated solutions as these, and the data clearly establish the approximate magnitude of the rate of hydrolysis.

Discussion

Ethylene Phosphate.—The structure of ethylene phosphate (I) is established by the following facts. The analyses of the barium and cyclohexylammonium salts, and the molecular weight of the barium salt, correspond to the molecular formulas shown. Electrometric titration shows no inflection in the region around *p*H 7; the compound is therefore a secondary ester. Further, the infrared spectrum of the barium salt, pressed in a KI window, is typical of that found for secondary esters. The spectrum of the salt determined in a KBr window or in a Nujol mull shows no absorption whatever in the hydroxyl region. On hydrolysis of barium ethylene phosphate, the barium salt of hydroxyethyl phosphate is formed, according to equation 2. Additional evidence for the structure of the anion I can be obtained from the method of synthesis of its barium salt from bromoethyl phosphate, equation 1. Since the rate at which bromide ion is formed greatly exceeds that at which hydrogen ion is produced, the process cannot be hydrolysis, but must be a displacement reaction. Since the half-time for the production of bromide ion is independent of the concentration of bromoethyl phosphate, the reaction is unimolecular, and therefore must be ring-closure rather than polymerization.

Rate Factor.—The activation energy for the hydrolysis of dimethyl phosphate, estimated from the data (Table II) at 115 and 125°, is about 28.2 kcal./mole. A simple extrapolation suggests that the rate constant, at 25° and an ionic strength of 1, is about 3×10^{-11} sec.$^{-1}$ (mole)$^{-1}$; ethylene phosphate, then, shows a rate constant for alkaline hydrolysis which exceeds that for dimethyl phosphate by almost seven powers of ten. Even when a correction for the difference in ionic strength is applied, the factor must still be of the order of five million. The simplest cyclic ester, like those previously[1,2] synthesized, hydrolyzes with extraordinary ease. The reasons for this large difference in rate between cyclic and open-chain esters have not yet been elucidated.[11]

Barium Ion Catalysis.—The data of Table I show that the hydrolysis of ethylene phosphate, like that of diphenyl phosphate,[12] is an example of homogeneous metal ion promoted hydrolysis of a phosphate ester. This and several other facets of this problem are currently under investigation.

Acknowledgment.—The authors wish to express their appreciation to the National Science Foundation for a grant which made this work possible and for a fellowship for one of us (J.R.C.).

CAMBRIDGE, MASS.

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(11) V. Schomaker and J. S. Rollett, in private communications, have suggested that the phosphate rings may be subjected to a new type of strain. The data (J. D. Dunitz and J. S. Rollett, *Acta Cryst.*, **9**, 327 (1956)) on the X-ray diffraction pattern of crystals of dibenzylphosphoric acid show a dihedral angle between the two C–O–P planes of 90°. If this angle proves to be an inherent property of phosphate esters, it would supply sufficient strain in the five ring esters to account for rapid hydrolysis. Neither this hypothesis nor any of several others we have examined has been established.

(12) C. W. Helleiner and G. C. Butler, *Can. J. Chem.*, **33**, 705 (1955).