716. *The Reactions of Organic Phosphates. Part I. The Hydrolysis of Methyl Dihydrogen Phosphate.*

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The hydrolysis of methyl dihydrogen phosphate has been investigated, by kinetic and isotope techniques, over the range of acidities pH 7 to 10M-perchloric acid. Four hydrolytic reactions have been identified. These involve the decomposition of (*a*) the monoanion with phosphorus–oxygen bond fission, (*b*) the neutral species with carbon–oxygen bond fission, and (*c*) the conjugate acid with both carbon–oxygen and phosphorus–oxygen bond fission. An oxygen exchange between methyl dihydrogen phosphate and solvent in strong acid solution has also been found. The mechanisms of all the reactions are discussed.

Nomenclature.—Throughout this and subsequent papers we describe accurately substances used in experimental work, *e.g.*, methyl disodium phosphate; names such as monomethyl phosphate, dimethyl phosphate, α-D-glucose 1-phosphate, etc., are used as collective designations for the various species, ionic and/or non-ionic, present in solution.

The mechanisms of the reactions of phosphate esters, although of interest to both chemists and biochemists, have not, until recent years, been systematically investigated. In this series of papers we propose to give an account, in mechanistic terms, of nucleophilic displacement processes, and especially those involving hydrolysis, as they occur in a group of relatively simple organic esters, of orthophosphoric acid. This may serve as a starting point for the study of the mechanisms of those more complex reactions, such as phosphate transfer, which are of direct interest to biochemists. Preliminary accounts of some of our work have already been published.[1, 2]

The hydrolysis of organic phosphates is complicated by several factors. First, there is the problem of determining which of the possible forms of the substrate is the reactive species under specified conditions. For example, a phosphate monoester could undergo reaction as a dianion, a monoanion, a neutral molecule, or one of the conjugate acids. As will be seen, identification of the true factor can usually be made from the pH–rate profile and the dissociation constants of the substrate. Secondly, for each species reaction may occur with carbon–oxygen or with phosphorus–oxygen bond fission. Distinction between these two possibilities is conveniently made by using the isotope ^{18}O as a tracer, although, as will be seen below, complications may arise owing to concomitant exchange occurring in reactants and products. Thirdly, most of the reactions involve water (as distinct from hydroxide ions) as nucleophilic reagent and consequently the molecularity cannot be determined by the use of a simple-order criterion. In these cases, molecularity can be assigned with fair certainty only for reactions which are acid-catalysed and where the Zucker–Hammett criterion is applicable.

The essential features of the pH–rate profile for simple monoalkyl phosphates have been known for some time. Typically the rate of hydrolysis is very small in alkaline solution,[3, 4] increases to a maximum at about pH 4, then falls to a minimum at about pH 0·5 and rises again in strongly acid solutions.[5, 6, 7] The French workers, Bailly [5] and Desjobert,[7] who largely pioneered this field, suggested that the maximum rate at about pH 4 was due to reaction of the monoanion, by some especially readily occurring mechanism.

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[1] Barnard, Bunton, Llewellyn, Oldham, Silver, and Vernon, *Chem. and Ind.*, 1955, 760.

[2] Vernon, *Chem. Soc. Special Publ.*, No. 8, 1957, 17.

[3] Cavalier, *Compt. rend.*, 1898, **127**, 60.

[4] Plimmer and Scott, *J.*, 1908, **93**, 1699.

[5] Bailly, *Bull. Soc. chim. France*, 1942, **9**, 340, 405.

[6] Fleury, *Compt. rend.*, 1945, **221**, 416.

[7] Desjobert, *Bull. Soc. chim. France*, 1947, 809; *Compt. rend.*, 1947, **224**, 575; *Bull. Soc. Chim. biol.*, 1954, **36**. 475.

This view has recently been supported by the detailed investigations of Westheimer and his collaborators.[8, 9] They found, for example, that the hydrolysis of optically active mono-2-methoxy-1-methylethyl phosphate had a maximum rate at pH 4 where the monoanion was the bulk component and that, under these conditions, hydrolysis proceeded with complete retention of configuration, indicating phosphorus–oxygen bond fission. Consistently, isotopically normal alcohol was recovered from a reaction run in water enriched with the isotope ^{18}O. It was suggested that reaction of the monoanion proceeded through some intermediate in which monoanion and water were linked by hydrogen bonds, the special reactivity of the monoanionic species of phosphate monoesters, compared with the corresponding neutral or dianionic species or with the monoanions of phosphate diesters, being due to its special ability to form such a complex. In the strongly acid region, mono-2-methoxy-1-methylethyl phosphate was found to undergo acid-catalysed hydrolysis, and the occurrence of carbon–oxygen bond fission was inferred from the fact that the alcohol recovered from the products was, under these conditions, considerably racemised.

In the present paper an investigation, by kinetic and isotope techniques, of the hydrolysis of monomethyl phosphate is reported. The results make possible an analysis of the mechanisms of hydrolysis more complete than any hitherto reported for a phosphate ester.

EXPERIMENTAL

Methyl dihydrogen phosphate was prepared by treatment of trisodium phosphate with dimethyl sulphate.[10] It was isolated as the barium salt (Found: Ba, 55·5. Calc. for CH_3O_4PBa: Ba, 55·5%). This was converted into the disodium salt which was used throughout the investigation. Its equivalent weight and water of crystallisation content were determined by titration and drying to constant weight respectively (Found: equiv., 263·3; H_2O, 41·2. Calc. for $CH_3O_4PNa_2,6H_2O$: equiv., 263·9; H_2O, 40·9%). Conventional tests for phosphate, sulphate, and barium ions gave negative results.

Determination of Dissociation Constants.—The values of the dissociation constants at 100° (the temperature of most of the kinetic runs) are required. It is clearly difficult to obtain accurate values at such a relatively high temperature: the following procedures, which yield approximate values, were therefore adopted:

(*a*) The second dissociation constant (pK_2) was determined from measurements of pH, by means of a standardised glass-electrode system, made in the course of titration of a solution of methyl disodium phosphate of known strength with standard 0·1M-hydrochloric acid. Let q and q' be the number of c.c. of acid added at equivalence (one sodium atom) and at any other point before equivalence, respectively. Then q' and $(q - q')$ are proportional to the concentrations of $MeHPO_4^-$ and $MePO_4^{2-}$ respectively. Activity effects being ignored, pK_2 is given by the Henderson equation:[11] $pK_2 = pH + \log [q'/(q - q')]$. At 1·05°, 11·0°, 22·5°, 34·2°, 58·0°, and 74·5°, the following values for pK_2 were obtained: 6·75, 6·61, 6·58, 6·59, 6·70, 6·71. The plot of pK_2 against temperature is roughly parabolic and simple extrapolation gave the value $pK_2 = 6{\cdot}85$ at 100°. Harned and Embree[12] have pointed out that the variation of dissociation constant of weak acids with temperature can be reasonably well represented by the equation:

$$pK_T = pK_M + 5{\cdot}0 \times 10^{-5}(T - \theta)^2$$

where K_M is the maximum value of the dissociation constant K_T and θ is the corresponding temperature. Application of this equation ($pK_M = 6{\cdot}58$, $\theta = 26{\cdot}0°$) gave the value $pK_2 = 6{\cdot}85$ at 100°.

