236. *Studies on Phosphorylation. Part XIX.* The* P¹P¹-*Diethyl-Pyrophosphate Ion.*

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The hydrolysis of tetraethyl pyrophosphate (TEPP) (I) has been studied kinetically and the catalytic effects of several added bases including the monohydrogen phosphate anion have been investigated. Evidence is presented that P^1P^1-diethyl pyrophosphate ion (II) is formed as an intermediate with the anion named, but is extremely unstable. It can act as a phosphorylating agent towards alcohols, and, when formed in concentrated aqueous or anhydrous solution, yields polymetaphosphate.

A considerable number of syntheses of organic pyrophosphates and triphosphates has been recorded from this and other laboratories and much general information is available on the dependence of stability on the degree of substitution and on the nature of the substituents.[1,2] Yet from the comparative viewpoint quantitative evidence is scanty. Precise knowledge of the hydrolytic chemistry of these compounds is at present only available for a few mono-[3] and tetra-esters.[4-7] The present study arose out of an interest in the possible reactions of P^1P^1-dialkyl pyrophosphates. Avison[8] has indicated that the dimethyl compound is formed in the reaction between tetramethyl pyrophosphate and orthophosphate but that it is very unstable. We find evidence, detailed below, for the

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* Part XVIII, *J.*, 1958, 3039.

[1] Corby, Kenner, and Todd, *J.*, 1952, 1234; Christie, Elmore, Kenner, Todd, and Weymouth, *J.*, 1953, 2947.

[2] Todd, *Proc. Nat. Acad. Sci. U.S.A.*, 1959, 1389.

[3] Holbrook and Ouellet, *Canad. J. Chem.*, 1957, **35**, 1496; Liebécq and Jacquemotte-Louis, *Bull. Soc. Chim. biol.*, 1958, **40**, 67, 759.

[4] Toy, *J. Amer. Chem. Soc.*. 1948, **70**, 3882.

[5] Jacobson and Hall, *Ind. Eng. Chem.*, 1948, **40**, 694.

[6] Ketelaar and Bloksman, *Rec. Trav. chim.*, 1948, **67**, 665.

[7] Cherbuliez, Lieber, and Stucki, *Helv. Chim. Acta*, 1953, **36**, 537.

[8] Avison, *J.*, 1955, 732.

formation of the corresponding diethyl pyrophosphate in the reaction between tetraethyl pyrophosphate (TEPP) and orthophosphate.

The hydrolysis in aqueous solution of several tetra-alkyl pyrophosphates has been studied.[4–7] While acid was shown to have little effect on the rate of hydrolysis of the tetraethyl ester, the rate in dilute sodium hydroxide was so fast as to be immeasurable with accuracy.[6] The effect of pH on the rate of hydrolysis of tetraethyl pyrophosphate in water at 57·0° is shown in Table 1. Good first-order plots were obtained in all cases,

TABLE 1. *Effect of* pH.

pH	10^4k (sec.$^{-1}$)		pH	10^4k (sec.$^{-1}$)
4·80	1·9	k_0	8·80	3·3
5·80	1·9		9·10	4·6
6·80	1·9		9·40	8·0
7·80	1·9			
8·30	2·3		9·80	16

though with a small deviation towards the end of the run at pH 9·80 almost certainly due to atmospheric carbon dioxide. Experimental rather than theoretical infinity values were used although the discrepancy between these was only of the order of 2%. Chromatography showed that the sole product of the hydrolysis was diethyl phosphate and this was assumed when calculating theoretical infinity values.

When k_0 is taken as the rate constant for hydrolysis by neutral water and K_W at 57° as $8{\cdot}3 \times 10^{-14}$,[8a] a plot of $\log_{10}(k - k_0)$ against log $[OH^-]$ gives the linear expression:

$$\log_{10}(k - k_0) = 1{\cdot}02 \log_{10}[OH^-] + 0{\cdot}52$$

whence

$$k_{OH^-} = 3{\cdot}3 \text{ l. mol.}^{-1} \text{ sec.}^{-1}$$

Thus the overall reaction between tetraethyl pyrophosphate and hydroxide ion is probably of the second order and proceeds by an S_N2 displacement on phosphorus followed by loss of a proton. This would be consistent with the mechanism of reaction of hydroxide ion and some other bases with phosphorohalidates.[9]

When alkali phosphates were added a definite catalytic effect on the hydrolysis was noted. The effect of phosphate at 57·0° is illustrated in Table 2.

