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GEL CHROMATOGRAPHIC INVESTIGATION OF THE SUBSTITUTION REACTION BETWEEN DIPHOSPHONATE AND HYPOPHOSPHATE

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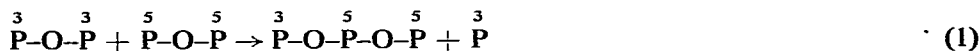
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SUMMARY

Gel chromatography was used to characterize the substitution reaction between diphosphonate and hypophosphate. In addition to a well-known trimeric oxo acid, $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}$, a hitherto unknown tetrameric oxo acid, $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$, was detected. The stability of the tetramer in aqueous solution was examined.

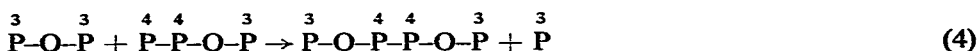
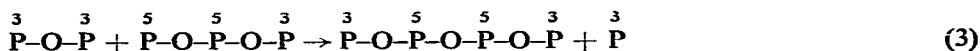
INTRODUCTION

It has been shown^{1,2} that diphosphate, $\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{P}}$, and hypophosphate, $\overset{4}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}$, react with diphosphonate, $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$, in aqueous solution to give the respective trimeric oxo acids of phosphorus as follows³:



The rationale for eqns. 1 and 2 is that they are S_N2 substitutions in which diphosphate and hypophosphate act as nucleophilic agents^{1,4}.

Since diphosphate and hypophosphate both have two identical terminal groups there is the possibility that further addition of $\overset{3}{\text{P}}$ -units to the trimeric products in eqns. 1 and 2 may take place as shown in eqns. 3 and 4 to give the tetrameric oxo acids with two terminal $\overset{3}{\text{P}}$ -units^{4,5}.



* The abbreviated notations³ and structural formulae for the oxo acids in the above equations are shown in Table I, as are those of other compounds referred to later.

TABLE I
 ABBREVIATED NOTATIONS FOR OXO ACIDS OF PHOSPHORUS

<i>Abbreviated notation*</i>	<i>Structural formula</i>
⁵ P	$\begin{array}{c} \text{O} \\ \\ \text{HO}-\text{P}-\text{OH} \\ \\ \text{OH} \end{array}$
³ P	$\begin{array}{c} \text{O} \\ \\ \text{H}-\text{P}-\text{OH} \\ \\ \text{OH} \end{array}$
⁵ P-O- ⁵ P	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{HO}-\text{P}-\text{O}-\text{P}-\text{OH} \\ \quad \\ \text{OH} \quad \text{OH} \end{array}$
⁵ P-O- ⁵ P	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{H}-\text{P}-\text{O}-\text{P}-\text{OH} \\ \quad \\ \text{OH} \quad \text{OH} \end{array}$
³ P-O- ³ P	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{H}-\text{P}-\text{O}-\text{P}-\text{H} \\ \quad \\ \text{OH} \quad \text{OH} \end{array}$
⁴ P-P	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \text{HO}-\text{P}-\text{P}-\text{OH} \\ \quad \\ \text{HO} \quad \text{OH} \end{array}$
⁵ P-O- ⁵ P-O- ⁵ P	$\begin{array}{c} \text{O} \quad \text{O} \quad \text{O} \\ \quad \quad \\ \text{HO}-\text{P}-\text{O}-\text{P}-\text{O}-\text{P}-\text{OH} \\ \quad \quad \\ \text{OH} \quad \text{OH} \quad \text{OH} \end{array}$
³ P-O- ⁵ P-O- ⁵ P	$\begin{array}{c} \text{O} \quad \text{O} \quad \text{O} \\ \quad \quad \\ \text{H}-\text{P}-\text{O}-\text{P}-\text{O}-\text{P}-\text{OH} \\ \quad \quad \\ \text{OH} \quad \text{OH} \quad \text{OH} \end{array}$
³ P-O- ⁵ P-O- ³ P	$\begin{array}{c} \text{O} \quad \text{O} \quad \text{O} \\ \quad \quad \\ \text{H}-\text{P}-\text{O}-\text{P}-\text{O}-\text{P}-\text{H} \\ \quad \quad \\ \text{OH} \quad \text{OH} \quad \text{OH} \end{array}$

