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GEL AND ION-EXCHANGE CHROMATOGRAPHIC PURIFICATION OF THE REACTION PRODUCTS BETWEEN DIPHOSPHATE AND DIPHOSPHONATE

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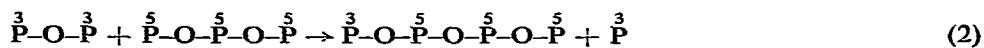
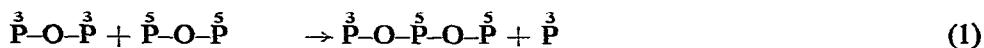
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SUMMARY

Gel and ion-exchange chromatographic techniques were applied to the separation of lower oxo acids of phosphorus, $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ and $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{3}{\text{P}}$, produced by the substitution reaction between $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ (diphosphonate) and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ (diphosphate). Sephadex G-25 and Bio-Rad AG 1-X8 columns were used. Hydrolysis of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ in the process of preparative separation is also discussed.

INTRODUCTION

Much interest has recently been focused on the chemistry of a series of lower oxo acids of phosphorus that contain one or two phosphorus atoms with an oxidation number of 3 at the terminal positions¹⁻³. Of these lower oxo acids, shown in Table I, only three compounds, $\overset{3}{\text{P}}$ (phosphonate), $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ (diphosphonate) and $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ (isohypophosphate), have been isolated and well characterized^{4,5}. The synthesis of polymers higher than dimers is our major concern. The formation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ and $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ in solution according to eqns. 1 and 2 has been suggested on the basis of a gel chromatographic investigation¹. In these substitution reactions (S_N2), $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ (diphosphate) and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ (triphosphate) are assumed to act as nucleophilic agents, respectively²:



No appropriate single-step separation method has been developed in our laboratory for the selective isolation of these compounds from the reaction mixtures represented by eqns. 1 and 2. Therefore, it is important to employ chromatographic techniques in order to obtain $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ or $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ of high purity before

TABLE I

LOWER OXO ACIDS OF PHOSPHORUS WITH ONE OR TWO PHOSPHORUS ATOMS OF OXIDATION NUMBER 3 AT THE TERMINAL POSITIONS

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

* Based on ref. 6.

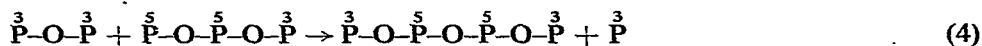
the samples are subjected to subsequent treatment by methods such as precipitation and lyophilization.

This paper describes the gel and ion-exchange chromatographic purification of $\overset{3}{\text{P}}-\text{O}-\overset{5}{\text{P}}-\text{O}-\overset{5}{\text{P}}$ on a preparative scale from the products of the reaction between $\overset{3}{\text{P}}-\text{O}-\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}-\text{O}-\overset{5}{\text{P}}$. The chromatographic separation of $\overset{3}{\text{P}}-\text{O}-\overset{5}{\text{P}}-\text{O}-\overset{5}{\text{P}}$ was easily achieved with satisfactory resolution, the purity of the product being greater than 90%.

Although $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ in solution was stable in a refrigerator (-5°), it tended to decompose according to eqn. 3 on storage at room temperature or in the process of chromatographic desalting:



In the course of the gel chromatographic purification of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$, evidence for the formation of a hypothetical tetramer, $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{3}{\text{P}}$, was obtained, which was assumed to be based on the substitution reaction in eqn. 4:



EXPERIMENTAL

Reagents

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

One of the starting materials, $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$, for the synthesis of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ was prepared according to the literature⁷ with a slight modification. In an erlenmeyer flask with a glass stopper, 108 ml of acetic anhydride was added to a mixture of 21.6 g of disodium phosphonate pentahydrate ($\text{Na}_2\text{PHO}_3 \cdot 5\text{H}_2\text{O}$) and 8.2 g of phosphonic acid (H_2PHO_3). The resulting mixture was agitated for 5 days to give a white precipitate, which was suspended in acetone and centrifuged several times in order to remove acetic anhydride. The air-dried precipitate was dissolved in a minimum volume of water and recrystallized from ethanol. The purity of the disodium diphosphonate ($\text{Na}_2\text{P}_2\text{H}_2\text{O}_5$) thus obtained was checked by gel chromatography to be 75–95% (as P).

