127. *The Synthesis and Hydrolysis of t-Butyl Phosphate.*

By A. LAPIDOT, DAVID SAMUEL, and M. WEISS-BRODAY.

t-Butyl phosphate has been prepared by oxidation of t-butyl phosphite. The hydrolysis of this compound was studied by following the rate of formation of inorganic phosphate in solutions from pH 7 to 3N-perchloric acid. The organic product is mainly t-butyl alcohol, with very little isobutene. The hydrolysis is strongly acid-catalysed, the rate being proportional to the H_0 function. The rates of hydrolysis of the neutral molecule and of the monoanion have been calculated from the measured equilibrium constants between each species and the total rate of dephosphorylation at various pH's. t-Butyl phosphate is hydrolysed with predominantly P–O bond fission in the pH range 5—7 and with exclusively C–O bond fission in acid solution. t-[$^2H_9$]Butyl phosphate is hydrolysed at the same rate as the non-deuterated compound at pH 7, and 2·8 times slower in more acidic conditions. It is suggested that the monoanion of this compound is hydrolysed with transient formation of metaphosphate whereas the neutral molecule and conjugate acid are hydrolysed by a unimolecular, nucleophilic S_N1 reaction.

THE nucleophilic reactions of alkyl dihydrogen phosphates are of great interest on account of the many functions of such compounds in biological systems. Several groups of workers [1,2] have studied reactions of monoalkyl phosphates in aqueous solution but have confined their attention to primary and secondary alkyl phosphates such as methyl,[3] ethyl,[4] glycerol-1 and -2,[5] α-D-glucose,[6] and 2-methoxy-1-methylethyl phosphate.[7] The main features are that these compounds are stable in basic solution and undergo hydrolysis at pH <7 with P–O bond fission. In most cases the active species is the monoanion, resulting in a maximum in the rate of hydrolysis at pH ~ 4. However, even at this pH, primary alkyl phosphates are relatively stable, methyl phosphate, for instance, having a half-life of about 2·3 hours at 100°. It has been found, on the other hand, that phosphate esters of biological interest appear to be much more reactive. In certain cases these compounds undergo ready elimination [8] in alkaline solution, caused by the presence of certain structural features in the alkyl radical. In other compounds, such as phosphate derivatives of mevalonic acid [9] and αβ-dihydroxybutyric acid phosphate,[10] the fact that the phosphate group is linked to a tertiary carbon atom may account for their reactivity. Since the mechanism of reaction of compounds of this type has not yet been investigated, we have synthesised the simplest member of this series, t-butyl dihydrogen phosphate, and studied its hydrolysis. The difficulty in preparing this compound has been considered in the past [11] to be due to the ease of cleavage of the alkyl group during phosphorylation or working-up. We have, however, prepared it by oxidation of t-butyl phosphite with alkaline permanganate and characterised it as the dicyclohexylammonium salt. We now report a study of its hydrolysis in aqueous solution, made by kinetic and isotopic techniques.

Whilst this work was in progress, Cramer *et al.*[12] synthesised t-butyl phosphate by another method and reported that it is stable in N-sodium hydroxide and is totally hydrolysed on boiling at pH 4 in 15 minutes and at pH 2 in <10 seconds. The products were

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

[2] Vernon, *op. cit.*, p. 17.

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

[4] Desjobert, *Compt. rend.*, 1947, **224**, 575; *Bull. Soc. chim. France*, 1947, **14**, 809.

[5] Bailly, *Bull. Soc. chim. France*, 1942, **9**, 340, 405; Swoboda and Crook, *Biochem. J.*, 1955, **59**, 24.

[6] Bunton, Llewellyn, Oldham, and Vernon, *J.*, 1958, 3588.

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

[8] (*a*) Lapidot, Samuel, and Silver, *Chem. and Ind.*, 1963, 468; (*b*) Halmann, Lapidot, and Samuel, *J.*, 1962, 1944.

[9] Lindberg, Yuan, de Waard, and Bloch, *Biochemistry*, 1962, **1**, 182, citing previous references.

[10] Wold and Ballou, *J. Amer. Chem. Soc.*, 1959, **81**, 2368.

