

Hydrolysis of Benzoylphosphates *

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The mixed anhydrides of phosphoric acid have in recent years attracted the interest of several biochemists, and their work ¹⁻³ has brought forward several facts about their reactivities which are of considerable interest also from the point of view of pure chemistry.

Chantrenne ³ has shown that at pH = 7.4 dibenzoylphosphate and phenylbenzoylphosphate will readily benzoylise glycine, ammonia and aniline, while monobenzoylphosphate will not. The latter, however, reacts much faster with water than the disubstituted compounds. Lipman ¹ has investigated monoacetylphosphate and Lynen ² has hydrolysed both mono-, di- and triacetylphosphate. The triacetyl compound is destroyed almost momentarily by water and the behaviour of the two others seems closely analogous to that of the benzoyl compounds.

Bentley ⁴ has hydrolysed acetylphosphate in nearly neutral and in strongly alkaline solutions in ¹⁸O enriched water and he traces the water's oxygen to the phosphate ions in the former case and to the acetate in the latter.

The purpose of the present investigation is to find the variation with pH of the rate of hydrolysis of mono- and dibenzoylphosphate in order to get a better basis for speculations about the mechanism of these interesting reactions.

EXPERIMENTAL

Preparation of dibenzoylphosphate was done as described by Chantrenne ³, but not in a "cold room". Possibly for this reason no monobenzoylphosphate could be isolated.

Reaction took place in test tubes in thermostat at 37.0° C and samples were withdrawn at intervals and analysed photometrically through the formation of a red ferric ion complex with hydroxamic acid, formed by reaction of the residual amount of mixed an-

* The results of this work were communicated at the *Syvende nordiske kemikermøde* in Helsinki, August 1950.

hydride with hydroxylamine. The method was a slight modification of Lipmans procedure: Three stock solutions were prepared. A: 28 % (4 *M*) hydroxylamine hydrochloride; B: 14 % (3.5 *M*) sodium hydroxide and C: 1.66 % ferric chloride in 1 *N* HCl. Just before use equal volumes of A and B were mixed and 1 ml of the mixture was added to a 1 ml sample in a 10 ml measuring flask. After standing for an hour in thermostat at 37° C 2 ml C-solution was added and the volume adjusted to 10 ml with 1 *N* HCl. The optical density was measured in 10 mm cells in a Spekker photometer with green filters (604). The modification in the methods of Lipman and Tuttle⁵ and Chantrenne³ were necessitated partly by the wider range of pH throughout which a uniform procedure was desired, and partly by the fact that the undiluted solution was not stable; the colour faded and bubbles were formed on the windows of the cell. Diluted according to the above procedure, the solution kept so well that it faded only about 2 % in an hour.

On the Spekker photometer the optical density is read directly, and so long as the same cell is used, the reading is proportional to the concentration of unhydrolysed anhydride bonds, *i. e.*

$$\epsilon = \text{const} \cdot (2 [\text{B}_2\text{P}] + [\text{B}_1\text{P}]) \quad (1)$$

where $[\text{B}_2\text{P}]$ and $[\text{B}_1\text{P}]$ are the concentrations of dibenzoyl- and monobenzoylphosphate, respectively.

This analytical method is not accurate and convenient enough to make it feasible to investigate the variation of rate constants with temperature and ionic strength, but it is quite accurate enough for an orientation with regard to the dependence on pH.

At high pH-values it is convenient to follow the reaction by automatic titration of the liberated acid, and a few runs were made with a "pH-stat", built in this institute by Mr. Arne E. Nielsen, chemical engineer. In acid solution this method is not so suitable, as the buffering capacity of the weak acids formed makes the readings less accurate, and eventually under the *pK* of benzoic acid the consumption of base becomes negligible.

Calculation of the Rate Constants presents the familiar difficulties in cases where the two steps of the reaction proceed with comparable velocities. At constant pH both steps should be expected to proceed as first-order reactions, and no deviations from this could be detected.