Preparation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$

This preparation was carried out according to eqn. 1. Tetrasodium diphosphate decahydrate ($\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$) (22.3 g) was dissolved in 50 ml of water by heating on a water-bath. To the $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution, 19.5 g of disodium phosphonate ($\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$) were added and mixed well. The mixture was maintained in a thermostat at 50° for 5 h, then allowed to stand for 1 h in a refrigerator in order to precipitate the remaining $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. An aliquot of the filtrate was subjected to the subsequent chromatographic experiments.

Colorimetric determination and identification of phosphorus compounds

The colorimetric determination was carried out according to the orthophosphoric heteropoly-blue method, by which phosphorus of oxidation numbers 3 and 5 can be determined differentially⁸. The mixed molybdenum(V)–molybdenum(VI) solution used was prepared according to the method of Hosokawa and Oshima⁹, in which the use of metallic zinc was recommended for reduction of molybdenum(VI) to molybdenum(V), instead of metallic mercury as reported by Lucena-Conde and Prat¹⁰.

A 1 *M* molybdenum(VI) solution was prepared by dissolving 35 g of ammonium molybdate tetrahydrate [(NH₄)₆Mo₇O₂₄·4H₂O] in 400 ml of 6 *M* hydrochloric acid. The subsequent procedure for the preparation of molybdenum(V)–molybdenum(VI) reagent should be carried out at low temperature with ice cooling. About 3 g of metallic zinc were then added to this 1 *M* molybdenum(VI) solution. When the zinc had completely dissolved, part of the molybdenum(VI) was reduced to molybdenum(V), giving a change in the colour of the solution from light yellow to brown. To the resulting mixed solution, 200 ml of concentrated hydrochloric acid and then 400 ml of concentrated sulphuric acid were slowly added. The solution was diluted to 1 l with deionized water to give a green molybdenum(V)–molybdenum(VI) reagent solution that is stable for at least several months at room temperature.

To an aliquot of sample solution in a 25-ml volumetric flask, 2 ml of the molybdenum(V)–molybdenum(VI) reagent were added for the determination of phosphorus of oxidation number 5, *i.e.*, the $\overset{5}{\text{P}}$ unit (procedure II in ref. 8). If total phosphorus ($\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}$ units) was to be determined, both 1 ml of 1 *M* sodium hydrogen sulfite solution and 2 ml of the molybdenum(V)–molybdenum(VI) reagent were added (procedure III⁸). The subsequent treatment was common to the two procedures⁸. The mixed solution was then diluted to about 20 ml with water and allowed to stand for 1 h at about 95° in a water-bath. After the volume of the solution had been adjusted to the mark with water at room temperature, the absorbance was measured with a Hitachi 101 spectrophotometer at 830 nm (orthophosphoric heteropoly-blue).

By means of these two procedures⁸, the amounts of $\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}$ units can be determined differentially. In chromatographic experiments, each fraction, diluted with water if necessary, was subjected to the determination of $\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}$ units in order to identify the structural compositions of the oxo anions of phosphorus in the fractions.

Anion-exchange chromatography

The gradient elution technique was employed to separate $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ by anion-exchange chromatography. The elution conditions were as follows: column, 87 × 2.7 cm I.D., Bio-Rad AG 1-X8 (100–200 mesh); mixing bottle, 0.1 *M* sodium chloride solution, 750 ml; reservoir, 0.35 *M* sodium chloride solution; one fraction, 20 ml; sample volume, 20 ml.

Gel chromatography

Unless otherwise stated, the experimental conditions for gel chromatography were as follows: column, 90 × 1.5 cm I.D., Sephadex G-25 Fine (Pharmacia, Uppsala, Sweden); eluent, 0.1 *M* sodium chloride solution; one fraction, 12 drops by a drop counter (about 1 ml); sample volume, 1 ml.