[11] Mirbach, Ph.D. Thesis, Marburg University, 1961.

[12] Cramer, Rittersdorf, and Bohm, *Annalen*, 1962, **654**, 180.

not identified and no mechanism of hydrolysis was suggested. We have obtained the same rates of hydrolysis for t-butyl phosphate synthesised by the two methods.

RESULTS

Products.—Butyl phosphate breaks down in aqueous solution to orthophosphate, t-butyl alcohol, and traces of isobutene. t-Butyl alcohol was identified mass-spectrometrically, and isobutene by both mass spectrometry and vapour phase chromatography (see Experimental section).

In order to determine the ratio of olefin to alcohol, the rate of formation of isobutene was determined as by Leffek [13] *et al.* (see Experimental section).

We have found that the amount of olefin produced is $<2\%$ of the total organic product formed in these conditions.

Effect of Acidity.—We have confirmed that t-butyl phosphate resists hydrolysis above pH 7, no inorganic phosphate being detected after refluxing a N-sodium hydroxide solution of this compound for 24 hours. The rate of hydrolysis can be determined from pH 7 to nearly 3N-perchloric acid by following the rate of formation of inorganic phosphate colorimetrically. The rate of liberation of phosphate rises steadily with increasing acidity, as shown in Table 1. Fig. 1 shows that, unlike the hydrolysis of most esters [1,2] and *N*-substituted amides [14] of phosphoric acid, the hydrolysis of t-butyl phosphate does not have a maximum rate at pH 4.

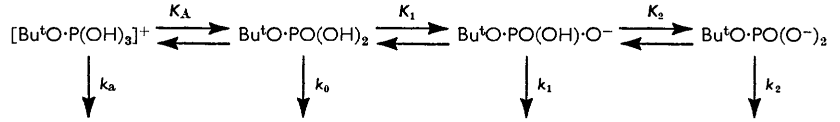
TABLE 1.

Partial and overall rate constants for hydrolysis of t-butyl phosphate at 73·9°.

pH	$[H_2A]/C$	$[HA^-]/C$	$[HA^{2-}]/C$	10^4k_{obs} (sec.$^{-1}$)
2·0	0·852	0·148	—	70
2·6	0·593	0·407	—	28·5 †
3·6	0·126	0·874	—	6·9
3·8	0·083	0·917	—	4·6
4·0	0·054	0·946	—	3·1 (1·87) *
4·2	0·035	0·965	—	1·8
4·7	0·011	0·988	0·001	0·77
5·9	0·0007	0·972	0·027	0·105 ‡
7·0	0·000001	0·212	0·788	0·015

$C = [H_2A] + [HA^-] + [HA^{2-}]$. * In D_2O solution. † $k_0 = 48·3$. ‡ $k_1 = 0·07$.

At each pH, the conjugate acid, neutral molecule, monoanion, or dianion, can be present, each of which can be hydrolysed with a specific rate constant as follows:



$$\begin{tikzcd}[Bu^tO{\cdot}P(OH)_3]^+ \arrow[r, shift left, "K_A"] \arrow[d, "k_a"] & Bu^tO{\cdot}PO(OH)_2 \arrow[l, shift left] \arrow[r, shift left, "K_1"] \arrow[d, "k_0"] & Bu^tO{\cdot}PO(OH){\cdot}O^- \arrow[l, shift left] \arrow[r, shift left, "K_2"] \arrow[d, "k_1"] & Bu^tO{\cdot}PO(O^-)_2 \arrow[l, shift left] \arrow[d, "k_2"] \\ {} & {} & {} & {}\end{tikzcd}$$

In solutions more acid than pH 0 the predominant species are the neutral molecule and conjugate acid, whereas in the pH range 2—7 both neutral species and monoanion are present.

