852. *The Existence of the* P^1P^1*-Diethyl Pyrophosphate Ion.*

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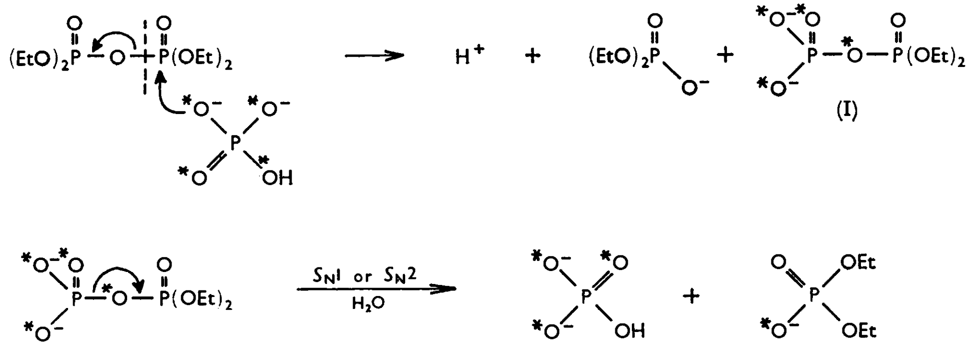
The phosphate-catalysed hydrolysis of tetraethyl pyrophosphate has been studied with the aid of oxygen-18. It is shown to proceed *via* the P^1P^1-diethyl pyrophosphate ion, whose existence had been suggested previously on the basis of kinetic results.

THE P^1P^1-dialkyl pyrophosphate ion has been postulated as an intermediate in the hydrolysis of tetramethyl [1] and tetraethyl pyrophosphate,[2] catalysed by phosphate ions, in the pH range 7·9—9·2. An analogous ion has been suggested as intermediate in the synthesis of nucleotides when tetra-*p*-nitrophenyl phosphate is used as phosphorylating agent.[3] The only products of the hydrolyses are the corresponding dialkyl phosphate and phosphate ion. The mechanism proposed [2] involves nucleophilic (S_N2) attack by the phosphate ion on a phosphorus atom of the pyrophosphate to form the hypothetical P^1P^1-dialkyl phosphate ion, subsequent hydrolysis of which could proceed by either an S_N1 or an S_N2 mechanism.

The P^1P^1-dialkylpyrophosphate ion (I; not labelled) was not isolated, its formation being inferred mainly from kinetic evidence.[1,2] When Avison [2] suggested that this reaction may serve as a model for phosphate transfer in biological systems, the possibility of an ion (I) acquired additional interest. The present work describes the use of oxygen-18 as a tracer which confirms its existence.

The hydrolysis of tetraethyl pyrosphate in the presence of [^{18}O]phosphate ion is represented in the annexed scheme. By the catalytic path each molecule of tetraethyl pyrophosphate (TEPP) produces 2 molecules of diethyl phosphate (DEP) containing a total of 8 oxygen atoms, one of which will be enriched: the simultaneous non-catalysed



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[1] Brown and Hamer, *J.*, 1960, 1155.
[2] Avison, *J.*, 1955, 732.
[3] Hampton and Maguire, *J. Amer. Chem. Soc.*, 1961, **83**, 150.

hydrolysis produces phosphate that is not enriched. The percentage enrichment of diethyl phosphate therefore depends on the relative rates of the catalysed and the non-catalysed reaction:

$$\text{Catalysed: } \mathrm{d[DEP]}_t/\mathrm{d}t = k[\mathrm{P}][\mathrm{TEPP}]_t = -\mathrm{d[TEPP]}_t/\mathrm{d}t. \qquad (1)$$

$$\text{Not catalysed: } \mathrm{d[DEP]}_t/\mathrm{d}t = k'[\mathrm{TEPP}]_t = -\mathrm{d[TEPP]}_t/\mathrm{d}t. \qquad (2)$$

From (1) and (2) we have

$$[\mathrm{TEPP}]_t = [\mathrm{TEPP}]_0 \exp\{-(k[\mathrm{P}] + k')t\},$$

where

$$[\mathrm{P}] = [\mathrm{HPO_4}^{2-}].$$

The final enrichment of diethyl phosphate may be calculated as follows: We define

$$\alpha_t = \frac{\text{Total excess moles } ^{18}\text{O in phosphate ion}}{\text{Total number of moles of phosphate ion}} \text{ at time } t.$$