TABLE I (continued)

Abbreviated notation*	Structural formula
³ P-O- ⁴ P- ⁴ P	$\begin{array}{c} \text{O} \quad \text{O} \quad \text{O} \\ \quad \quad \\ \text{H}-\text{P}-\text{O}-\text{P}-\text{P}-\text{OH} \\ \quad \quad \\ \text{HO} \quad \text{HO} \quad \text{OH} \end{array}$
³ P-O- ⁵ P-O- ⁵ P-O- ³ P	$\begin{array}{c} \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \\ \quad \quad \quad \\ \text{H}-\text{P}-\text{O}-\text{P}-\text{O}-\text{P}-\text{O}-\text{P}-\text{H} \\ \quad \quad \quad \\ \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{OH} \end{array}$
³ P-O- ⁴ P- ⁴ P-O- ³ P	$\begin{array}{c} \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \\ \quad \quad \quad \\ \text{H}-\text{P}-\text{O}-\text{P}-\text{P}-\text{O}-\text{P}-\text{H} \\ \quad \quad \quad \\ \text{HO} \quad \text{HO} \quad \text{OH} \quad \text{OH} \end{array}$

* Based on ref. 3.

Formation of ³P-O-⁵P-O-⁵P-O-³P in solution has been suggested on the basis of a gel chromatographic investigation¹. The present work was undertaken to obtain positive evidence for the formation of ³P-O-⁴P-⁴P-O-³P by the reaction of hypophosphate with diphosphonate. Gel chromatographic separation of ³P-O-⁴P-⁴P-O-³P was easily achieved with satisfactory resolution. The structural composition of this new species was identified using a colorimetric method by which ³P-units and ⁴P-units can be differentially determined.

The stability of ³P-O-⁴P-⁴P-O-³P in solution was also examined. It tended to decompose during storage at room temperature according to eqns. 5 and 6.



The nomenclature used in this paper, monomer, dimer, trimer, etc., is based on the number of phosphorus atoms in the molecule, e.g., ⁴P-⁴P and ³P-O-⁴P-⁴P-O-³P are dimer and tetramer, respectively.

EXPERIMENTAL

Reagents

Unless otherwise stated, guaranteed reagents from Wako (Osaka, Japan) were used without further purification.

Disodium diphosphonate ($\overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}}$) was prepared by chemical dehydration of a mixture of disodium phosphonate and phosphonic acid^{1,6}. Disodium dihydrogen hypophosphate hexahydrate ($\overset{4}{\text{P}}-\overset{4}{\text{P}}$) was prepared by oxidation of red phosphorus with 30% hydrogen peroxide⁷.

Gel chromatography

The experimental conditions for gel chromatography were as follows: column, 95×1.5 cm I.D., Sephadex G-25 (Pharmacia, Uppsala, Sweden); eluent, 0.1 M sodium chloride; one fraction, 12 drops by a drop counter (*ca.* 1 ml); sample volume, 1 ml.

Colorimetric determinations

The colorimetric determination of phosphorus compounds was carried out using a mixed molybdenum(V)–molybdenum(VI) reagent¹. The following procedures are recommended to permit the differential analysis of $\overset{3}{\text{P}}$ -units, $\overset{5}{\text{P}}$ -units and $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units by a heteropoly-blue method^{1,8}.

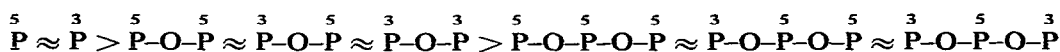
Procedure I: to an aliquot of sample solution in a 25 ml volumetric flask 2 ml of the molybdenum(V)–molybdenum(VI) reagent was added. The solution was allowed to stand for 30 min at room temperature. The absorbance at 830 nm was measured. $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units can be selectively detected by this procedure.

Procedure II: (see reference 1) $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units and $\overset{5}{\text{P}}$ -units can be determined by this procedure.

Procedure III: (see reference 1) total absorbance due to $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units, $\overset{5}{\text{P}}$ -units and $\overset{3}{\text{P}}$ -units can be determined by this procedure.