In order to calculate the concentrations of the various species at a given pH, the equilibrium constants (K_1 and K_2) must be measured. The dissociation constants of dicyclohexylammonium t-butyl phosphate were determined by titration with hydrochloric acid at 25° (see Experimental section) and found to be $pK_1 = 2·76 \pm 0·02$ and $pK_2 = 7·43 \pm 0·05$ ($K_1 = 1·74 \times 10^{-3}$ and $K_2 = 3·72 \times 10^{-8}$). The values are somewhat higher than those observed for methyl and other simple phosphates.[3] However, owing to the rapid breakdown of the compound in strongly acidic solution the equilibrium constant between neutral molecule and conjugate acid (K_A) cannot be determined.

[13] Leffek, Llewellyn, and Robertson, *Canad. J. Chem.*, 1960, **38**, 2171.

[14] Halmann, Lapidot, and Samuel, *J.*, 1960, 4672; 1961, 3158; Chanley and Feageson, *J. Amer. Chem. Soc.*, 1958, **80**, 2686.

By using the calculated concentrations of the various ionic species and the observed total rate constant, the partial rate constants k_0, k_1 were calculated[15] (see Table 1), as follows: Since at pH 2·6, the proportion of the neutral species is 0·593 and if, for these conditions, the contribution of the monoanion to the central rate is assumed to be negligible, the value of the first-order rate coefficient for the total species is $k_0 = 2{\cdot}85 \times 10^{-3}/0{\cdot}593 = 4{\cdot}83 \times 10^{-3}$ sec.$^{-1}$. At pH 5·9 the proportion of the neutral species is very small, namely, $[HA] = 0{\cdot}0007$. Hence the rate coefficient (k_1) of decomposition of the monoanion can be obtained from the equation $k_{obs} = [HA]/C + k_1\ [HA^-]/C$ (where $C = [H_2A] + [HA^-] + [HA^{2-}]$), whence $k_1 = 7 \times 10^{-5}$ sec.$^{-1}$.

The values of k_0 and k_1 can now be used for calculating the total rate constants at other pH's and comparing them with the observed rates. As seen from Table 1 and Fig. 1 good agreement is obtained between the observed and the calculated rate constants.

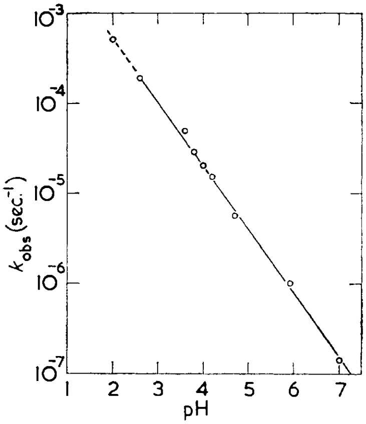


Fig. 1. Dependence of rate constant for hydrolysis of t-butyl phosphate on pH at 73·9°.

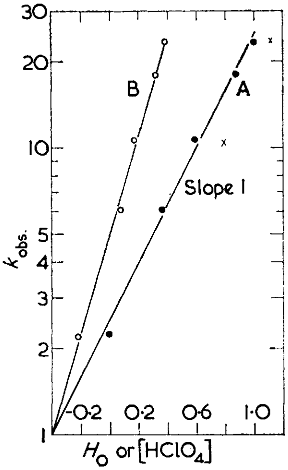


Fig. 2. Dependence of log k for the acid-catalysed hydrolysis of t-butyl phosphate on (A) acidity function (H_0) or (B) concentration of perchloric acid at 10·0°; ○ ● In H_2O. × In D_2O.

This agreement also indicates that the hydrolysis of the dianion does not contribute to the total rate and, indeed, in basic solution, where the dianion is the only species, hydrolysis does not occur.

Table 2.

Calculated rate constant for hydrolysis of neutral molecule at 73·9°.

pH	2	2·6	3·6	3·8	4·0	4·2
$10^3 k_0$ (sec.$^{-1}$)	8·2	4·83	5·45	5·55	5·7	5·15

It is also seen from Table 1 that in the range pH ~2·6—4 hydrolysis of the neutral molecule is predominant. The rate constant (k_0) for the hydrolysis of this species at various pH's is shown in Table 2. The constancy of the calculated results over this range indicates that this assumption is valid.

The hydrolysis of *t*-butyl phosphate in the acid range of 0·1M—2·74 M-perchloric acid increases rapidly with acidity, the rate being proportional to the Hammett acidity function (H_0) with a slope of 1·0 (see Fig. 2 and Table 3).