Then, the number of moles of ^{18}O lost per second from phosphate ion at time t is

$$-[\mathrm{P}]\frac{\mathrm{d}\alpha_t}{\mathrm{d}t} = -\frac{\alpha_t}{4}\cdot\frac{\mathrm{d[TEPP]}_t}{\mathrm{d}t}\text{ (catalysed)} = -\frac{\alpha_t}{4}k[\mathrm{P}][\mathrm{TEPP}]_t$$

$$= -\frac{\alpha_t}{4}k[\mathrm{P}][\mathrm{TEPP}]_0 \exp\{-(k[\mathrm{P}] + k')t\}.$$

Rearranging and integrating this expression gives

$$\int_0 \frac{\mathrm{d}\alpha_t}{\mathrm{d}t} = \frac{k}{4}\cdot[\mathrm{TEPP}]_0 \int_0^\infty \exp\{-(k[\mathrm{P}] + k')t\}\,.\,\mathrm{d}t$$

Then

$$\ln\frac{\alpha_0}{\alpha_\infty} = \frac{k}{4}\cdot\frac{[\mathrm{TEPP}]_t}{k[\mathrm{P}] + k'}.$$

I.e.,

$$\alpha_0/\alpha_\infty = \exp\left[\frac{k[\mathrm{TEPP}]_0}{4(k[\mathrm{P}] + k')}\right].$$

The total loss of oxygen-18 from phosphate ion after complete hydrolysis of the pyrophosphate is $(\alpha_0 - \alpha_\infty)$ (mol. of phosphate ion). This is also the number of mols. of diethyl phosphate each containing one atom of oxygen-18; so the excess percentage enrichment of the diethyl phosphate after complete hydrolysis is:

$$\frac{(\text{mol. of phosphate ion})\,(\alpha_0 - \alpha_\infty)}{\text{mol. of DEP formed}} \times \tfrac{1}{4} \times 100$$

(the factor $\frac{1}{4}$ is to allow for the four oxygen atoms in the DEP molecule)

$$= \frac{(\text{mol. of phosphate ion})}{4\,(\text{mol. of DEP formed})}\cdot 100\alpha_0\left(1 - \exp - \left[\frac{k[\mathrm{TEPP}]}{4(k[\mathrm{P}] + k')}\right]\right). \qquad (3)$$

In applying equation (3) to the present work, values of k and k', as defined in equations (1) and (2), were taken from Brown and Hamer's work[1] ($k = 8{\cdot}8 \times 10^{-3}$ l. mole^{-1} sec.$^{-1}$; $k' = 3{\cdot}3 \times 10^{-4}$ sec.$^{-1}$).

Results.—Tetraethyl pyrophosphate was hydrolyzed at 58—60° at pH 8·8 in the presence of [^{18}O]phosphate ion. The pH and temperature correspond to those used by Brown and Hamer.[1] When hydrolysis was complete, diethyl hydrogen phosphate was recovered and analysed for oxygen-18. The results are in Table 1 for two experiments.

TABLE 1. *Hydrolysis of tetraethyl pyrophosphate in the presence of* [^{18}O]*phosphate ion, at* pH 8·8 *and* 59° ± 1°.

[TEPP] (mole l.$^{-1}$)	[P] (mole l.$^{-1}$)	Initial excess atom % ^{18}O in phosphate ion	Excess atom % ^{18}O in DEP	Excess atom % ^{18}O in DEP, calc. from eqn. (3)
0·033	0·1	12·1	0·915, 0·908	1·05

Inorganic phosphate was recovered quantitatively by precipitation as barium phosphate and was analysed for oxygen-18. The total amounts of oxygen-18 in the products are shown in Table 2.

TABLE 2.