RESULTS AND DISCUSSION

As gel chromatography plays an important role in characterizing the phosphorus compounds produced in these substitution reactions, the efficiency of the gel column must be examined. Gel chromatographic separation is based on the difference in molecular sizes of the sample components, and is useful for the group separation of monomeric, dimeric and trimeric oxo acids of phosphorus with no P–P linkages¹. For example, it has been shown that the elution volumes of orthophosphate, phosphonate, diphosphate, isohypophosphate, diphosphonate, triphosphate and two new trimeric oxo acids, $\overset{3}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{O}}-\overset{3}{\text{P}}$ and $\overset{3}{\text{P}}-\overset{5}{\text{O}}-\overset{3}{\text{P}}$, decrease in the increasing order of their degrees of polymerization, regardless of the oxidation states of phosphorus atoms^{1,9}:



The Sephadex G-25 column used in this work was confirmed to give satisfactory separation of $\overset{5}{\text{P}}$, $\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{O}}-\overset{5}{\text{O}}-\overset{3}{\text{P}}$ and $\overset{3}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{O}}-\overset{5}{\text{O}}-\overset{3}{\text{P}}$, as reported previously¹.

Eqns. 2 and 4 include phosphorus oxo acids with $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units. Therefore, it is also necessary to establish an analytical technique by which various phosphorus oxo acids with $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units can be separated and determined. An elution profile for an equimolar mixture of phosphonate, diphosphonate and hypophosphate on the Sephadex G-25 column is shown in Fig. 1. Diphosphonate, $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$, and hypophosphate, $\overset{4}{\text{P}}-\overset{4}{\text{P}}$, are the starting materials in eqns. 2 and 4, but they do not react to an appreciable extent at room temperature. Procedures I and III were used to permit the differential analysis of $\overset{3}{\text{P}}$ -units and $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units in each fraction. By procedure I the $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units are selectively detected to give the broken line in Fig. 1. The solid line represents the distribution of $\overset{3}{\text{P}}$ -units plus $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units obtained from procedure III. It is noted that $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$ is hydrolysed during procedure III to produce $\overset{3}{\text{P}}$, detected as orthophosphoric heteropoly-blue, whereas $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units remain unhydrolysed during procedures I and III and can be detected as hypophosphoric heteropoly-blue.

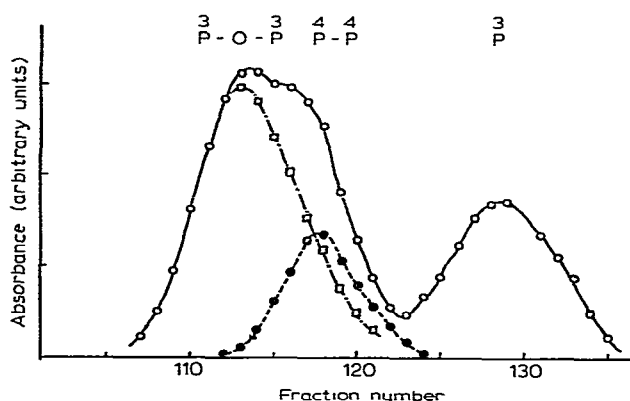


Fig. 1. Gel chromatographic separation of phosphonate, diphosphonate and hypophosphate; each $20 \mu\text{mol}$ of $\overset{3}{\text{P}}$, $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$ and $\overset{4}{\text{P}}-\overset{4}{\text{P}}$.

The difference in height between the solid and broken lines in Fig. 1 represents the distribution of $\overset{3}{\text{P}}$ -units. A well-defined peak of $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$ thus can be obtained, the trailing part of which is shown by a broken line. The fact that dimeric $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ is eluted later than dimeric $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$ is of interest and will be useful in predicting the elution positions of trimers and tetramers with $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units, e.g. $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{4}{\text{P}}-\overset{4}{\text{P}}$ and $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{4}{\text{P}}-\overset{4}{\text{P}}-\overset{3}{\text{O}}-\overset{4}{\text{P}}$.

Formation of $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}-\overset{4}{\text{P}}$ and $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}-\overset{4}{\text{P}}-\overset{3}{\text{O}}-\overset{4}{\text{P}}$

The following experiment was carried out to obtain the gel chromatographic

pattern for the reaction products formed by the method of Blaser and Worms² which is based on eqn. 2.