Effect of Temperature.—The rate of formation of phosphate was determined at three different temperatures (see Table 4), from which the energy and entropy of activation were calculated. The energy of activation appears to be slightly higher than that found in

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[15] Halmann and Lapidot, *J.*, 1960, 419.

TABLE 3.

Hydrolysis of t-butyl phosphate in acid solution at 10·0° and constant ionic strength (μ = 3).

$HClO_4$ (M)	0·62	1·25	1·52	2·06 *	2·14	2·45	2·74 *
10^4k (sec.$^{-1}$)..................	2·25	6·1	10·8	10·3	18·2	23·1	24·0

* In D_2O.

TABLE 4.

Rate constants for hydrolysis of t-butyl phosphate at several temperatures at pH 2·6.

Temp.	44·0°	52·3°	73·9°	ΔE (kcal. mole^{-1})	ΔS (e.u.)
10^4k_{obs} (sec.$^{-1}$)	0·343	3·32	28·5	33·3	+7·2

the hydrolysis of other alkyl phosphates which are usually in the range of 28—30 kcal. mole^{-1}.

Point of Bond Fission.—Hydrolysis of t-butyl phosphate in aqueous solution can occur with breakage of the bond between carbon and oxygen or between oxygen and phosphorus. The point of bond fission was determined at various pH's by using a solvent enriched in oxygen-18. In each case the phosphate formed on total hydrolysis was isolated as the barium salt and analysed for its oxygen-18 content. The results, calculated as percentage C–O bond fission, are presented in Table 5. They indicate that in solutions more acid than pH 5 only C–O bond fission occurs, whereas in the range pH 5—7 both P–O and C–O bond fission occur. In the last column of Table 1 the percentages of C–O bond fission calculated from the rate of hydrolysis of the neutral molecule at each pH at listed. The agreement between observed and calculated results is good.

TABLE 5.

Point of bond fission in hydrolysis of t-butyl phosphate at 100°.

pH	Buffer soln.	*A*, water (atom % excess)	*B*, phosphate (atom % excess)	*B*/*A*	C–O bond fission (%) found	C–O bond fission (%) calc.
—	N-$HClO_4$	4·501	0·006	0·001	99·5	100
4·0	0·2M-(K H phthalate)	4·94	0·055	0·011	95	100
5·9	0·2M-Acetate	4·14	0·65	0·157	36·5	34·5
7·0	0·2M-Diethylbarbiturate	4·55	0·87	0·191	23·5	25

Deuterium Isotope Effects.—The effect of substituting deuterium oxide for water in the solvent is summarised in Table 6 (from Fig. 2 and Tables 1 and 3). The deuterium solvent isotope effects are similar in magnitude to those in the hydrolysis of t-butyl chloride in water.[16] The effect of deuterium substitution in the alkyl group was examined by synthesising t-[$^2H_9$]butyl phosphate from the fully deuterated alcohol by the method of Cramer *et al.*[12] and measuring the rate of liberation of inorganic phosphate as before. Replacement of all the hydrogen in the t-butyl group leads to an appreciable reduction in the rate of hydrolysis of the phosphate ester in acid solution, as shown in Table 7.

TABLE 6.

Solvent isotope effect on the hydrolysis of t-butyl phosphate.

pH	Buffer	Temp.	K_{H_2O}/k_{D_2O}
4·0	0·05N-K H phthalate	73·9°	1·66
	2·06N-$HClO_4$	10·0	1·55
	2·74N-$HClO_4$	10·0	1·37

The rates of hydrolysis of undeuterated t-butyl phosphate prepared by our method and

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[16] Swain, Cardinaud, and Ketley, *J. Amer. Chem. Soc.*, 1955, **77**, 934; Robertson and Laughton, *Canad. J. Chem.*, 1957, **35**, 1319.

TABLE 7.

Rate [$10^4 k_{obs}$ (sec.$^{-1}$)] of decomposition of t-butyl and t-[$^2H_9$]butyl phosphate.