RESULTS AND DISCUSSION

Efficiency of gel chromatographic column

The gel chromatographic separation technique is based on differences in the

molecular sizes of the sample components, and is useful for the group separation of monomeric, dimeric and trimeric oxo acids of phosphorus, regardless of the oxidation states of the phosphorus atoms^{11,12}. In order to demonstrate the efficiency of the Sephadex G-25 column, an elution pattern is shown in Fig. 1 for an equimolar mixture of three homologous species, $\overset{5}{\text{P}}$, $\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{P}}$ and $\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{P}}$, whose chemical properties and gel chromatographic behaviour have been well characterized. Either procedure II or III⁸ can be employed to obtain similar elution profiles.

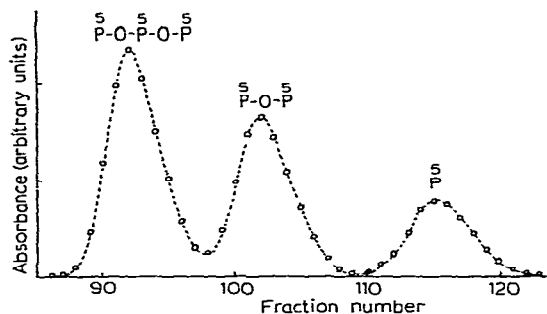


Fig. 1. Gel chromatographic separation of orthophosphate, diphosphate and triphosphate. Sample: 3 μmole each of $\overset{5}{\text{P}}$, $\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{P}}$ and $\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{P}}-\overset{5}{\text{O}}-\overset{5}{\text{P}}$.

Fig. 2 shows an elution curve for a mixture of $\overset{3}{\text{P}}$ and $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$. Procedure III⁸ was employed to determine the $\overset{3}{\text{P}}$ unit. It should be noted that if Procedure II⁸ is applied to the determination of $\overset{3}{\text{P}}$ compounds (Fig. 2), no peaks are observed, because Procedure II⁸ is unable to detect the $\overset{3}{\text{P}}$ unit. The $\overset{3}{\text{P}}$ unit can be detected only in the presence of sodium hydrogen sulphite (Procedure III⁸), which is based on the oxidation of the $\overset{3}{\text{P}}$ unit to the $\overset{5}{\text{P}}$ unit.

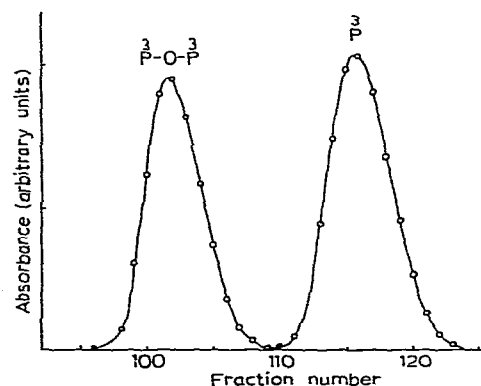


Fig. 2. Gel chromatographic separation of phosphonate and diphosphonate. Sample: 16 μmole of $\overset{3}{\text{P}}$ and 8 μmole of $\overset{3}{\text{P}}-\overset{3}{\text{O}}-\overset{3}{\text{P}}$.

Gel chromatographic separation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$

The gel chromatographic technique described above was applied to the characterization of the products of the reaction between $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. From eqn. 1, one can expect the presence of $\overset{3}{\text{P}}$ and $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ in the reaction mixture, in addition to the two starting materials. In such instances the differential analysis of $\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}$ units in the effluent is required in order to identify the components in the sample.

The reaction mixture (filtrate) obtained by the procedure described under Experimental was diluted with water (about 10-fold) and 1 ml of the solution was applied to the Sephadex G-25 column. Each fraction was subjected to the colorimetric differential analysis by both procedures II and III⁸, and an elution profile obtained is shown in Fig. 3. The solid line represents the distribution of $\overset{3}{\text{P}}$ plus $\overset{5}{\text{P}}$ units (procedure III⁸) in the fractions and the broken line represents only the $\overset{5}{\text{P}}$ unit (procedure II⁸). The difference in height between the two distribution curves represents the $\overset{3}{\text{P}}$ unit.