The differential equations,

$$d[\text{B}_2\text{P}]/dt = -k_2[\text{B}_2\text{P}] \quad \text{and} \quad (2)$$

$$d[\text{B}_1\text{P}]/dt = -k_1[\text{B}_1\text{P}] + k_2[\text{B}_2\text{P}] \quad (3)$$

can be solved and give:

$$[\text{B}_2\text{P}] = [\text{B}_2\text{P}]^0 \exp(-k_2t) \quad \text{and} \quad (4)$$

$$[\text{B}_1\text{P}] = \left\{ \exp(-k_2t) - \exp(-k_1t) \right\} [\text{B}_2\text{P}]^0 k_2/(k_1 - k_2) \quad (5)$$

(4) and (5) can be inserted in (1) to give an equation for ϵ as a function of time.

Fig. 1 shows as an example a plot of $\ln \epsilon$ against time for an experiment in a buffer of pH = 9.8. The later part of the curve forms a straight line as it should do, when all B_2P has disappeared and only B_1P is being hydrolysed. From the equations above we deduce for the asymptote of the curve for $t \rightarrow \infty$, when $k_2 > k_1$:

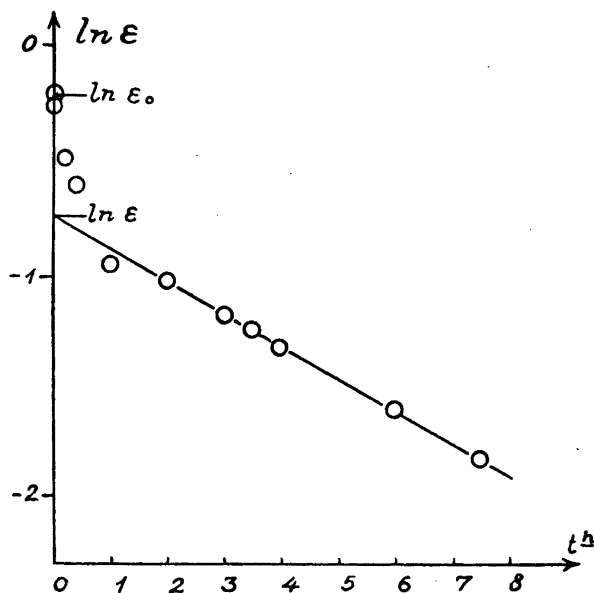


Fig. 1. Dibenzoylphosphate (B_2P) hydrolysis at $pH = 9.8$.

$$y = \ln \epsilon' - k_1 t \quad (6)$$

$$\text{where } \epsilon' = \epsilon_0 \cdot k_2 / 2(k_2 - k_1) \quad (7)$$

Taking the best straight line through the last points of the curve to represent the asymptote, we find a value for ϵ' from its point of intersection with the ordinate-axis and a value for k_1 from its slope. k_2 can then be found either from (7) or from the slope of the tangent of the curve at $t = 0$. With a small amount of trial and error a set of values is obtained with an accuracy corresponding to that of the analytical method.

When $k_2 \ll k_1$, the $\ln \epsilon$ curve becomes a straight line after a short while, during which the "stationary" concentration of B_1P is building up. From the slope of this line k_2 is easily inferred.

k_1 was determined by letting B_2P react for an hour with 0.1 mol glycine at $pH = 9.5$, which is approximately pK_2 for glycine, and subsequently following the hydrolysis of the formed monobenzoylphosphate after adjustment of the desired pH .

All pH -values are given as read on a valve potentiometer with a glass electrode standardized on Sørensen-phosphatebuffer.