Temp.	Solvent	Deuterio-compound *	Normal compound	k_H/k_D
27·0°	0·625N-HClO$_4$	4·35	12·2 *	2·8
		4·41	12·3	2·8
73·9	0·05N-K H phthalate	1·14	3·1 *	2·72
	(pH 4)	1·15	3·16	2·75

* Compounds prepared by the method of Cramer *et al.*[12]

by that of Cramer *et al.*[12] are the same. However, the rate of hydrolysis of the deuterio-compound at both pH 4 and in strong acid is 2·8 times as slow as that of the normal compound. On the other hand, after 3 hours at 100° in borate buffer (pH 7·05) 80·5% of the deuterio-compound and 81% of the normal compound had been hydrolysed; this indicates that in neutral solution, where P–O bond fission is predominant, there appears to be no difference in the rate of hydrolysis between the normal and the deuterio-compound.

DISCUSSION

The stability of t-butyl phosphate in basic solution indicates that the dianion is unreactive, in common with the dianions of other alkyl phosphates.[7] This is probably due to electrostatic repulsion. No ready elimination [8] in base occurs.

In the range pH 5—7, where the monoanion predominates, t-butyl phosphate is hydrolysed mostly with P–O bond fission, and at pH 7 no difference between the rates of breakdown of the deuterated and the undeuterated compounds is observed. This indicates, that, in this range, the usual type of hydrolysis of the monoanion of a phosphate ester takes place, with the formation of a metaphosphate intermediate.[1,2] However, the maximum at pH 4 is no longer present (see Fig. 1) since it is obscured by the more rapid breakdown of another highly reactive species. This is the neutral molecule which is present below pH 5 in increasing amounts and yields inorganic phosphate at a rate (k_0) which is *ca.* 10^3 times faster than that of hydrolysis of the monoanion (k_1) (see Table 1).

A similar situation has been observed in the hydrolysis of α-D-glucose 1-phosphate,[6] whose neutral molecule is also hydrolysed about 10^3 times faster than the monoanion, with only a small plateau at pH 5. With t-butyl phosphate, even this plateau no longer exists and a steady rise in rate occurs with increasing acidity. In the conditions used (see Experimental section) hardly any olefin is hydrated to t-butyl alcohol. Leffek *et al.*[13] found that only 2·9 ± 0·2% of olefin was formed in the hydrolysis of t-butyl chloride in water at 25°.

Further evidence that an unusual mechanism operates in acid solution is the fact that from pH 1 to nearly 3N-perchloric acid, almost complete C–O bond fission occurs (see Table 5). However, since less than 2% of olefin is formed, this reaction is not a β-elimination.[8] It is unlikely that any of the olefin has been hydrated to t-butyl alcohol, since in these experiments the isobutene was swept out as soon as it was formed and the amount that could react within a few seconds at 100° is very small. Further evidence against any large degree of elimination is the great stability of t-butyl phosphate in basic solution, although here the negative charge on phosphate may also alter the tendency to lose a proton.

We therefore consider that t-butyl phosphate is hydrolysed by an S_N1 reaction of the neutral molecule and of the conjugate acid. This mechanism is similar to that found for α-D-glucose 1-phosphate.[6]

Neutral molecule:

$$\mathrm{Bu^tO{\cdot}PO_3H_2} \xrightarrow[\text{Slow}]{} \mathrm{Bu^{t+}} + \mathrm{H_2PO_4^-} \xrightarrow[\text{Fast}]{} \text{Products}$$

Conjugate acid:

$$\mathrm{Bu^tO{\cdot}PO_3H_2} + \mathrm{H_3O^+} \rightleftharpoons [\mathrm{Bu^tO{\cdot}P(OH)_3}]^+ + \mathrm{H_2O} \xrightarrow[\text{Slow}]{} \mathrm{Bu^{t+}} + \mathrm{H_3PO_4} \xrightarrow[\mathrm{H_2O}]{\text{Fast}} \text{Products}$$

An interesting feature of this reaction is the large isotope effect when all the hydrogen atoms of the butyl group are replaced by deuterium.