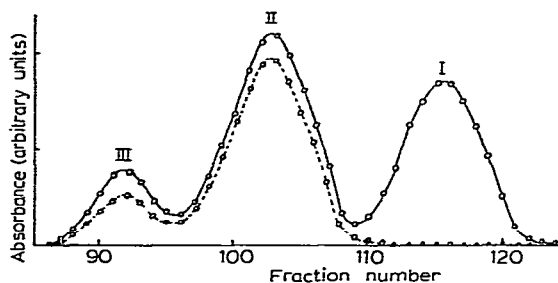


Fig. 3. Gel chromatographic profile of the products of the reaction between $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. Solid line, $\overset{3}{\text{P}} + \overset{5}{\text{P}}$ units; broken line, $\overset{5}{\text{P}}$ unit.

As expected, the monomer peak (I) in Fig. 3 is composed only of the $\overset{3}{\text{P}}$ unit, *i.e.*, $\overset{3}{\text{P}}$, which may arise not only from the substitution reaction in eqn. 1 but also from the two hydrolytic reactions in eqns. 3 and 5:



The dimer peak (II) can be assigned to the overlapping peaks of the two remaining reactants, $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$.

The ratio of $\overset{5}{\text{P}}$ to $\overset{3}{\text{P}}$ units in the trimer peak (III) was confirmed to be 2.0, as has been suggested in a previous paper¹, which can be expected from the composition of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. The relative amount of the trimer to the total phosphorus in Fig. 3 was calculated to be 14% (as P). This value is not strictly reproducible under the experimental conditions employed because of the difficulty in controlling the pH

of the solution, which decreases with the progress of the hydrolytic reactions (eqns. 3 and 5). The reaction temperature is another important factor that controls the extent of the hydrolytic reactions and, consequently, affects the yield of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. For example, when the reaction was carried out at 100° only a trace amount of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ was formed, while at room temperature a yield of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ of about 30% has been suggested¹.

Anion-exchange chromatographic separation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$

The ion-exchange chromatographic separation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ on the Bio-Rad AG 1-X8 column was also used to examine the products of the reaction between $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. A sample was prepared according to the procedure described for the gel chromatographic separation. As shown in Fig. 4, three peaks were obtained. The two starting materials gave an overlapping peak (II), while peak I represents $\overset{3}{\text{P}}$. In addition, a well resolved peak (III), assigned to $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$, was observed behind the elution position of the starting materials. The ratio of the $\overset{5}{\text{P}}$ to the $\overset{3}{\text{P}}$ units in peak III was confirmed to be 2.0, which is in accordance with the conclusion drawn from the gel chromatographic characterization.

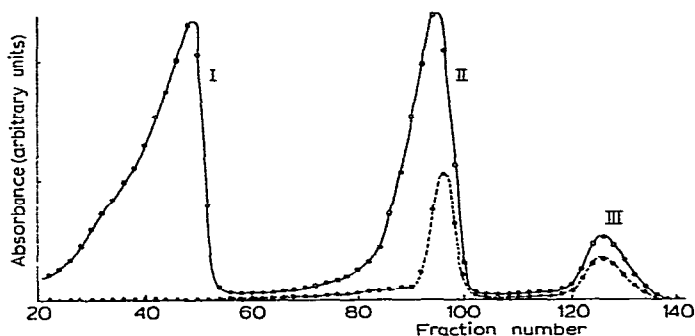


Fig. 4. Anion-exchange chromatographic profile of the products of the reaction between $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. Solid line, $\overset{3}{\text{P}} + \overset{5}{\text{P}}$ units; broken line, $\overset{5}{\text{P}}$ unit.