TABLE 2. *Effect of phosphate at* 57·0°.

Salt	pH	10^4k (sec.$^{-1}$)	Salt	pH	10^4k (sec.$^{-1}$)
0	8·80	3·3	0·100M-Na_2HPO_4	8·80	12·1
0·025M-Na_2HPO_4	8·80	5·3	0·050M-Na_2HPO_4	9·30	10·7
0·050M-Na_2HPO_4	8·80	7·8	0·050M-NaH_2PO_4	4·80	2·3
0·075M-Na_2HPO_4	8·80	9·9	0·100M-Na_2SO_4	8·80	3·3

Good first-order plots were again obtained in all cases and the sole phosphorus-containing products were diethyl phosphate and orthophosphate. This was shown by chromatography and by the fact that the experimental and theoretical alkali uptakes were in good agreement. The results are shown in the Figure and indicate that the increase in rate is proportional to the concentration of disodium hydrogen phosphate since at pH 8·80 about 95% of the total secondary phosphoryl dissociation will be ionised (pK_a of $H_2PO_4^-$ being 7·2 at 57·0° [10]).

The following argument shows that the catalysis at pH 8·8 must be due to the HPO_4^{2-} ion. From Table 2 it is evident that the $H_2PO_4^-$ ion has little effect. Moreover, the value of k obtained at pH 9·30 with added disodium hydrogen phosphate may be calculated from the aqueous rate at pH 9·30 (obtained by interpolation) together with the increase due to

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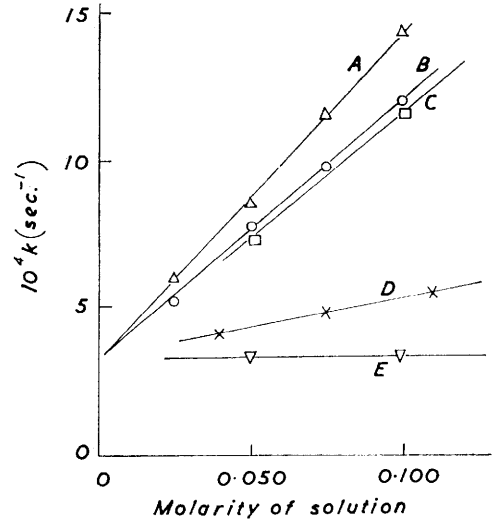
[8a] Harned and Hamer, *J. Amer. Chem. Soc.*, 1933, **55**, 2294.

[9] Dostrovsky and Halman, *J.*, 1953, 508; Hudson and Harper, *J.*, 1958, 1356; Heath, *J.*, 1956, 3796, 3804.

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

phosphate catalysis at pH 8·80 ($10^4k = 10{\cdot}9$ calc.; 10·7 obs.). Since at the higher pH $[HPO_4^{2-}]$ has changed very little whereas $[PO_4^{3-}]$ has increased by a factor of approximately 3 [pK_a of HPO_4^{2-} ~12·5 (ref. 11)] it is clear that in this pH range the increased rate of hydrolysis is due essentially to the HPO_4^{2-} ion.

It seems reasonable to assume that the first step in the catalysed hydrolysis involved a nucleophilic displacement on phosphorus by the HPO_4^{2-} ion, followed by hydrolysis of the intermediate (II) produced. Further evidence recorded below strongly supports this view.



Effect of added salts on the hydrolysis of tetraethyl pyrophosphate at 57·0° *and* pH 8·80.
A, Na_2SO_3. *B*, Na_2HPO_4. *C, Pyridine. D* Na_2MePO_4. *E*, 2,6-*Lutidine.*

$$\begin{tikzcd}
\text{(I)}\ (EtO)_2PO{\cdot}O{\cdot}PO(OEt)_2 \arrow[r, "k_0", "H_2O"'] \arrow[d, "k_1"', "HPO_4^{2-}"] & 2(EtO)_2PO_2^- + 2H^+ & \\
\text{(II)}\ (EtO)_2PO{\cdot}O{\cdot}PO_3^{2-} \arrow[r, "H_2O", "k_2"'] \arrow[dr, "k_3"] & (EtO)_2PO_2^- + HPO_4^{2-} + H^+ \quad \ldots \quad (A) & \\
+ (EtO)_2PO_2^- + H^+ & (EtO)_2PO_2^- + PO_3^- \arrow[r, "k_4", "H_2O"'] & HPO_4^{2-} + H^+ \quad . \quad (B)
\end{tikzcd}$$