In 6 ml of hot water 1.9 g of disodium dihydrogen hypophosphate hexahydrate ($\text{Na}_2\text{H}_2\text{P}_2\text{O}_6 \cdot 6\text{H}_2\text{O}$) was dissolved and then 3.3 ml of 2 M sodium hydroxide was added. While the solution was stirred, 0.47 g of disodium diphosphonate ($\text{Na}_2\text{H}_2\text{P}_2\text{O}_5$) was added and the mixture heated for 5 min at *ca.* 100° in a water bath.

The unchanged $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ was precipitated by rapid cooling of the solution and filtered out. An aliquot of the filtrate was separated by gel chromatography on the Sephadex G-25 column.

$\overset{3}{\text{P}}$ -units and $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units were differentially determined (Fig. 2) as indicated above. In addition to the peaks for two starting oxo acids, $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$ and $\overset{4}{\text{P}}-\overset{4}{\text{P}}$, three peaks appeared at the elution positions of monomer, trimer and tetramer. Only the leading edge of the peak for $\overset{3}{\text{P}}$ is shown. It is evident that both trimer and tetramer contain not only $\overset{3}{\text{P}}$ -units but also $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units. The main component, with equal amounts of $\overset{3}{\text{P}}$ -units and $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units, at the trimer position can be assigned to $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}-\overset{3}{\text{P}}$. The ratio of $\overset{3}{\text{P}}$ -units to $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -units for the tetramer was found subsequently to be 2:1, which suggests the formation of a hitherto unknown tetramer, $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}-\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{3}{\text{P}}$.

As with $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{5}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$ and $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{3}{\text{P}}$, which have two terminal $\overset{3}{\text{P}}$ -units¹, $\overset{3}{\text{P}}-\overset{4}{\text{O}}-\overset{4}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$ is likely to be formed by substitution according to eqn. 4 and/or 7.

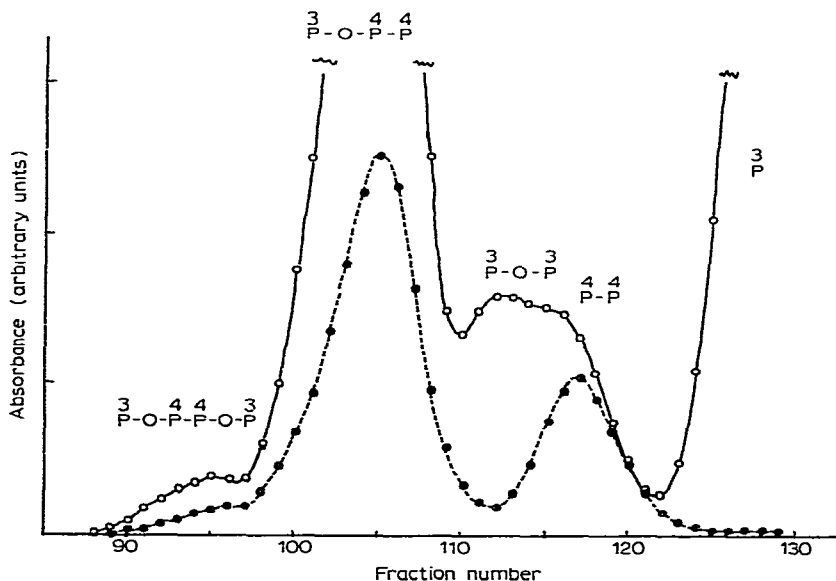


Fig. 2. Gel chromatographic profile for the reaction products between diphosphonate and hypophosphate. Solid line, $\overset{3}{\text{P}}$ -unit + $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -unit; broken line, $\overset{4}{\text{P}}-\overset{4}{\text{P}}$ -unit.