Stability of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ in aqueous solution

The $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution fractionated by anion-exchange chromatography was re-chromatographed on the Sephadex G-25 column in order to examine the purity of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. The main peak appeared at the elution position of trimer. The purity was confirmed to be as high as 98% (as P) if the $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ fraction was fresh. An aqueous solution of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ remained stable for at least 4 weeks at pH 7 in a refrigerator (about -5°). However, it was not as stable as the well known trimer

$\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$, and tended to decompose, the decomposition being greatly dependent on the temperature and pH of the solution. Although $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ seems to be decomposed mainly according to eqn. 3 to form $\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$, there is no satisfactory explanation for the facts that the increase in the amount of $\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ is different from that expected from only the hydrolysis of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ in eqn. 3 and the relative amount of the $\overset{3}{\text{P}}$ unit at the elution position of the dimers tends to increase gradually with time. As it is not likely that $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ is formed by the polymerization of $\overset{3}{\text{P}}$ (ref. 2), more complicated reactions may be involved in the decomposition of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. The formation of $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ by the reverse reaction of eqn. 1 and the formation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ by eqn. 6 are less probable, but are not completely excluded.



A mechanistic investigation is in progress and details will be presented elsewhere.

Desalting and lyophilization

As the $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ fractions obtained by anion-exchange chromatography contain large amounts of sodium chloride used as the eluting agent, it is necessary first to remove the sodium chloride by desalting before the $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution is subjected to lyophilization or precipitation. A sample solution to be desalted (sample A) was prepared by concentrating $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ fractions (about 200 ml) to about 10 ml under reduced pressure at room temperature.

A large Sephadex G-10 column (2.7 cm I.D.) was employed, instead of the Sephadex G-25 column used in the analytical separations, to accomplish the gel chromatographic desalting of 5 ml of sample A using deionized water as the eluent. By this procedure, sodium chloride can easily be separated from phosphorus compounds (Fig. 5). The elution curve for phosphorus compounds shown in Fig. 5 was obtained by the colorimetric procedure III⁸, while for sodium chloride the gravimetric method was employed.

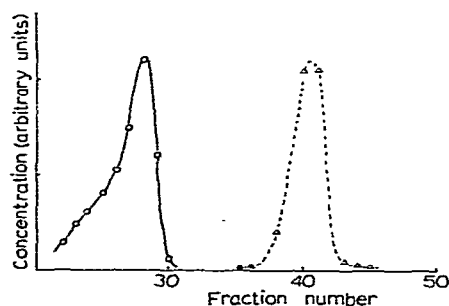


Fig. 5. Gel chromatographic desalting diagram for $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution. Column, 75 × 2.7 cm I.D. Sephadex G-10; eluent, water; fraction volume, 5 ml. Solid line, distribution of phosphorus compounds; broken line, distribution of sodium chloride.

The fractions containing phosphorus compounds shown in Fig. 5 were collected and concentrated to about 10 ml under reduced pressure at room temperature. An aliquot of this desalted and concentrated solution (sample B) was chromatographed on the Sephadex G-25 column in order to examine the decomposition of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ in the course of desalting. As shown in Fig. 6, the relative amount of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ was 66% (as P), which was considerably lower than the 82% in sample A before desalting (Fig. 7) and the 98% in the original $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution obtained by anion-exchange fractionation.

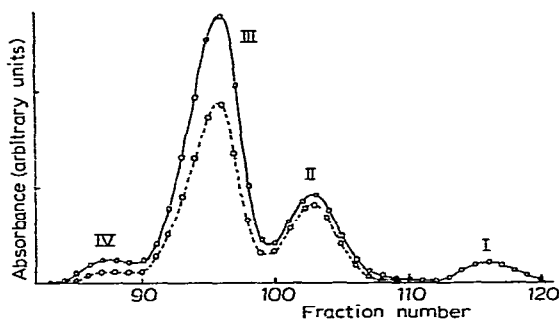


Fig. 6. Gel chromatographic profile for $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution after desalting. Solid line, $\overset{3}{\text{P}} + \overset{5}{\text{P}}$ units; broken line, $\overset{5}{\text{P}}$ unit. Peak III corresponds to $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$.