If we consider pathway (*A*) in the annexed reaction scheme, the rate equations are as follows (the concentration of the intermediate being denoted as [II]):

$$d[H^+]/dt = 2k_0[TEPP] + k_1[TEPP][HPO_4^{2-}] + k_2[II] \quad . \quad . \quad (1)$$

$$d[II]/dt = k_1[TEPP][HPO_4^{2-}] - k_2[II] \quad . \quad . \quad . \quad . \quad . \quad (2)$$

$$d[HPO_4^{2-}]/dt = -k_1[TEPP][HPO_4^{2-}] + k_2[II] \quad . \quad . \quad . \quad . \quad (3)$$

If $k_2 \gg k_1$, we may apply the stationary-state approximation, giving $d[II]/dt = 0$, and from (3) we then have $d[HPO_4^{2-}]/dt = 0$, *i.e.*, $[HPO_4^{2-}] = C^0$, and, assuming that [II] is extremely small at any time, we obtain:

$$d[TEPP]/dt = -(k_0 + k_1C^0)[TEPP]$$

i.e., an overall first-order reaction, whose rate constant is a linear function of the initial concentration of HPO_4^{2-} ion, C^0, as is found in practice (see Figure). Pathway (*B*) gives similar results if both k_3 and $k_4 \gg k_1$.

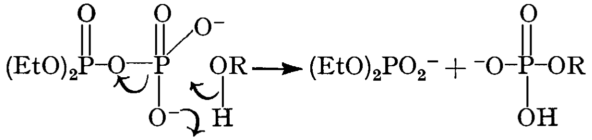
The kinetic evidence strongly favours the formation of an intermediate P^1P^1-diethyl pyrophosphate (II). Additional grounds for believing that an intermediate is formed come from the effect of sulphite, pyridine, and 2,6-lutidine on the hydrolysis of tetraethyl pyrophosphate. In each case, the reaction followed strictly first-order kinetics with negligible discrepancy between the theoretical and observed infinity alkali titres; diethyl phosphate was the sole phosphorus-containing product. It is seen (see Figure) that sulphite (pK_a of $HSO_3^- \sim 7{\cdot}2$ [11]) has an effect very similar to that of the HPO_4^{2-} ion. An intermediate of the type (III), if formed, would be expected to break down with extreme rapidity, as it has been shown [12] that in the alkaline hydrolysis of cyclic sulphites, intermediates of the type (IV; R = Alkyl) which must presumably be formed are too unstable

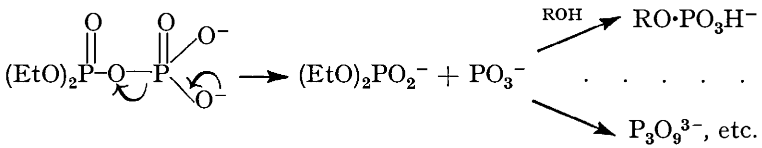
(III) $(EtO)_2PO{\cdot}O{\cdot}SO_2^-$ $RO{\cdot}SO_2^-$ (IV)

to be detected. Replacement of R by a strongly electron-attracting group such as diethyl phosphoryl, as in (III), should lead to even greater instability. Pyridine has an effect comparable to that of the HPO_4^{2-} and HSO_3^- ions, but lutidine has virtually none (see Figure). Since the sterically hindered lutidine is the stronger base (pK_a for the lutidinium ion is 6·9 [13] compared with 5·4 for the pyridinium ion [14]), one may reasonably conclude that the reaction involves a nucleophilic displacement by the base on phosphorus. These results are comparable with those obtained by Westheimer and his co-workers in their study of the solvolysis of tetrabenzyl pyrophosphate in presence of tertiary bases.[15]

However, decisive evidence that the ion (II) is an intermediate in the phosphate-catalysed hydrolysis of tetraethyl pyrophosphate comes from the fact that the phosphate is activated so as to phosphorylate added alcohols. When the hydrolysis was conducted in a saturated solution of disodium hydrogen phosphate in 50% methanol or 75% ethylene glycol, paper chromatography showed the presence of methyl dihydrogen phosphate and 2-hydroxyethyl dihydrogen phosphate respectively. The yields were low, presumably owing to the presence of water and the low concentration of phosphate. Alteration of the system to include salts of orthophosphate soluble in anhydrous solvents will be necessary if the method is to have practical value, but experiments along these lines have not been encouraging.