(*b*) The first dissociation constant (pK_1) was also determined from pH measurements. To a solution of the disodium salt enough standard hydrochloric acid solution was added to ensure

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[8] Butcher and Westheimer, *J. Amer. Chem. Soc.*, 1955, **77**, 2420.

[9] Kumamoto and Westheimer, *ibid.*, p. 2515.

[10] Bailly, *Compt. rend.*, 1920, **170**, 1061.

[11] Glasstone, " Introduction to Electrochemistry," D. van Nostrand and Co. Inc., New York, 1942, p. 390.

[12] Harned and Embree, *J. Amer. Chem. Soc.*, 1934, **56**, 1050.

that the phosphate existed as a mixture of $MeHPO_4^-$ (salt) and MeH_2PO_4 (acid). The pH of this solution is approximately related to pK_1 by the equation

$$pK_1 = pH + \log [(C_{salt} + C_{H^+})/(C_{acid} - C_{H^+})]$$

where the concentrations C_{salt} and C_{acid} are derived from the initial concentration of methyl disodium phosphate and the amount of hydrochloric acid added, and C_{H^+} is calculated from the pH. The procedure is less precise than that for the determination of pK_2. At 22·0°, 35·0°, 58·0°, 60·0°, and 72·0°, the following values for pK_1 were obtained: 1·52, 1·45, 1·46, 1·43, 1·60. Simple graphical extrapolation gave the value $pK_1 = 1{\cdot}61$ at 100°. Use of the Harned–Embree equation ($pK_M = 1{\cdot}44$, $\theta = 42{\cdot}0°$) gave the value $pK_1 = 1{\cdot}65$.

Buffer Solutions.—The buffer solutions used for the kinetic experiments were those for which pH values have been given at 20° and 150° by Stene.[13] The values of the pH at intermediate temperatures were found by simple interpolation, and are given, together with the composition of the buffers, in Table 1.

Colorimetric Analysis of Phosphate Ions.—This was carried out by Allen's modified method,[14] with a Hilger " Spekker " Photoelectric Absorptiometer fitted with a red filter (Kodak 608). In mixtures containing perchloric acid, the amount of acid present in the aliquot part taken for analysis was allowed for when adding the perchloric acid necessary to develop the blue colour. This is necessary since the final intensity of colour developed is diminished by the presence of excess of perchloric acid.

The method of analysis was found to be insensitive to the presence of extraneous impurities with the exception of silicate. To prevent interference due to silicate the kinetic experiments were carried out in steamed-out Monax test-tubes.

Kinetic Experiments.—The following experimental details of a run at 100·1° and with $1{\cdot}332 \times 10^{-3}$M-methyl disodium phosphate and 1·00M-perchloric acid are typical:

Aliquot parts (6 c.c.) of mixture were sealed in glass tubes, placed in the thermostat until thermal equilibrium was reached, removed at appropriate intervals, and cooled. Before analysis the tubes were allowed to reach a temperature of 20°. Aliquot parts (5 c.c.) were removed from each tube and the inorganic phosphate was determined colorimetrically. Since all the reactions were of first-order and since the intensity of colour developed in the phosphate determination was found to obey Beer's law, first-order rate coefficients (k_0) were calculated from the formula: $k_0 = (2{\cdot}303/t) \log [(D_\infty - D_0)/(D_\infty - D_t)]$, where D_0, D_t, and D_∞ are the optical densities of the coloured solutions, obtained in the determination of phosphate, derived from aliquot parts taken at zero-time, time t, and complete reaction respectively. The results were:

Time (min.)	0	180	445	840	1440	2070	2795	3690	5130	∞
D	0·006	0·052	0·116	0·198	0·311	0·402	0·492	0·574	0·681	0·858
10^6k_0 (sec.$^{-1}$)	—	5·15	5·20	5·07	5·13	5·03	5·05	4·93	5·12	—

The value, D_∞, was calculated from the known initial concentration of methyl disodium phosphate. In other experiments it was shown that the theoretical and the experimental infinity readings agreed within experimental error.

Table 1 gives the kinetic results obtained in the region pH 0—7·5. It can be seen that, in the region of pH 4, at the rate maximum, increase in buffer strength by some 50% or increase in total ionic strength is without appreciable effect on the rate. Similarly, change of solvent to deuterium oxide is, in so far as the water pH-scale is approximately applicable, also without effect. Arrhenius parameters for the reaction in the region of pH 4 were calculated by the method of least squares, from data at different temperatures, as $E = 30{\cdot}6 \pm 0{\cdot}2$ kcal. mole^{-1}, $A = 6{\cdot}47 \times 10^{12}$ sec.$^{-1}$. Table 2 gives the results of kinetic runs carried out in strongly acidic solutions at 100·1°. The concentrations refer to room temperature.

Isotope Experiments.—A. *Isolation and isotopic analyses of products.* The hydrolysis of monomethyl phosphate was carried out, under a variety of conditions, in water containing an excess abundance of the isotope ^{18}O. Isotopic analyses of the products, *i.e.*, methanol and inorganic phosphate, were then made. The techniques were as follows:

(*a*) Methanol. The reaction mixture, usually about 250 c.c. and containing enough monomethyl phosphate to yield approximately 4 c.c. of methanol at complete reaction, was sealed in a

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[13] Stene, *Rec. Trav. chim.*, 1930, **49**, 1133.

[14] Allen, *Biochem. J.*, 1940, **34**, 858.

glass ampoule and immersed in a thermostat until about 50% reaction had occurred. The ampoule was then cooled and opened. The contents were, where necessary, neutralised with solid potassium hydroxide and distilled, the first 20 c.c. of distillate being collected. This contained about 2 c.c. of methanol and was carefully fractionated through a small vacuum-jacketed column containing a metal spiral and having a low hold-up. Several samples of methanol were collected and the two middle samples (S_1 and S_2) were used for isotopic analysis. The remaining samples were mixed with isotopically normal water and refractionated, two middle samples (S_3 and S_4) again being analysed. Comparison of the results from the two pairs of samples provides a check on the efficiency of the distillation. The methanol samples were pyrolysed *in vacuo* to carbon monoxide over carbon heated by a radio-frequency heater. The carbon monoxide was analysed mass-spectrometrically. The following details are typical: a solution of methyl disodium phosphate (17·2 g. in 250 c.c.) in 5M-perchloric acid was heated for 9 hr. at 100·1°. The solvent contained 1·87 atom % excess abundance * of ^{18}O. The analysis of carbon monoxide obtained from the methanol isolated from the reaction gave S_1 1·40, S_2 1·47, S_3 1·41, S_4 1·52 atom % of ^{18}O.