^{18}O in DEP (μ mole)	^{18}O in phosphate after reaction (μ mole)	O^{18} recovered (μ mole)	Initial O^{18} in phosphate (μ mole)	Recovery of ^{18}O (%)
146·4	2832	2978	2904	102·6
145·3	2829	2974	2904	102·4

It is necessary to consider alternative paths whereby oxygen-18 could be incorporated into diethyl phosphate. Preliminary exchange between phosphate ion and the solvent water and subsequent exchange between enriched water and diethyl phosphate may be excluded on two grounds. Oxygen-18 exchange between phosphate ion and water at pH 9 is virtually undetectable under the experimental conditions,[4] and even if exchange had been complete the final excess percentage enrichment of the water would be less than 0·1%. Subsequent exchange between water and diethyl phosphate could not account for the observed enrichment of 0·91% in diethyl phosphate. Another path for oxygen-18 transfer to diethyl phosphate might be an exchange between labelled phosphate ion and diethyl phosphate; two solutions, each containing diethyl hydrogen phosphate (0·1M) and disodium hydrogen phosphate (0·4M) (12·1 excess atom % of ^{18}O), were adjusted to pH 8·8 by sodium hydroxide and kept at 60° for 2 and 4 hr. severally; the recovered diethyl hydrogen phosphate contained 0·004 and 0·000 excess atom % of ^{18}O, which excludes this mode of exchange.

Experimental.—The hydrolyses were carried out, with stirring, in a beaker kept at 59° ± 1°. Tetraethyl pyrophosphate (2 μmoles) was added rapidly to a solution of disodium hydrogen phosphate (6 μmoles) (12·1 excess atom % of O^{18}) in water (60 ml.). Carbon dioxide-free N-sodium hydroxide was run in at a rate sufficient to maintain the pH at 8·7—8·9 (as measured by a glass electrode). Isolation of the products was begun after 90 min. (~9 half-lives). The isolation procedures given below were also used in obtaining the results of the exchange experiments mentioned in the preceding paragraph.

Isolation of inorganic phosphate. Inorganic phosphate was precipitated with a slight excess of barium hydroxide solution. The precipitate was filtered off, washed in water, and dissolved in N-hydrochloric acid, and the solution was filtered. Barium phosphate was reprecipitated by the addition of sodium hydroxide solution, and the precipitate centrifuged off and washed with water before being dried in a vacuum oven.

Isolation of diethyl hydrogen phosphate. The filtrate from the first barium phosphate precipitate was treated as follows: To ensure complete removal of [^{18}O]phosphate ion, an isotopic dilution was carried out. The pH of the solution was adjusted to 2—3 with N-hydrochloric acid, and disodium hydrogen phosphate (2·5 μmoles) and barium chloride (3·8 μmoles) were dissolved in it. The pH was adjusted to 8—9 with aqueous sodium hydroxide, and the resulting precipitate of barium phosphate was filtered off. A solution of barium hydroxide (2 μmoles) was added to the filtrate which was then concentrated to a final volume of ~3 ml. in a vacuum at room temperature. Addition of 4 volumes of 95% ethanol gave a precipitate of the barium diethyl phosphate, which was centrifuged off, washed with 80% ethanol, acetone, and ether, and dried in a vacuum at about 70° (Found: P, 13·8. Calc. for $C_4H_{10}Ba_{\frac{1}{2}}O_4P$: P, 14·0%).

Oxygen-18 analyses of the barium phosphate and barium diethyl phosphate were carried out by the method given by Anbar and Guttmann.[5]

Discussion.—The results show that the hydrolysis of tetraethyl pyrophosphate in the presence of [^{18}O]phosphate ion produces diethyl hydrogen [^{18}O]phosphate. The percentage enrichment of this agrees reasonably with that expected on the basis of a catalysed path involving the formation of the P^1P^1-diethyl pyrophosphate ion as an intermediate. Alternative paths for oxygen-18 transfer have been excluded by showing the absence of oxygen-18 exchange between diethyl hydrogen phosphate and phosphate ion under the experimental conditions obtaining during the hydrolysis.

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[4] Silver, unpublished results.

[5] Anbar and Guttmann, *J. Appl. Rad. Isotopes*, 1958, **3**, 131.

In conjunction with the mainly kinetic results of previous studies, this work strongly supports the existence of the P^1P^1-diethyl pyrophosphate ion.

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