We next consider the question of whether the reactions of the ion (II) are to be ascribed to S_N2 displacements generating the stable $(EtO)_2PO_2^-$ anion, as in equation (4), or to unimolecular breakdown to metaphosphate anion (V) followed by further reaction


(4)


(5)

of the latter (5). The kinetic experiments provide no means of differentiation. Reactions with neutral molecules, *e.g.*, alcohols, could be accommodated on either mechanism. We nevertheless favour mechanism (5) in which monomeric metaphosphate ion (V) is generated,

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[11] Moeller, " Inorganic Chemistry," Chapman and Hall, London, 1952, p. 314.

[12] Bunton, de la Mare, Lennard, Llewellyn, Pearson, Pritchard, and Tillett, *J.*, 1958, 4761.

[13] Golumbic and Orchin, *J. Amer. Chem. Soc.*, 1950, **72**, 4145.

[14] Barron, *J. Biol. Chem.*, 1937, **12**, 313.

[15] Westheimer, *Chem. Soc. Special Publ.*, No. 8, 1957, p. 1.

on the following grounds. In the first place the remarkable instability of the ion (II) suggests that a special mechanism is involved. There seems no good reason for supposing the doubly charged P^2-phosphoryl group to be a reactive electrophilic centre, although there is the possibility that it behaves as an internal base during reaction. Secondly, when tetraethyl pyrophosphate was heated at 100° with a *concentrated* solution of disodium hydrogen phosphate considerable quantities of polymetaphosphate were produced. Moreover, when the ester was added to a solution of *NNN*-trimethylanilinium dihydrogen phosphate in anhydrous formamide, with an excess of 2,6-lutidine to maintain a reasonable concentration of HPO_4^{2-} ion, conversion of the phosphate into trimetaphosphate was almost complete. Significantly, only traces of pyrophosphate were formed.

That the problem is not necessarily simple can be seen when other reactions are considered, in which metaphosphate elimination has been invoked.[2] The phenyl hydrogen phosphate anion is comparatively unstable in aqueous solution,[16] a property it shares with many other monoaryl and monoalkyl hydrogen phosphates,[17] and the suggestion has been made, *inter alia,* that hydrolysis involves the formation and then rapid hydration of metaphosphate.[16,18] We have noticed, apparently for the first time, that when potassium phenyl hydrogen phosphate was heated in concentrated aqueous solution trimetaphosphate was formed and, again, pyrophosphate appeared to be absent. The solid salt was quite stable at 100°, as was a solution of dicyclohexylammonium phenyl hydrogen phosphate at 101° in dioxan, either anhydrous or containing one or two molar proportions of water. The observations, although crude, provide some support for the view that the decomposition of phenyl hydrogen phosphate involves elimination of metaphosphate, but not by a simple unimolecular reaction since solvent molecules are almost certainly involved either directly or, perhaps, by affecting the dielectric properties of the medium. Metaphosphate has also been discussed as a reaction intermediate in the breakdown of phosphoramidic acids to account for formation of pyrophosphates and polymetaphosphates.[19-21]

Incidental to the above experiments the effect of methyl phosphate on the rate of hydrolysis of tetraethyl pyrophosphate was investigated (Table 3). It caused a marked increase in rate, and first-order rate constants were calculated for the earlier part of the hydrolysis (5—60%) and are shown in the Figure. The logarithmic plot slowly deviated from linearity after about 60% hydrolysis. There was, too, a small but significant discrepancy (5—8%) between the theoretical and the observed infinity values for alkali uptake, indicating that a stable pyrophosphate was still present at the completion of the run.

TABLE 3.

[$MePO_4^{2-}$] 0·110M. pH 8·80

TEPP hydrolysed (%)	0	25	50	75
First-order rate const. ($\times 10^4$ sec.$^{-1}$)	5·25	5·18	4·80	4·05

Chromatography showed the presence of traces of a substance, slower-running than diethyl phosphate, which was identified as P^1P^2-dimethyl dihydrogen pyrophosphate by comparison with an authentic sample prepared by rearrangement of methyl hydrogen *N*-cyclohexylphosphoramidate.[22] The detection of P^1P^1-diethyl P^2-methyl hydrogen pyrophosphate as an end-product was prevented by the presence of very large amounts of diethyl phosphate. Although a sample of this triester was not available, the closely related trimethyl hydrogen pyrophosphate was prepared and found to be comparatively stable in water at pH 8·80 ($t_{\frac{1}{2}} \sim 30$ hr.). The dimethyl dihydrogen pyrophosphate must

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[16] Bunton, Llewellyn, Oldham, and Vernon, *J.*, 1958, 3574.