TABLE 1. *Rates of hydrolysis of monomethyl phosphate at* pH 0—7·5 (100·1° *unless specified*).

pH	10^6k_0 (sec.$^{-1}$)	Composition *	pH	10^6k_0 (sec.$^{-1}$)	Composition *
0·02	5·09	1·0M-$HClO_4$	6·43	5·17	0·05M-P, 0·0455M-NaOH
0·32	3·85	0·5M-$HClO_4$	7·46	1·33	0·05M-H_3BO_3, 0·00261M-NaOH, 0·05M-KCl
0·72	3·25	0·2M-$HClO_4$	—		
1·02	3·45	0·1M-$HClO_4$	4·08	8·27	0·075M-P
1·24	3·52	0·0645M-HCl, 0·05M-KCl	3·67	8·67	0·05M-P, 0·95M-$NaClO_4$
1·61	4·60	0·0263M-HCl, 0·05M-KCl	(4·17)	9·49 †	0·05M-P
2·20	6·72	0·0067M-HCl, 0·05M-KCl	4·12 [1]	0·815	0·05M-P
2·50	6·99	0·05M-P, 0·0396M-HCl	4·14 [2]	2·29	0·05M-P
4·17	8·23	0·05M-P	4·19 [3]	23·9	0·05M-P
5·60	7·38	0·05M-P, 0·030M-NaOH	4·21 [4]	50·0	0·05M-P

* P = potassium hydrogen phthalate. † In 95% deuterium oxide at 101·0°.
[1, 2, 3, 4] At 80·0, 89·0, 109·7, and 117·2° respectively.

TABLE 2. *Hydrolysis of monomethyl phosphate in strongly acidic solutions* (100·1°).

M-$HClO_4$	10^6k_0 (sec.$^{-1}$)	M-$HClO_4$	M-$NaClO_4$	10^6k_0 (sec.$^{-1}$)	M-$HClO_4$	M-$NaClO_4$	10^6k_0 (sec.$^{-1}$
1·00	5·08	2·00 [1]	6·00	20·8	6·00 [2]	1·00	26·8
2·00	7·77	4·00 [1]	4·00	26·7	1·50 [3]	2·50	7·53
3·00	11·3	6·00 [1]	2·00	33·0	2·50 [3]	1·50	10·3
4·00 [3]	15·0	7·00 [1]	1·00	35·3	3·00 [3]	1·00	11·6
4·00 *	15·2	2·00 [2]	5·00	15·4	3·50 [3]	0·50	12·5
5·00	18·5	4·00 [2]	3·00	21·8			
6·00	24·2						
7·00 [2]	31·5						
8·00 [1]	41·5						
9·00	54·7						
10·00	78·5						

[1, 2, 3] Solutions of total ionic strength (μ) 8·00, 7·00, and 4·00M respectively.
* In 67·6% deuterium oxide.

(*b*) Inorganic phosphate. A modification of the method originally given by Cohn was used.[15] The solution remaining after the distillation of methanol, as described above, was centrifuged, if necessary, to remove potassium perchlorate and adjusted to *ca.* pH 10. Sufficient barium chloride solution was added to precipitate all the inorganic phosphate as barium phosphate [$Ba_3(PO_4)_2$]. The precipitate was centrifuged off and washed several times with ice-cold water (in which methyl barium phosphate is relatively soluble). The barium phosphate was then dissolved in the minimum amount of 2M-hydrochloric acid and reprecipitated by adding 2M-sodium hydroxide until the pH was *ca.* 10. The precipitate was removed by centrifugation and washed with cold water. This procedure was repeated four times to ensure complete removal of adsorbed impurities. The barium phosphate was again dissolved in the minimum amount of 2M-hydrochloric acid, and a solution of potassium sulphate added dropwise until no more

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* Subsequent figures also refer to atom % excess over the normal abundance.

[15] Cohn, *J. Biol. Chem.*, 1949, **180**, 771.

barium sulphate was precipitated. (It is important to avoid excess of potassium sulphate.) The barium sulphate was removed by centrifugation and the supernatant liquid adjusted to pH 4·5 with potassium hydroxide solution. Two volumes of ethanol were then added and the precipitated potassium dihydrogen phosphate was centrifuged off, washed with ethanol and then with ether, and dried at 100° for 2 hr. Samples (*ca.* 0·3 g.) were then sealed in glass tubes (vol. *ca.* 5 c.c.) with carbon dioxide at *ca.* 8 cm. pressure, and were heated at 200° for 24 hr.* The carbon dioxide was then analysed mass-spectrometrically. A typical result is that obtained from the experiment described in the last section: the ^{18}O contents of the carbon dioxide from two samples were 0·278 and 0·280 atom % of ^{18}O.

B. *Calculation of the position of bond fission.* It being assumed that no exchange reactions involve the oxygen atoms of the solvent and of the factors and products, then if N_S, N_M, and N_P are the excess abundance of ^{18}O in the solvent, methanol, and inorganic phosphate respectively, the percentage of the total reaction proceeding by a process involving carbon–oxygen bond fission is $100 \times N_M/N_S$, and the corresponding function giving the percentage of phosphorus–oxygen bond fission $100 \times 4N_P/N_S$.† Further, the two percentages, which are obtained independently, should, for hydrolysis under specified conditions, total 100, within experimental error.

However, methanol, in aqueous acid, undergoes oxygen exchange with the solvent,[16] and phosphate ions undergo a similar exchange both in acidic solution and in the range pH 0—7.[17] Consequently, except for the case of methanol isolated from reactions run in neutral or slightly acidic conditions, the above simple way of dealing with the isotopic data is inapplicable.

The proportion of carbon–oxygen bond fission, Q_C, can be calculated from the isotopic enrichment, N_M, of the isolated methanol, and of the solvent N_S, by the following equation which takes account of the exchange reaction between methanol (M) and the solvent:

$$Q_C = \frac{(N_M R/N_S - 1)(k_0 - k_E{}^M) - (k_E{}^M \exp - k_0 t) + (k_0 \exp - k_E{}^M t)}{k_0 (\exp - k_E{}^M t - \exp - k_0 t)}$$

where R is the fraction of monomethyl phosphate which has undergone hydrolysis in time t and $k_E{}^M$ is the first-order rate coefficient for the oxygen exchange of methanol under the reaction conditions.

The proportion of phosphorus–oxygen bond fission, Q_P, can be calculated from the isotopic enrichment, N_P, of the isolated inorganic phosphate by a similar equation:

$$Q_P = \frac{4[N_P R/N_S - 1)(k_0 - k_E{}^P) - (k_E{}^P \exp - k_0 t) + (k_0 \exp - k_E{}^P t)]}{k_0 (\exp - k_E{}^P t - \exp - k_0 t)}$$

where $k_E{}^P$ is the first-order rate coefficient for the oxygen exchange of inorganic phosphate (all four oxygen atoms) under the reaction conditions. The derivation of these equations is given in the Appendix.

Table 3 gives the results obtained at 100·1°.