Since so little olefin is formed this cannot be a primary isotope effect, in which hydrogen is removed in the rate-determining step. However, secondary β-deuterium isotope effects of this magnitude have been observed by Shiner *et al.*[17] in the hydrolysis of t-pentyl chloride ($k_H/k_D = 2{\cdot}35$ at 25°) and by Lewis and Coppinger[18] for that of t-butyl chloride (2·56 at 2°). Leffek *et al.*[13] suggested that the effect of β-deuterium in such cases is increased as the transition state of the reaction tends to be more ionic. The large degree of ionisation is due to three factors: (*a*) the presence of a t-butyl group; (*b*) a phosphate leaving group; and (*c*) a solvent of high dielectric constant. In the present case the effect, $k_H/k_D = 2{\cdot}7$—2·8, is slightly larger than for halides and is what one may expect for a more ionic transition state.

Our results confirm the view that the elimination suggested[9] for phosphate derivates of mevalonic acid is associated with a simultaneous decarboxylation. The present study shows that, in the absence of a β-carboxyl group, very little elimination occurs from a tertiary alkyl monophosphate over a wide range of conditions.

Experimental

Di-t-butyl phosphite was prepared by the method of Goldwhite and Saunders[19] from t-butyl alcohol (93·7 ml.) and phosphorus trichloride (45·75 g.) in ether in the presence of pyridine. The product, b. p. 70—75°/1 mm. (lit.,[1] 66·68°/0·5 mm.), was obtained in 40% yield (Found: C, 50·2; H, 9·6. $C_8H_{19}O_3P$ requires C, 49·4; H, 9·8%).

The proton magnetic resonance spectrum contains a P–H doublet, a single large peak for all eighteen protons of the two butyl groups, and a very small peak due to some hydrogen-containing, high-boiling impurity, probably polyisobutene.

Cyclohexylammonium t-Butyl Phosphite.—Di-t-butyl phosphite (3·88 g.) was dissolved in a small quantity of 1 : 4 aqueous ethanol, and a concentrated solution of sodium hydroxide (0·8 g.) in water was added drop by drop with shaking until the colour of an added drop of phenolphthalein persisted. The solution was brought to pH $\sim$2 with concentrated hydrochloric acid, and 20% aqueous ferric chloride was added drop by drop until the orange colour persisted. The solutions should be as concentrated as possible to ensure complete precipitation of the ferric salt; this was centrifuged off and washed with water and acetone until free from chloride. Cyclohexylamine was added to a suspension of the ferric salt in a little water until the mixture was just basic. The precipitate of ferric hydroxide was centrifuged off and the supernatant solution evaporated to dryness *in vacuo* at room temperature. The white residue was washed with acetone; recrystallised from water–acetone, it had m. p. 160—163° (lit.,[20] 170°) (yield 45%), R_F (in propan-2-ol–water–ammonia) 0·78 (Found: C, 50·2; H, 9·9; N, 5·7. Calc. for $C_{16}H_{24}NO_3P$. C, 50·7; H, 10·1; N, 5·9%).

Dicyclohexylammonium t-Butyl Phosphate.—Dicyclohexylammonium t-butyl phosphite (0·273 g.), dissolved in water (10 ml.) containing sodium hydrogen carbonate (0·2 g.), was oxidised by Brown and Hammond's method,[21] a solution of potassium permanganate (0·105 g.) being added drop by drop with cooling and stirring. After several hours the manganese dioxide was filtered off, and the filtrate treated with Dowex 50(H^+) and poured into an excess of cyclohexylamine. The solvent was removed *in vacuo* and the white residue recrystallised from ethanol containing a little cyclohexylamine, as white needles, m. p. 191—193° (lit.,[12] 191—193°) (Found: C, 51·6; H, 10·0; N, 7·1; P, 8·6. Calc. for $C_{16}H_{37}N_2O_4P$: C, 54·5; H, 10·6; N, 7·95; P, 8·8%).

Dilithium t-butyl Phosphate.—An aqueous solution dicyclohexylammonium t-butyl phosphate was shaken for 2 min. with Dowex 50W(H^+). After addition of lithium hydroxide to pH 7, followed by two volumes of ethanol, the dilithium salt was precipitated, centrifuged off, and dried *in vacuo.*

[17] Shiner, Mahler, Baker, and Hiatt, *Ann. N.Y. Acad. Sci.*, 1960, **84**, 583.