[17] Desjobert, *Bull. Soc. chim. France*, 1947, **14**, 809; Thesis, Sorbonne, 1951.

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

[19] Clark and Todd, *J.*, 1950, 2031.

[20] Stokes, *Amer. Chem. J.*, 1893, **15**, 198; Goehring and Sambeth, *Chem. Ber.*, 1957, **90**, 232.

[21] Cf. also Samuel and Westheimer, *Chem. and Ind.*, 1959, 51.

[22] Clark, Kirby, and Todd, *J.*, 1957, 1497.

have been formed by exchange reactions, presumably through diethyl methyl hydrogen pyrophosphate, but the amount of reaction proceeding this way was small, as shown by the titration result, and so leaves at least the major part of the increase in hydrolysis rate unaccounted for. Evidently the rate of attack of methyl phosphate (pK_a of $MeHPO_4^-$ is 6·7 at 57°)[16] on tetraethyl pyrophosphate is much lower than that of orthophosphate, indicating that it is a considerably weaker nucleophilic agent.

EXPERIMENTAL

Paper chromatograms were run on Whatman No. 1 paper with propan-2-ol–ammonia–water (7 : 1 : 2).

Materials.—Tetraethyl pyrophosphate was commercial, " pure " material (n_D 1·4181). In some experiments material (n_D 1·4184), prepared by Toy's method,[4] was used. In each case titrimetric assay with alkali indicated a purity of 95—98%. There was no fast-hydrolysing impurity as in material prepared from phosphoric oxide and triethyl phosphate.[6]

Methyl phosphate was prepared from methanol and phosphoryl chloride and isolated as the crystalline barium salt. This was converted into the sodium salt by treatment with the calculated amount of sodium sulphate. The recrystallised salt gave one spot on chromatograms (R_F 0·15) and showed no reaction for chloride or phosphate ions and only a very faint positive test for sulphate (Found: equiv., 265. Calc. for $CH_3Na_2PO_4,6H_2O$: equiv., 264).

Disodium phosphate and sodium sulphite were of " AnalaR " quality.

Sodium Trimethyl Pyrophosphate.—Tetramethyl pyrophosphate was prepared by Toy's method[4] and had b. p. 108—110°/0·3 mm., n_D 1·4124. Alkali assay gave a purity of 85%.

To anhydrous sodium iodide (0·5 g.) in dry acetone (5 ml.) was added tetramethyl pyrophosphate (0·7 ml., 1·1 mol.), and the mixture was set aside at room temperature for 8 hr. The crystalline *product* (0·72 g.) separated and was collected and washed well with acetone. It had R_F 0·65 and gave no test for iodide (Found, in material dried at 0·2 mm./12 hr. at room temperature: C, 15·2; H, 4·1; P, 14·4. $C_3H_9O_7P_2Na$ requires C, 14·9; H, 3·8; P, 14·7%). The crystalline salt, on storage for 8 months, absorbed water. A solution of the material was strongly acid and paper chromatography showed that almost complete conversion into dimethyl phosphate and P^1P^2-dimethyl dihydrogen pyrophosphate had occurred.

Methyl Hydrogen N-*Cyclohexylphosphoramidate.*—Benzyl hydrogen *N*-cyclohexylphosphoramidate (2·0 g.) was dissolved in dioxan (20 ml.) and treated at 0° with excess of ethereal diazomethane. After 15 min. ether and diazomethane were removed *in vacuo* and the dioxan solution of benzyl methyl *N*-cyclohexylphosphoramidate was hydrogenated over 10% palladised charcoal. Uptake of hydrogen was rapid. The solvent was removed under reduced pressure and the residual oil taken up in ether. From this solution at 0° the *product* separated as fine needles, m. p. 90—91° (Found: C, 43·3; H, 8·7; N, 7·3%; equiv., 193. $C_7H_{16}O_3NP$ requires C, 43·3; H, 8·3; N, 7·2%; equiv., 191). In air the crystals collapsed in one month to a viscous mass consisting almost entirely of dicyclohexylammonium dimethyl pyrophosphate.

Dicyclohexylammonium Dimethyl Pyrophosphate.—After the hydrogenation step described above, the dioxan solution was heated at 100° for 4 hr. and then cooled. The *product* separated overnight in plates (0·62 g.), m. p. 205—206°, R_F 0·45 (Found: C, 41·1; H, 8·5; N, 6·8. $C_{14}H_{34}O_7N_2P_2$ requires C, 41·4; H, 8·5; N, 6·9%).