TABLE 3. *Isotope experiments.*

Conditions	Time (hr.)	$10^6 k_0$ (sec.$^{-1}$)	$10^6 k_E{}^M$ (sec.$^{-1}$)	$10^6 k_E{}^P$ (sec.$^{-1}$)	N_S	N_M	N_P	Q_C	Q_P
5M-$HClO_4$	9	18·5	7·58 [16]	2·50 [17]	1·87	1·45	0·279	73	44·5
pH 4·0	48	8·23	—	0·92 [17]	0·78	0·015	0·26	1·9	107
pH 6·5	175	5·17	—	—	0·97	0·01	—	1·0	—
ca. pH 1, 9M-$NaClO_4$...	16	—	—	—	0·60	0·50	—	84·0	—

(C) *Isotopic analysis of substrate.* It will be seen from Table 3 that, for hydrolysis in strongly acid solution, the sum of the percentages of reaction proceeding by phosphorus–oxygen and carbon–oxygen bond fission significantly exceeds 100. This discrepancy must mean that some

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* Isotopic fractionation in the dehydration of the phosphate has been ignored.

† The factor four arises because, whereas hydrolysis can isotopically enrich only one of the four oxygen atoms of the phosphate, all four contribute to the isotope content of the water obtained by heating the potassium dihydrogen phosphate. Analysis of the carbon dioxide after equilibration with this water effectively gives the average isotopic enrichment of all the oxygen atoms of the inorganic phosphate.

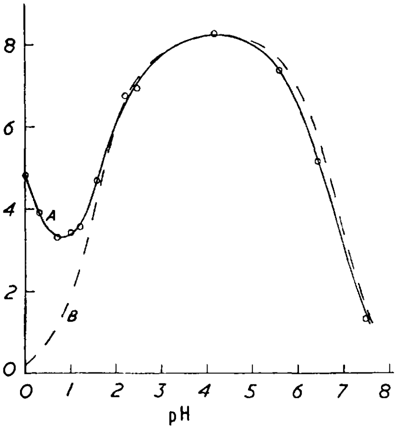
[16] Oldham, unpublished experiments.

[17] Welch, unpublished experiments.

process exists whereby the substrate can become isotopically enriched. The following experiment was designed to measure the extent of this enrichment. A solution of methyl disodium phosphate (17 g. in 250 c.c. of 5M-perchloric acid) was allowed to hydrolyse for 9 hr. at 100·1°; the solvent contained 1·87 atom % of ^{18}O. Inorganic phosphate was then removed as already described. To the remaining solution enough barium chloride was added to convert the monomethyl phosphate into its barium salt. The mixture was set aside and then centrifuged. The supernatant liquid was heated to the b. p. and the precipitated methyl barium phosphate filtered off. The precipitate was washed several times with hot water and finally with acetone. Colorimetric analysis showed it to contain less than 1% of inorganic phosphate. It was then necessary to hydrolyse the isolated monomethyl phosphate under conditions in which no exchange of either organic or inorganic phosphate would occur. The following two procedures were used:

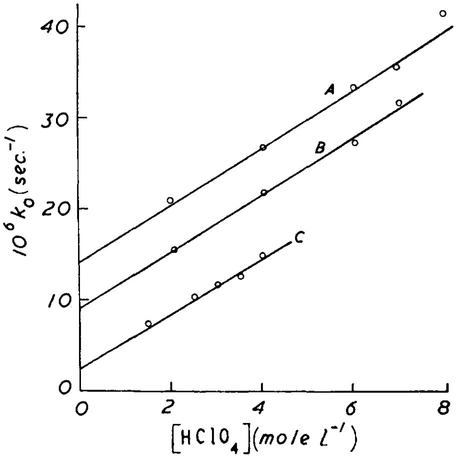
(*a*) The barium salt (1·9 g.) was dissolved in the minimum quantity of 2M-hydrochloric acid, and the barium was removed by quantitative precipitation with potassium sulphate solution. The remaining solution was mixed with three volumes of 4M-hydriodic acid and heated for 20 min. at 100°. Inorganic phosphate was then isolated and analysed as described before, giving N_P 0·075, 0·050% of ^{18}O. The procedure is satisfactory because iodide ion enormously speeds the hydrolysis of monomethyl phosphate,[16] but does not catalyse the exchange reaction of inorganic phosphate.[17]

FIG. 1. *Hydrolysis of monomethyl phosphate at 100·1°.*



A, Experimental. B, Calculated.

FIG. 2. *Acid-catalysed hydrolysis of monomethyl phosphate at constant ionic strength at* 100·1°.



A, μ = 8·0, gradient = 3·10 × 10^{-6}. *B*, μ = 7·0, gradient = 3·12 × 10^{-6}. *C*, μ = 4·0, gradient = 3·02 × 10^{-6}.

(*b*) The barium salt (1·8 g.) was converted into the potassium salt as described above. To the resulting solution were added 200 c.c. of acetate buffer (0·20M; pH 5·0) and 10 c.c. of a concentrated solution of human prostatic acid phosphatase. The mixture was kept for 20 hr. at 20°, after which the inorganic phosphate which had been formed was isolated and analysed, giving N_P 0·065 atom % of ^{18}O. The validity of this procedure depends on the fact that exchange between phosphate and the solvent does not occur under the specified conditions.[18]

One possible explanation for the isotopic enrichment observed in the substrate is that it arises from the reverse reaction, *i.e.*, from formation of monomethyl phosphate from the hydrolysis products. To test this possibility a solution containing inorganic phosphate and methanol (0·5M) in 5M-perchloric acid which had been heated at 100° for a prolonged period was analysed and found to contain more than 98% of the original inorganic phosphate. A simple calculation now eliminates the possibility. The equilibrium constant, $[MeH_2PO_4]/[MeOH][H_3PO_4]$ is, from the analytical figure, less than 0·04; hence the second-order rate coefficient for the

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[18] Bunton, Silver, and Vernon, *Proc. Chem. Soc.*, 1957, 348.

hypothetical reaction between phosphate and methanol would be less than $7{\cdot}3 \times 10^{-7}$ l. mole^{-1} sec.$^{-1}$. Under the conditions of the partial hydrolysis experiment this means that less than 2% of the monomethyl phosphate isolated could have been formed by recombination of products and this could not account for the observed enrichment.

DISCUSSION

(a) *Hydrolysis of the Monoanion.*—The variation of rate of hydrolysis with pH in the region pH 1—7 is, as other workers have pointed out,[5, 7-9] paralleled by the variation in concentration of the monoanion species. From the dissociation constants, simple calculation shows that, at pH 4·17, 99·9% of the monomethyl phosphate is present as the monoanion. The first-order rate coefficient (k_0) at any other pH is, therefore, if the monoanion is the only reactive species, given by:

$$k_0 = k_M C_M / C_P \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where k_M is the specific first-order rate coefficient, $8{\cdot}23 \times 10^{-6}$ sec.$^{-1}$, for the monoanion and C_M and C_P refer to the monoanion and stoicheiometric concentrations respectively. Since the two dissociation constants are relatively widely separated, the ratio, C_M/C_P, can be easily calculated. Fig. 1 shows a plot of values of k_0 (obtained in this way), against pH: in the region pH 1—7 the agreement between expected and observed rate is good. In media more acidic than pH 1, however, the observed rate is greater than that expected and the discrepancy becomes larger as the media become more acidic. The greater part of this discrepancy is clearly due to the emergence of an acid-catalysed reaction, *i.e.*, one involving one or more of the conjugate acids of monomethyl phosphate. Part of the effect, however, might be due to reaction proceeding *via* the neutral species, which below pH 1 is the bulk component of the solution, and we shall show below that such a reaction does in fact occur. From the results obtained in the pH range 1—7, it may, therefore, safely be concluded that (i) reaction proceeds mainly *via* the monoanion which is more reactive than either the dianion or the neutral species and that (ii) in more acidic media reactions involving species other than the monoanion become important.