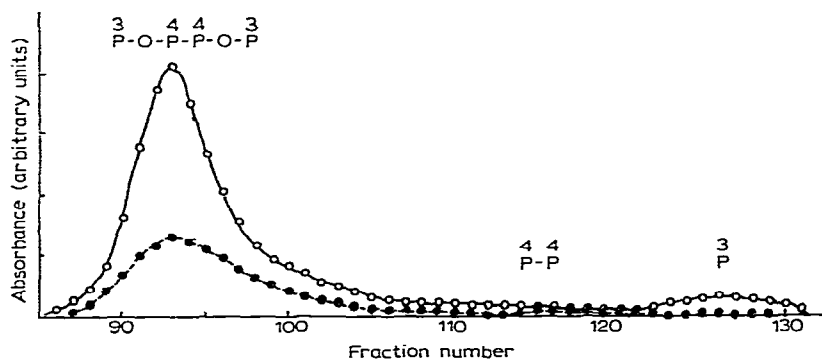
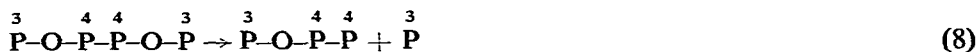


Fig. 4. Gel chromatographic evaluation of the decomposition of $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-O-}\overset{3}{\text{P}}$: immediately after fractionation.

$\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-}\overset{3}{\text{P}}$ (Fig. 5). After 7 days $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}$ had also decomposed to an appreciable extent (Fig. 6). The overall indication is that $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ is probably hydrolysed according to eqns. 8 and 9 to give $\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}$ and $\overset{3}{\text{P}}$ as final products.



$\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}$ is very stable in aqueous solution and is hydrolysed according to eqn. 10 only at high acid concentration¹⁰. Therefore, decomposition of $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ by $\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}$ bond cleavage (eqn. 11) seems less probable.

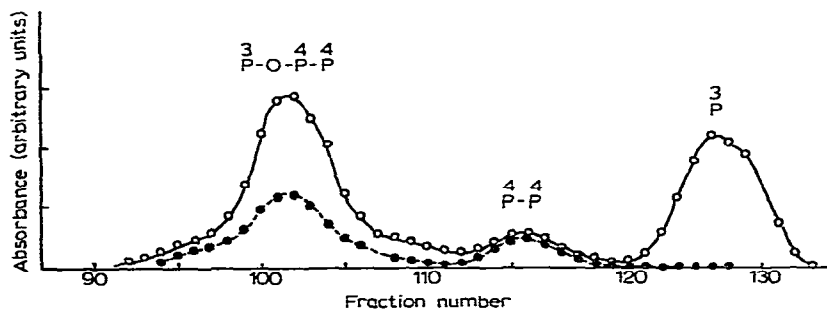


Fig. 5. Gel chromatographic evaluation of the decomposition of $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-O-}\overset{3}{\text{P}}$: after 3 days.

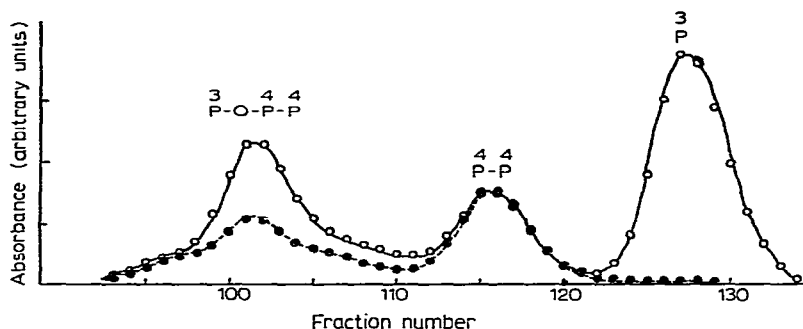


Fig. 6. Gel chromatographic evaluation of the decomposition of $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-O-}\overset{3}{\text{P}}$: after 7 days.

The amounts of $\overset{3}{\text{P}}$ -units at the elution positions of dimers tend to increase during decomposition of $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}\text{-O-}\overset{3}{\text{P}}$, which cannot be explained by eqns. 8 and 9. By analogy with the formation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ according to eqn. 12 (ref. 2), the formation of $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ through eqn. 13, the reverse of eqn. 2, seemed not unlikely.



This reaction was investigated by allowing a mixed solution composed of 0.07 M $\overset{3}{\text{P}}$ and 0.01 M $\overset{3}{\text{P}}\text{-O-}\overset{4}{\text{P}}\text{-}\overset{4}{\text{P}}$ to stand at room temperature. After two weeks evidence was obtained that dimer with $\overset{3}{\text{P}}$ -units had formed, but in insufficient amount to permit the quantitative analysis. Further mechanistic investigations are required if such complex behaviour is to be explained.

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