The substance was also prepared by heating tetramethyl pyrophosphate in dry acetone with sodium iodide (2 mol.), but then always contained traces of dimethyl phosphate due presumably to trimethyl phosphate present as an impurity in the pyrophosphate.

Hydrolyses of Tetraethyl Pyrophosphate.—These were carried out in a vessel fitted with a mechanical stirrer, containing 100 ml. of water or the appropriate aqueous salt solution maintained thermostatically at 57·0°. During the faster reactions the temperature tended to rise during the early part of the hydrolysis; the greatest rise (observed in the fastest reaction) amounted to 0·3°. Tetraethyl pyrophosphate (0·5 ml.) was added and then carbon dioxide-free N-sodium hydroxide was run in, an automatic titrator being used to maintain the pH at the required value. Volume changes were neglected since the final titre was *ca.* 4 ml. and evaporation losses were 1—3 ml. depending on the length of the run.

Reactions were, in general, followed over the range 0—80% hydrolysis, but values could not be obtained accurately in the first minute. For the slower reactions infinity values were obtained by finally heating the reaction solution at 85° for 30 min. and did not differ significantly from those measured at 57·0°. In all cases the experimentally determined infinity value was used rather than the theoretical value since in some cases an appreciable discrepancy was noticed. The random error in the calculated rate constants was less than ±5%.

Reaction of Tetraethyl Pyrophosphate with Orthophosphate in Formamide.—*NNN*-Trimethylanilinium dihydrogen phosphate (0·5 g.) was made by passing the anilinium iodide solution through a Dowex-2 column in the dihydrogen phosphate form and evaporating the effluent to dryness, giving white crystals. These were dissolved in dry formamide (15 ml.), and 2,6-lutidine (4 ml.) was added to maintain the concentration of HPO_4^{2-}. Tetraethyl pyrophosphate (0·7 ml., 1·5 mol.) was added and the solution heated at 50° for 12 hr. Chromatography in propan-2-ol–trichloroacetic acid–water–ammonia (Ebel [23]) showed, by comparison with authentic specimens, that diethyl phosphate and trimetaphosphate alone were present, the latter being confirmed by the ability of the solution to coagulate egg albumin in dilute acetic acid. The solution gave a slight precipitate with cadmium chloride in dilute acetic acid, suggesting traces of pyrophosphate.

When tetraethyl pyrophosphate was heated at 100° with concentrated disodium hydrogen phosphate solution positive tests for meta- but not pyro-phosphate were obtained.

Phenyl Dihydrogen Phosphate.—This was prepared by the method of Chanley and Feageson [24] and after recrystallisation had m. p. 100°. The monopotassium and the mono(dicyclohexylamine) salt were prepared by treating the free acid in ethanol–water with 1 mol. of potassium hydrogen carbonate and dicyclohexylamine respectively and removing the solvent under a vacuum at room temperature. *Dicyclohexylammonium hydrogen phenyl phosphate* formed needles from ethanol–ether (Found: C, 60·95; H, 8·6; N, 4·1. $C_{18}H_{30}O_4NP$ requires C, 60·85; H, 8·5; N, 4·0%).

Potassium hydrogen phenyl phosphate (0·5 g.) was heated in water (0·5 ml.) at 100° for 30 min. The presence of phenol was shown by a violet colour in ferric chloride solution and a white precipitate with bromine water. Chromatography in the Ebel system indicated the presence of trimetaphosphate, as did the ability of the solution to coagulate egg albumin.

Dicyclohexylammonium hydrogen phenyl phosphate (200 mg.) was heated in dry dioxan (8 ml.) at 100°. Liberated phenol was estimated by treating aliquot portions with an excess of standard bromate–bromide solution and back-titrating the excess, after addition of potassium iodide, against standard thiosulphate solution. The starting material was found to consume 1 mol. of bromine, compared with 3 mol. consumed by the liberated phenol. Even after 8 hr. at 100° only traces ($\ll 5\%$) of phenol were detectable. The addition of 1 and 2 mols. of water to the reaction solution had no significant effect.

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[23] Ebel, *Bull. Soc. chim. France*, 1953, 991.

[24] Chanley and Feageson, *J. Amer. Chem. Soc.*, 1955, **77**, 4002.