The above analysis does not exclude the possibility that, as Kumamoto and Westheimer have pointed out,[9] the true factors are the neutral species and hydroxide ions, since such a reaction would have the same kinetic form as that involving the monoanion and a water molecule. In this case the specific rate coefficient for this hypothetical reaction would be

$$k_M \,.\, C_P/(C_{OH^-} \,.\, C_N) = ca.\ 10^7 \text{ l. mole}^{-1} \text{ sec.}^{-1}$$

where C_N and C_{OH^-} are the concentrations of neutral species and hydroxide ions respectively at pH 4·17. This value should be compared with the value $3{\cdot}3 \times 10^{-2}$ l. mole^{-1} sec.$^{-1}$ for the known second-order reaction between trimethyl phosphate and hydroxide ions [19] at 100°. Such a large rate difference would be inexplicable and the view that the reaction is between the neutral species and hydroxide ions is, therefore, to be rejected.

The isotope experiments (Table 3) show that hydrolysis of the monoanion proceeds with phosphorus–oxygen bond fission. The value of Q_P (percentage of phosphorus–oxygen bond fission) actually obtained (107%) shows that there was, in fact, rather more tracer in the inorganic phosphate product than could be accounted for by simple hydrolysis involving phosphorus–oxygen bond fission. (In calculating Q_P the exchange reaction of inorganic phosphate has already been allowed for.) The discrepancy, however, is probably just within the experimental uncertainties of the phosphate analysis and it is

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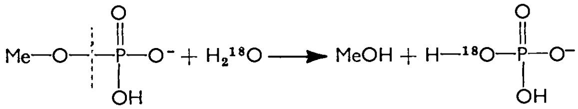
[19] Barnard, unpublished experiments.

not considered that it is indicative of a concomitant exchange involving monomethyl phosphate. The reaction may, therefore, be formulated:



$$\mathrm{Me{-}O{-}P({=}O)(OH){-}O^- + H_2{}^{18}O \longrightarrow MeOH + H{-}{}^{18}O{-}P({=}O)(OH){-}O^-}$$

Hydrolysis of monoanion species with phosphorus–oxygen bond fission has now been demonstrated in several cases, *viz.*, α-D-glucose-1 (following paper), phenyl,[20] *p*-tolyl,[20] *p*-nitrophenyl,[20] glycerol-1,[21] glycerol-2,[21] 2-methoxy-1-methylethyl,[8] and benzyl[9] phosphate monoesters. The reaction is probably a general one and it can be expected that it will be observed with any monosubstituted phosphate, in solutions of *ca.* pH 4, unless it is obscured by some especially ready reaction involving the neutral species and proceeding with carbon–oxygen bond fission.

It is difficult to specify the precise mechanism by which the monoanion of an orthophosphate monoester undergoes hydrolysis largely because, for the conditions under which reaction occurs, there is no appropriate experimental criterion of molecularity. Certain features of the mechanism can, however, be inferred from the available data, and and proposed mechanistic scheme must be consistent with these. First, the reaction is limited to those species containing an ionised and an un-ionised acidic group, *i.e.*, the groups OH and O^- must be present. This emerges from the following comparisons. The corresponding reaction for dimethyl phosphate, as will be shown in Part III, cannot have a first-order rate coefficient greater than $1{\cdot}6 \times 10^{-9}$ sec.$^{-1}$ (at 100°). Hence the rate factor between monomethyl and dimethyl phosphate for this reaction is at least 5000, and may, since the reaction has not actually been observed in the phosphate diester, be very much larger than this (cf. ref. 9). Similarly, the dianion $MePO_4{}^{2-}$, which like the monoanion $Me_2PO_4{}^-$, contains no hydroxyl group, is very resistant to hydrolysis. The neutral species MeH_2PO_4, as will be shown later, does undergo hydrolysis (k_{N} = *ca.* 5×10^{-7} sec.$^{-1}$ at 100·1°) but with carbon–oxygen fission. If we assume that a reaction involving phosphorus–oxygen bond fission and, therefore, analogous to the reaction of the monoanion, would not be experimentally distinguished if its rate coefficient were less than a tenth of the total for the neutral species, then an upper limit (*ca.* 5×10^{-8} sec.$^{-1}$) can be set for its rate coefficient. This means that the monoanion is at least a hundred times more reactive, and the true rate factor may be very much larger. Secondly, the summarised data[2] for a variety of compounds of the general formula, $RO{\cdot}PO(O^-)OH$, where R is alkyl, aryl, or pyranosyl, show that the rates of hydrolysis and the Arrhenius parameters are not very sensitive to the nature of R. Although there tends to be a correlation between rate of hydrolysis and stability of the anion RO^-, the spread in the values of the kinetic parameters is small. This might be taken as support for the view that the rate-determining step does not involve separation of the anion RO^-. Thirdly, as can be seen from Table 1, the reaction of monomethyl phosphate, and no doubt, by analogy, of other phosphate monoester monoanions, is not catalysed by general acids and is little influenced by large increases in ionic strength.

Three mechanisms are consistent with the observed facts, namely: (*a*) The negative charge on the O^- group is imagined as the "driving-force" of the reaction. At the same time hydrogen migration from the hydroxyl group to the ester-oxygen atom occurs (cf. refs. 8 and 9). The immediate products are methanol and the hypothetical metaphosphate ion. The latter has been postulated as an intermediate in some phosphorylations[22] and would presumably undergo rapid hydration to orthophosphoric acid.[23]

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[20] Silver, unpublished experiments.

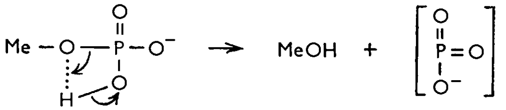
[21] Swoboda and Crook, *Biochem. J.*, 1955, **59**, xxiv.

[22] Todd, Arthur D. Little Lectures, Massachusetts Inst. Technology, 1954.

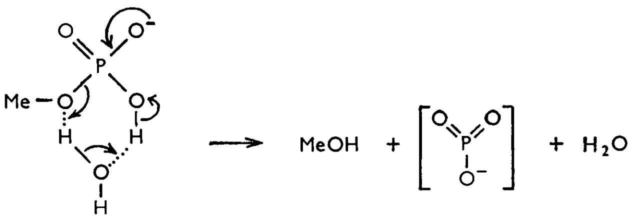
[23] Topley, *Quart. Rev.*, 1949, **3**, 345.