Discussion of experimental results. Within the not very great experimental accuracy the results show the usual appearance of reactions which proceed both acid- and base-catalysed as well as uncatalysed, except for a few values of k_2 at $pH = 7.0$ and 7.4 . The most probable explanation why these values are 3 or 4 times too high, is "general base catalysis" by HPO_4^- ions. If the "pH-stat" had been at hand at an earlier date, it would have been easy to investigate this discrepancy by using that instead of buffersolutions

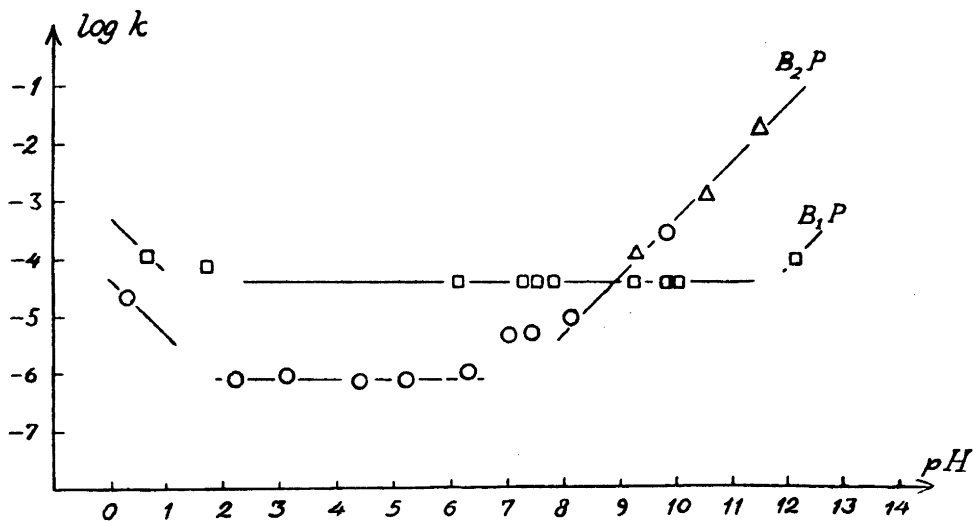


Fig. 2. Hydrolysis of benzoylphosphates.

Table 1. Determinations of k_1 (the unimolecular velocity constant of hydrolysis of mono-benzoylphosphate at 37° C). All experiments are carried out with 0.01–0.02 M sodium-dibenzoylphosphate in solutions of indicated composition: $\frac{1}{2}$ McI and $\frac{1}{2}$ Sør. mean buffer solutions according to Mc Ilvaine (citrate-phosphate) and Sørensen (phosphate) respectively, diluted to half concentrations. Glyc. = aminoacetic acid. The pH -values are given as read on a valve potentiometer with glass-electrode, standardized on Sørensen's phosphate buffer solution. Ionic strength is calculated approximately and with neglect of amfoions.

pH	Solution	Ionic strength	k_1 (sec ⁻¹)	$\log k_1$
0.65	NaCl/HCl 0.1 M Glyc.	0.4	$1.2 \cdot 10^{-4}$	- 3.92
1.7	NaCl/HCl 0.1 M Glyc.	~ 0.5	$7.8 \cdot 10^{-5}$	- 4.11
6.1	$\frac{1}{2}$ McI 0.1 M Glyc.	0.2	$3.9 \cdot 10^{-5}$	- 4.41
7.3	$\frac{1}{2}$ Sør. 0.2 Glyc.	0.1	$4.1 \cdot 10^{-5}$	- 4.39
7.5	$\frac{1}{2}$ Sør. 0.03 M Glyc.	0.1	$4.1 \cdot 10^{-5}$	- 4.39
7.8	$\frac{1}{2}$ Sør. 0.1 M Glyc.	0.1	$4.0 \cdot 10^{-5}$	- 4.39
9.0	0.1 M Na ₂ CO ₃ 0.1 M Glyc.	0.25	$4.0 \cdot 10^{-5}$	- 4.39
9.25	NaOH from pH-stat	<0.03	$3.5 \cdot 10^{-5}$	- 4.46
9.8	0.1 M Na ₂ CO ₃	0.25	$4.0 \cdot 10^{-5}$	- 4.39
10.0	0.1 M Glyc. + NaOH	0.05	$3.5 \cdot 10^{-5}$	- 4.46
12.1	NaOH	0.1	$9.1 \cdot 10^{-5}$	- 4.04

Table 2. Determinations of k_2 (the unimolecular velocity constant of the hydrolysis of dibenzoylphosphate at 37° C) see text of Table 1.