[18] Lewis and Coppinger, *J. Amer. Chem. Soc.*, 1954, **76**, 4495.

[19] Goldwhite and Saunders, *J.*, 1957, 2409.

[20] Tanaka, *Yakugaku Zasski*, 1959, **79**, 437 (*Chem. Abs.*, 1959, **53**, 16932.)

[21] Brown and Hammond, *J.*, 1960, 4229.

Dicyclohexylammonium t-[$^2H_9$]*Butyl Phosphate.*—This was synthesised from t-[$^2H_9$]butyl alcohol and phosphorylated trichloroacetonitrile by the method described by Cramer *et al.*; [12] it had m. p. 191—192° (lit.,[12] for normal compound 191—193°).

Deuterium oxide (99·8%) was used to prepare solvent for kinetic measurements. $DClO_4$ solutions were prepared by dissolving 72% perchloric acid in deuterium oxide. Water enriched in ^{18}O was obtained from the separation plant of the Weizmann Institute. Its ^{18}O content was determined by Anbar's [22a] hypobromite method.

Determination of the Position of Bond Breakage.—Dicyclohexylammonium t-butyl phosphate (*ca.* 200 mg.) was dissolved in aqueous acid or in buffer solutions containing an excess abundance of ^{18}O in the solvent water. After incubation at 100° for a time equivalent to complete decomposition, the orthophosphate was isolated as the barium salt, purified by reprecipitation at pH 8, and analysed [22b] for its ^{18}O content by conversion into carbon dioxide. The isotopic content of this gas was determined by means of a Consolidated Engineering Corp. model 21-401 mass spectrometer. Analyses were run at least in triplicate and are considered accurate to ±3%.

Identification of Organic Products.—Dicyclohexylammonium t-butyl phosphate was completely hydrolysed in aqueous acid or buffer solutions in closed vessels. For qualitative analysis the low-boiling fractions were transferred *in vacuo*, together with some water, into small bulbs. Isobutene and t-butyl alcohol were identified by gas chromatography and compared with authentic samples. Both isobutene and the alcohol were also identified mass spectrometrically, the former by means of peaks at 41 and the latter at 50 mass units. Quantitative analysis of the olefin was performed in an all-glass apparatus as described by Leffek *et al.*[13] Dilithium t-butyl phosphate was hydrolysed in sealed bulbs at pH 4, at pH 0, and in N-perchloric acid at room temperature. When hydrolysis was complete, the sealed bulbs were broken inside the apparatus and a stream of nitrogen passed through the solution whilst the temperature was rapidly raised to 100° by immersion in a boiling-water bath. The olefin was swept into aqueous bromine–bromide. Back-titration with thiosulphate indicated that less than 2% of olefin was formed. It can be calculated from the results of Lucas and Eberz [23] that the extent of hydration of isobutene in these conditions is very small. For example, the time for hydration of 50% of the isobutene is 4·9 hr. in 0·1N-nitric acid at 25°.

Kinetic Experiments.—The rate of formation of phosphate over the range from pH 7 to 3N-perchloric acid was followed by withdrawing aliquot parts from appropriate buffer or aqueous acid solutions kept in a thermostat-bath, as described by Fisk and Subbarow.[24] The concentration of the phosphomolybdate complex was measured in a Beckman D.U. spectrometer calibrated at 660 μ. The thermostat-baths were maintained at constant temperatures with an accuracy of ±0·1°.

We are grateful to Mr. Brian L. Silver for helpful discussions.

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[*Received, June* 19*th*, 1963.]

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[22] (*a*) Anbar and Guttmann, *Internat. J. Appl. Radiation Isotopes*, 1959, **3**, 233; (*b*) Anbar, *ibid.*, 1953, **3**, 131.

[23] Lucas and Eberz, *J. Amer. Chem. Soc.*, 1934, **56**, 640.

[24] Fiske and Subbarow, *J. Biol. Chem.*, 1925, **66**, 375.