(*b*) A mechanism involving bimolecular attack on the phosphorus with simultaneous hydrogen migration to the ester-oxygen atom in the rate-determining step is also acceptable, and has been discussed elsewhere.[2] (*c*) The suggestion due to Westheimer and his colleagues [8,9] that the reaction involves a cyclic intermediate formed by hydrogen-bonding between the monoanion and a water molecule is attractive and has recently been used as a basis for a model of the reaction when catalysed by the enzyme, prostatic acid phosphatase.[18] Each of these mechanisms involves (i) the monoanion specifically, (ii) phosphorus–oxygen bond fission, and (iii) hydrogen-transfer (either from the hydroxyl group of the phosphate or from a water molecule to the ester-oxygen atom). Distinction between them must await further relevant information.

(*a*)



(*c*)



(b) *Hydrolysis of the Neutral Species.*—In solutions containing M-perchloric acid the predicted first-order rate coefficient for a reaction proceeding entirely through the monoanion is $0{\cdot}183 \times 10^{-6}$ sec.$^{-1}$ (from equation 1). The observed rate is $5{\cdot}08 \times 10^{-6}$ sec.$^{-1}$ and hence for solutions of stoicheiometric acidity greater than M, the contribution from hydrolysis of the monoanion is negligible. There remains, however, the possibility that in strongly acidic solutions hydrolysis proceeds partly through the neutral species which is, under these conditions, the bulk component. That this is the case can be seen from Fig. 2 in which experimentally obtained first-order rate coefficients (k_0) are plotted against stoicheiometric acidity (C_{H^+}) for a series of constant ionic strengths, μ = ($NaClO_4 + HClO_4$). For each ionic strength the plot of k_0 against C_{H^+} is a straight line, the slope and the intercept (at $C_{\mathrm{H}^+} = 0$) of which measure, for a particular ionic strength, the rate coefficients for the reactions of the conjugate acid and of the neutral species respectively. Since each line has, within the experimental scatter, the same slope, corresponding to a specific rate coefficient k_{A} of $3{\cdot}08 \times 10^{-6}$ l. mole^{-1} sec.$^{-1}$, the acid-catalysed reaction is, clearly, not subject to a large salt effect. The rate coefficient for the reaction of the neutral species, however, as measured by the intercepts in Fig. 2, increases as μ increases; this reaction, therefore, exhibits a large positive salt effect.

It has been suggested [24] that, for reactions in not very dilute solutions involving uncharged reagents, the dependence of rate coefficient on ionic strength has the form:

$$k_{\mathrm{N}} = k_{\mathrm{N}}^{0} \exp b\mu \qquad (2)$$

where k_{N} and k_{N}^0 are the specific rate coefficients at ionic strengths μ and zero respectively, and b is a constant. The hydrolysis of the neutral species obeys this equation since a straight line is obtained by plotting values of log k_{N} (calculated from the intercepts of Fig. 2) against μ. From the slope and intercept of the line b and k_{N}^0 are found to be

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[24] Long and McDevit, *Chem. Rev.*, 1952, **51**, 119.

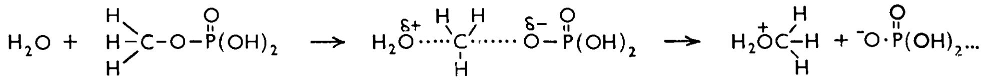
0·423 and 0·50 × 10^{-6} sec.$^{-1}$ respectively. Hence the first-order rate coefficient (k_N') for hydrolysis due to the neutral species under any particular condition is:

$$k_N' = \frac{C_N}{C_P} \cdot 0{\cdot}50 \times 10^{6} \exp 0{\cdot}423\mu \qquad . \quad . \quad . \quad . \quad . \quad (3)$$

where C_N/C_P is the proportion of monomethyl phosphate present as the neutral species.

The position of bond fission for the neutral species is not easy to determine since, in dilute solutions, there is no part of the pH–rate profile where its hydrolysis is predominant. For example, at pH 1, where the neutral species is the bulk component (*ca.* 80%), the rate coefficient for its hydrolysis is, as predicted by equation 3, *ca.* 0·40 × 10^{-6} sec.$^{-1}$, whereas the observed rate coefficient is 3·45 × 10^{-6} sec.$^{-1}$. In solutions containing 9M-sodium perchlorate, however, the rate coefficient for hydrolysis of the neutral species rises to *ca.* 1·3 × 10^{-5} sec.$^{-1}$, and under these conditions isotopic analysis should indicate, at least for the bulk of the reaction, the position of bond fission. Hydrolysis at *ca.* pH 1 in the presence of 9M-sodium perchlorate gave 84% of carbon–oxygen bond fission. Since, under these conditions, the reaction of the neutral species accounts for *ca.* 80% of the total rate and the remainder is mostly due to hydrolysis of the monoanion (phosphorus–oxygen bond fission) it may be concluded that hydrolysis of the neutral species proceeds predominantly with carbon–oxygen bond fission.

The molecularity of the reaction cannot be directly determined and two possible mechanisms must be considered. One involves the rate-determining formation of a methyl cation [S_N1(C)] and the other a bimolecular attack on carbon by a water molecule [S_N2(C)]. Since water is a relatively strong nucleophile towards a saturated carbon atom whereas the formation of a methyl cation is energetically difficult, the second mechanism is to be preferred and.can be formulated as in equation (4).



$$H_2O + H_3C{-}O{-}\overset{O}{\overset{\|}{P}}(OH)_2 \longrightarrow H_2\overset{\delta+}{O}\cdots CH_3 \cdots \overset{\delta-}{O}{-}\overset{O}{\overset{\|}{P}}(OH)_2 \longrightarrow H_2\overset{+}{O}CH_3 + {}^{-}O{\cdot}\overset{O}{\overset{\|}{P}}(OH)_2 \ldots(4)$$

(c) *Hydrolysis of the Conjugate Acid.**—It is possible, from equations (1) and (3), to calculate, for any particular acidity, the first-order rate coefficient for that proportion of the total reaction which proceeds *via* the monoanion and the neutral molecule. By subtraction from the experimentally observed value, the first-order rate coefficient (k_0') of the acid-catalysed hydrolysis (*i.e.*, that of the conjugate acid) can be found. Such calculations, made with results obtained from experiments in which no sodium perchlorate was added and given in Table 2, yield the values given in Table 4.

A plot of the first-order rate coefficients in Table 4 against acidity shows that, up to 7M-perchloric acid, k_0' increases in proportion with stoicheiometric acidity; in more acidic solutions, however, the rate of increase of k_0' is rather greater. The linear portion of the curve gives a value for the specific second-order rate coefficient for the acid-catalysed reaction (k_A) of 3·08 × 10^{-6} l. mole^{-1} sec.$^{-1}$, in close agreement with the value found from the experiments at constant ionic strengths (see section *b* above).