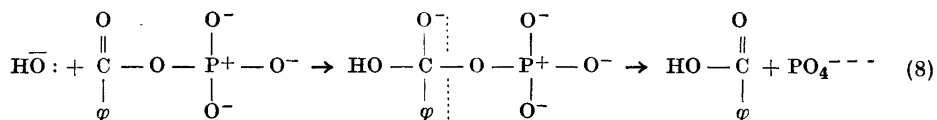
pH	Solution	Ionic strength	k_2 (sec ⁻¹)	log k_2
0.32	HCl	0.1	$1.9 \cdot 10^{-5}$	- 4.72
2.2	½ McI	0.02	$7.65 \cdot 10^{-7}$	- 6.12
3.1	½ McI	0.04	$9.05 \cdot 10^{-7}$	- 6.04
4.4	½ McI	0.1	$7.43 \cdot 10^{-7}$	- 6.13
5.2	½ McI	0.12	$7.49 \cdot 10^{-7}$	- 6.13
6.3	½ McI	0.2	$1.04 \cdot 10^{-6}$	- 5.98
7.0	½ McI	0.2	$4.22 \cdot 10^{-6}$	- 5.37
7.4	½ Ser	0.08	$9.5 \cdot 10^{-6}$	- 5.02
8.1	½ McI	0.3	$9.35 \cdot 10^{-6}$	- 5.03
9.25	NaOH from pH-stat	< 0.01	$1.2 \cdot 10^{-4}$	- 3.92
9.8	0.1 M Na ₂ CO ₃	0.25	$2.6 \cdot 10^{-4}$	- 3.59
10.5	NaOH from pH-stat	< 0.03	$1.22 \cdot 10^{-3}$	- 2.91
11.5	NaOH from pH-stat	< 0.03	$1.75 \cdot 10^{-2}$	- 1.76

also in ranges where the burette readings were of little or no use analytically. Working with buffer solutions we met considerable difficulties in finding suitable substances. All substances with amino-groups were ruled out *a priori* and also borate, for instance, turned out to falsify the results, reacting either with B₂P or with hydroxylamine.

Some effort was invested in attempts to refine the analytical technique, but with little success. We would not deem it worth while to attempt more accurate kinetic measurements with these substances unless it is possible to find some better analytical procedure.

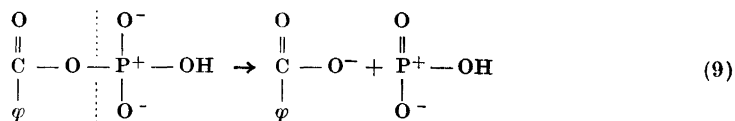
DISCUSSION

In conformity with the present views on the hydrolysis of carbonic esters, acetic anhydride etc., it is a natural assumption that reaction of the benzoylphosphates with bases takes place as displacement reaction at the carbon atom, the phosphate ion being displaced by a hydroxyl ion or another base. The mentioned tracer experiments by Bentley with acetylphosphate support this hypothesis:

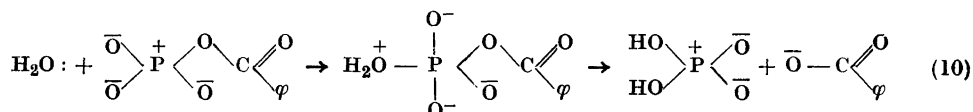


According to the tracer experiments the remarkably rapid reaction of B₁P in acid or neutral solution must proceed by some other path, however, and Bentley's results leave as the two most probable possibilities for the initial step:

a) fission of bond between O and P:



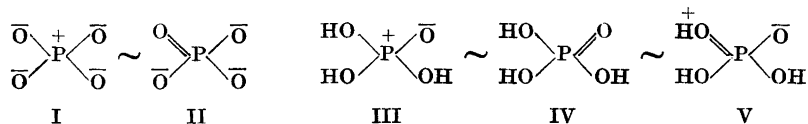
b) displacement at the P atom utilizing its fifth covalent orbital for the formation of an intermediate bond:



Now from this investigation we know that the reaction is virtually uncatalysed within a long range of pH-values, and it seems improbable that water-molecules should react so easily when much stronger bases as NH_3 are not known to be phosphorylated in this way.

a) then seems the more feasible, and probably it is a general feature that not too large *central atoms, surrounded by four tetrahedrally arranged oxygen atoms, are very well shielded against intruding bases*. We know for instance that sulfate and orthophosphate ions do not exchange ^{18}O in alkaline solution and also that pyrophosphate and hypophosphate ($\text{P}_2\text{O}_6^{4-}$) ions as well as ortho-carbonic esters are completely stable in alkaline solution, while the hydrolysis of such compounds is strongly catalysed by acids.

Another interesting phenomenon is the much greater reactivity of B_2P than of B_1P in reactions with bases, such as (8). Chantrenne's work^{3b} has made it probable that the power to acylise glycine, ammonia, aniline *etc.* in aqueous solution is a characteristic of the disubstituted phosphoric acids, no matter what the other substituent might be. This seems to indicate that we will have to look at the engagement of the fifth covalent bond orbital of the phosphorus atom for an explanation.



In the orthophosphate ion structures with pentacovalent phosphorus (II) are supposed to participate to some unknown extent, being responsible for the stability of the ortho-acid relative to the meta-acid in contradistinction to

nitric acid. In the ion the four oxygen atoms will obviously take an equal share of the fifth bond, and even in the partly or totally undissociated acid the OH groups will be able to take a considerable part of it due to the small electronegativity of hydrogen (V). When phenyl, alkyl or acyl groups are substituted for the proton this will not happen to any comparable degree, and consequently the remaining O^- and OH groups will get an increased share of the fifth bond, resulting in a relative stabilisation of the dissociated ion and hence in a stronger acid character. This may account for the fact that substituted phosphoric acids are stronger than orthophosphoric acid itself. Ethyl- and diethylphosphoric acids are reported to have first dissociation constants which are 3 and 9 times that of orthophosphoric acid.

Considering next the intermediate steps in the displacement reaction (8), we notice that it involves an analogous rupture of a bond from the acyl radical to oxygen and here again: the better chance the oxygen atom has for getting a double bond to phosphorus, the more readily will it let loose the acyl group.

It is in agreement with this hypothesis that acetyl metaphosphate is an even stronger acylating reagent than diacetylphosphate, as shown by Heyman and Rosenberg ⁶.

The acyl groups and the proton are "acids" in Lewis' sense or "antibases" in the language of J. Bjerrum ⁷ and the parallelism between the acylating potency and the normal acid strength is interesting from the point of view of generalized acid-base terminology. The extent to which such a parallelism will prove to exist, will determine the feasibility and applicability of such terminologies.

That the ability to react through fission as in (9) is reversed for B_2P and B_1P in comparison with the reactivity towards bases (8), is a natural consequence of the fact, that the P—O bonds are made stronger through the same superposed structures which loosen the O—R bond (cp. V).

Trisubstituted phosphoric esters and mixed anhydrides are known to be highly unstable in aqueous solution. According to the present hypothesis they should react by splitting one of the O—R bonds. Experiments with ^{18}O would prove or disprove this mechanism.

SUMMARY

The rates of hydrolysis of benzoylphosphate (B_1P) and of dibenzoylphosphate (B_2P) have been measured over the normal range of pH-values. The rapid hydrolysis of B_1P is shown to be virtually uncatalysed by acids and bases, while B_2P reacts slowly with water and rapidly with bases (Fig. 2).

Structural speculations lead to the possible explanation that substitution of a proton in orthophosphoric acid derivatives through the fifth covalent bond

of the P-atom influences the other bonds in such a way as to strengthen the P—O bonds and to loosen other O—R bonds.

This work was inspired mainly through discussions with Dr. Th. Rosenberg, formerly of this institute and now of *Nordisk Insulinlaboratorium*.

The Spekker photometer was generously presented to the institute by the maker, Adam Hilger Ltd. through the Royal Society of London.

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