The experiments with ^{18}O showed that, in strongly acid solution, both carbon–oxygen and phosphorus–oxygen fission occur. Isotopic analysis of the methanol produced indicated that the proportion of the total reaction proceeding (in 5M-perchloric acid) by phosphorus–oxygen bond fission is 0·27. Similar analysis of the inorganic phosphate produced, however, gave a value, for the same quantity, of 0·44. This discrepancy is not due to experimental error and immediately suggests the occurrence of an exchange between monomethyl phosphate and solvent. This explanation was confirmed by showing that monomethyl phosphate, recovered from a partial hydrolysis, contained significant amounts of tracer. Whatever the mechanism of this exchange, the ester-oxygen cannot

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* By conjugate acid is meant the sum of the possible structures obtained by adding a proton to uncharged monomethyl phosphate. The possible structures are differentiated only for purposes of detailed mechanistic discussion.

become isotopically enriched, and consequently isotopic analysis of the methanol, which can only contain the ester-oxygen atom or one derived from the solvent, must provide a true measure of the proportion in which the two possible types of bond fission occur. Hence, it may be concluded that, in 5M-perchloric acid, 27% of the molecules undergoing hydrolysis do so by phosphorus–oxygen bond fission.

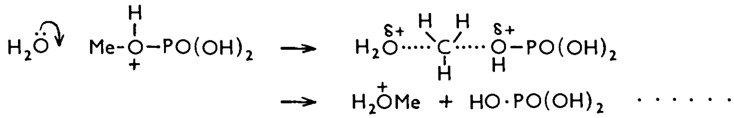
TABLE 4. *Rate coefficients for acid-catalysed hydrolysis of monomethyl phosphate after correction for hydrolysis of neutral molecule.*

$HClO_4$ (M)	$10^6k_0'$ (sec.$^{-1}$)	$10^6k_A = 10^6k_0'/C_{H^+}$ (l. mole^{-1} sec.$^{-1}$)	$HClO_4$ (M)	$10^6k_0'$ (sec.$^{-1}$)	$10^6k_A = 10^6k_0'/C_{H^+}$ (l. mole^{-1} sec.$^{-1}$)
1·0	4·12	4·12	6·0	17·7	2·93
2·0	6·48	3·23	7·0	21·7	3·10
3·0	9·42	3·13	8·0	26·4	3·30
4·0	12·2	3·05	9·0	31·8	3·53
5·0	14·3	2·85	10·0	43·3	4·33

Tables 2 and 4 shows that, whereas, in 5M-perchloric acid at 100·1°, the observed first-order rate coefficient is $18·5 \times 10^{-6}$ sec.$^{-1}$, that due to the acid-catalysed reaction is $14·3 \times 10^{-6}$ sec.$^{-1}$; the remainder of the reaction is due, as was shown in section (*b*), to hydrolysis of the neutral molecule. The latter process involves carbon–oxygen bond fission and hence the true proportion of phosphorus–oxygen bond fission for the acid-catalysed reaction must be greater than 0·27: simple calculation gives 0·35. The two processes which make up the acid-catalysed reaction, therefore, have specific second-order rate coefficients of $k_A(C) = 0·65k_A$ and $k_A(P) = 0·35k_A$ where the symbols in parentheses indicate the positions of bond fission.

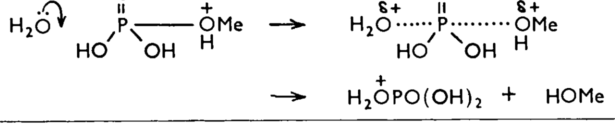
The formation of the conjugate acid involved in the acid-catalysed reaction is not rate-determining since (i) proton-transfers, in strongly acid solution, to an oxygen atom have invariably been found to be rapid and (ii) change of solvent to 67·6% deuterium oxide is associated with a small increase in reaction rate (see Table 2).* The rate-determining steps of the acid-catalysed reactions therefore involve equilibrium concentrations of conjugate acid, and, since proportionality between rate and stoicheiometric acidity is observed, both reactions should, on the Zucker–Hammett hypothesis, be bimolecular and involve a water molecule in their rate-determining steps.

The reaction which proceeds by carbon–oxygen bond fission may be formulated in conventional manner as a nucleophilic attack by a water molecule on the carbon atom of a monomethyl phosphate molecule containing a protonated ester-oxygen atom, *viz.*:



(5)

The reaction involving phosphorus–oxygen bond fission may be similarly formulated as a nucleophilic attack on the phosphorus atom, *viz.*:



(6)

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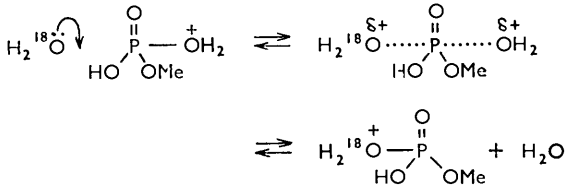
* It should be noted that the increase in rate would undoubtedly have been greater had the experiment been performed in 100% deuterium oxide since Wiberg [25] has shown that the effect of deuterium oxide on acid-catalysed rates increases more rapidly than does the mole fraction in the region of 100% deuterium oxide.

In the present case some 15% of the reaction involved bimolecular attack on the neutral species (*i.e.*, was not acid-catalysed) and since this reaction may well be slower in deuterium oxide,[26] the true increase for the acid-catalysed reaction may have been partially masked.

[25] Wiberg, *Chem. Rev.*, 1955, **55**, 713.

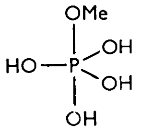
[26] Oldham, Ph.D. Thesis, London, 1957.

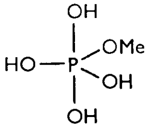
The exchange reaction, the molecularity of which has not been determined but which, by analogy, may be assumed to be bimolecular, is most simply formulated as involving an alternative conjugate acid (*i.e.*, one in which one of the hydroxyl groups is protonated), *viz.*:


$\cdots\cdots\cdots\cdots(7)$

The reactions (6) and (7) then involve displacement from the phosphorus atom of whichever attached group is protonated and their relative rates would depend partly on the relative concentrations of the two conjugate-acid forms.

Since phosphorus can use *d*-orbitals for covalent binding, nucleophilic displacement on phosphorus need not involve synchronous bond-making and bond-breaking. In alternative formulations we would suppose that the reactions proceed *via* a common intermediate containing five groups covalently bound to the phosphorus atom. Several structures, of which (I) and (II) are two, or their protonated forms may be written for such an intermediate. Hydrolysis or exchange will result if the intermediate, after protonation, loses respectively MeOH or H_2O. At the moment, however, no experimental test is available to distinguish between these possibilities.


(I)


(II)

(d) *General.*—Four hydrolytic mechanisms have been identified for monomethyl phosphate and their characteristics are summarised in Table 5.

TABLE 5. *Summary of reaction mechanisms for hydrolysis of monomethyl phosphate.*

Form	10^6 × Specific rate coefficient (100·1°)	Bond fission	Molecularity	Salt effect
(A) Monoanion	8·23 [a]	P–O	?	Nil
(B) Neutral species	0·50 [a, c]	C–O	2	Large +
(C) Conjugate acid	2·00 [b]	C–O	2	Nil
(D) Conjugate acid	1·08 [b]	P–O	2	Nil

[a] Sec.$^{-1}$. [b] l. mole^{-1} sec.$^{-1}$. [c] At $\mu = 0$.

The rate coefficient k_0 (sec.$^{-1}$) for hydrolysis at any acidity within the range pH 7·5 to 7M-perchloric acid is given by equation (8):

$$10^6 k_0 = \frac{C_M}{C_P}\cdot 8{\cdot}23 + \frac{C_N}{C_P}\cdot 0{\cdot}50\,.\,\exp\,(0{\cdot}423\mu) + \frac{C_N}{C_P}\cdot 3{\cdot}08C_{H^+} \quad . \quad . \quad . \quad (8)$$

and the percentage phosphorus–oxygen bond fission (Q_P) by:

$$Q_P = \frac{8{\cdot}23C_M + 1{\cdot}08C_{H^+}C_N}{8{\cdot}23C_M + 0{\cdot}50C_N \exp\,(0{\cdot}423\mu) + 308C_{H^+}C_N}$$

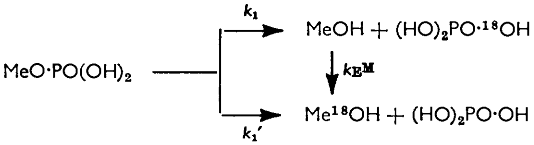
The fit of equation (8) is very good except in the region pH 1—0 where the uncertainties associated with the dissociation constants become relatively more important.

The behaviour of monomethyl phosphate may be taken as typical of that of primary monoalkyl phosphates. For groups other than methyl, reactions (B) and (C) will be relatively suppressed, especially if steric factors become important; the general character of the rate profile will, however, remain the same. For compounds containing a tertiary alkyl group unimolecular reactions of the neutral species and conjugate acid involving carbon–oxygen bond fission will become relatively important and the minimum in the pH–rate profile at about pH 0 may disappear [cf. glucose 1-phosphate (following paper)].

It should be stressed that the analysis presented in this paper applies only to solutions not containing strong nucleophiles such as halide ions. For example, monomethyl phosphate is known to be much more rapidly hydrolysed by halogen acids than by equivalent concentrations of perchloric or sulphuric acid. This, as we shall show in a later paper, is due to incursion of bimolecular reactions involving halide ions and the neutral or conjugate acid species.

APPENDIX

(a) *Calculation of* Q_C.—Let k_1 be the first-order rate coefficient for hydrolysis of monomethyl phosphate with phosphorus–oxygen bond fission,



k_1' the first-order rate coefficient for its hydrolysis with carbon–oxygen bond fission,

k_E^M the first-order rate coefficient for the isotopic equilibrium between the oxygen atoms of water and methanol,*

a_0 the initial concentration of monomethyl phosphate,

b the concentration of isotopically normal methanol at time t,

c the concentration of methanol having the same excess abundance of ^{18}O as the solvent at time t,

N_S the excess abundance of ^{18}O in the solvent,

N_M the excess abundance of ^{18}O in the methanol isolated from the reaction mixture, *i.e.*, $N_M/N_S = c/(b + c)$.

The total rate of hydrolysis, k_0, is:

$$k_0 = k_1 + k_1' \qquad (1)$$

and

$$Q_C = k_1'/k_0 \qquad (2)$$

and $(b + c)/a_0 = R$, where R is the fraction of monomethyl phosphate hydrolysed at time t.

Therefore,

$$c/a_0 = N_M R/N_S \qquad (3)$$

Now

$$dc/dt = k_1'[a_0 - (b + c)] + k_E^M b \qquad (4)$$

and, since the total reaction is of the first-order,

$$a_0 - (b + c) = a_0 \exp - k_0 t \qquad (5)$$

Eliminating b from (4) and (5) we have:

$$dc/dt + k_E^M c = k_E^M a_0 + a_0(k_1' - k_E^M) \exp - k_0 t \qquad (6)$$

Integration after multiplication by $\exp k_0 t$ gives:

$$c \exp k_0 t = a_0 \exp k_0 t - a_0 \frac{(k_1' - k_E^M)}{(k_0 - k_E^M)} \exp [- (k_0 - k_E^M)t] + I$$

where I, since when $t = 0$, $c = 0$, is found as:

$$I = a_0 k/(k_0 - k_E^M)$$

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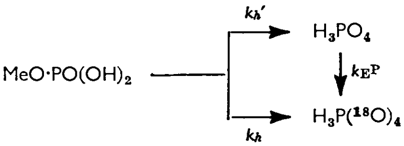
* In this particular case, because only one atom is exchanged and because the solvent is in large excess, k_E^M is also the first-order rate coefficient for the oxygen exchange irrespective of labelling.

Rearrangement after elimination of k_1 and k_1' by (1) and (2) gives:

$$\frac{c}{a_0} = \frac{N_M}{N_S} \cdot R = 1 - \frac{(Q_C k_0 - k_E{}^M) \exp - k_0 t + (1 - Q_C) k_0 \exp - k_E{}^M}{k_0 - k_E{}^M}$$

whence $$Q_C = \frac{(N_M R/N_S - 1)(k_0 - k_E{}^M) - (k_E{}^M \exp - k_0 t) + (k_0 \exp - k_E{}^M t)}{k_0[(\exp - k_E{}^M t) - (\exp - k_0 t)]} \quad . \quad (7)$$

(b) *Calculation of* Q_P.—The derivation of the expression for Q_P is a little more complicated because, whereas hydrolysis with phosphorus–oxygen bond fission leads to inorganic phosphate with only one oxygen atom enriched isotopically, the oxygen exchange of inorganic phosphate leads to isotopic enrichment of all four atoms. It is mathematically simpler to set up the equations in terms of the hypothetical process:



in which hydrolysis of monomethyl phosphate produces either isotopically normal inorganic phosphate (first-order rate coefficient k_h') or inorganic phosphate with all four oxygen atoms having the same excess abundance of ^{18}O as the solvent. The first-order rate coefficient for the isotopic equilibration of inorganic phosphate is $k_E{}^P$.* The coefficients k_h' and k_h are related to those previously defined (on the assumption of random distribution of tracer in the phosphate) by:

$$k_h = k_1/4 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

and $$(k_h' - 3k_h) = k_1' \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

Now $$Q_P = 4k_h/(k_h + k_h') = 4k_h/k_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

If b' and c' are the concentrations, at time t, of isotopically normal and isotopically equilibrated inorganic phosphate then,

$$dc'/dt = k_h[a_0 - (b' + c')] + b'k_E{}^P \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

and $$a_0 - (b' + c') = a_0 \exp - k_0 t \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

By eliminating b' between (11) and (12) a differential equation is obtained which can be integrated as for (6). Substitution by using (8), (9), and (10) then gives:

$$Q_P = \frac{4[(N_P \mathrm{R}/N_S - 1)(k_0 - k_E{}^P) - (k_E{}^P \exp - k_0 t) + (k_0 \exp - k_E{}^P t)]}{k_0[(\exp - k_E{}^P t) - (\exp - k_0 t)]} \quad . \quad (13)$$

When $k_E{}^M$ and $k_E{}^P$ are zero, equations (7) and (13) reduce to $Q_C = N_M/N_S$ and $Q_P = 4N_P/N_S$ respectively.

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Auckland University College, New Zealand.]

[*Received, March 24th,* 1958.]

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* In this particular case $k_E{}^P$ is one-quarter of the rate coefficient for the oxygen exchange irrespective of labelling.