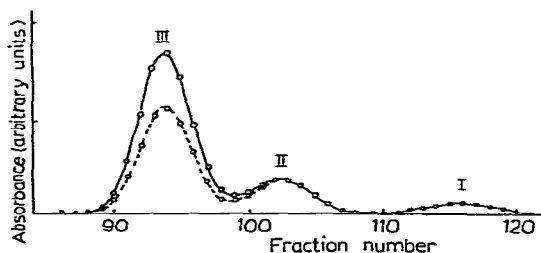
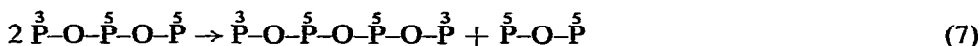


Fig. 7. Gel chromatographic profile for $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution before desalting. Solid line, $\overset{3}{\text{P}} + \overset{5}{\text{P}}$ units; broken line, $\overset{5}{\text{P}}$ unit. Peak III corresponds to $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$.

Sample B was evaporated to dryness under reduced pressure at room temperature in order to obtain solid $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$, but the solid obtained was confirmed by gel chromatography to be not $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$, but a mixture of $\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. On the other hand, if sample B was subjected to lyophilization (2 days) a gelatinous material was obtained in which the concentration of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ was 40% (as P).

An interesting observation in Fig. 6 is the appearance of a small peak (IV) before the peak of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. The peak corresponds to the elution position of a tetramer of the oxo anion of phosphorus. The ratio of the $\overset{5}{\text{P}}$ to the $\overset{3}{\text{P}}$ units in the tetramer was

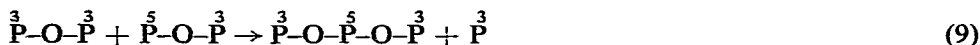
1:1, which could be expected from the composition $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{3}{\text{P}}$. This compound is an unknown one and is likely to be formed by eqn. 4 or 7:



Positive evidence for the formation of oxo acids such as $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ with two terminal $\overset{3}{\text{P}}$ units was also given by the formation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ in the reaction between $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ and $\overset{5}{\text{P}}$. A mixed solution of 1.25 M $\overset{3}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ and 0.25 M $\overset{5}{\text{P}}$ (disodium salt) was allowed to stand at room temperature. At an early stage of the reaction the formation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ according to the well characterized reaction in eqn. 8 was clearly indicated by the increase of the $\overset{5}{\text{P}}$ unit at the elution position of the dimer on the Sephadex G-25 column:



Before the elution position of the dimer, a well defined peak appeared, which at first increased with time and then tended to decrease again. After 2 days, the concentration of the trimer reached a maximum value of 1.8% (as P), which was less than the 16% of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}$. The ratio of the $\overset{5}{\text{P}}$ to the $\overset{3}{\text{P}}$ units of the trimer was 1:2, which suggests the formation of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{3}{\text{P}}$ according to eqn. 9 and/or eqn. 10:



Precipitation with metal ions

In order to obtain metal salts of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$, calcium or lanthanum ions were added to the $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ solution fractionated chromatographically. Unfortunately, $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ was catalytically decomposed in the presence of such metal ions to give precipitates of $\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$ and/or $\overset{3}{\text{P}}$, but not of $\overset{3}{\text{P}}\text{-O-}\overset{5}{\text{P}}\text{-O-}\overset{5}{\text{P}}$.

REFERENCES

- 1 N. Yoza, M. Tokuda, T. Inoue and S. Ohashi, *Inorg. Nucl. Chem. Lett.*, 9 (1973) 385.
- 2 N. Yoza and S. Ohashi, *Kagaku-no-Ryoiki*, 27 (1973) 897.
- 3 S. Ohashi, *Pure Appl. Chem.*, 44 (1975) 415.
- 4 J. R. Van Wazer, *Phosphorus and its Compounds*, Vol. 1, Interscience, New York, 1958, p. 345.
- 5 S. Ohashi, in H. Grayson and J. Griffith (Editors), *Topics in Phosphorus Chemistry*, Vol. 1, Interscience, New York, 1964, p. 114.
- 6 B. Blaser and K.-H. Worms, *Z. Anorg. Allg. Chem.*, 300 (1959) 225.
- 7 B. Blaser and K.-H. Worms, *Z. Anorg. Allg. Chem.*, 311 (1961) 313.
- 8 N. Yoza and S. Ohashi, *Bull. Chem. Soc. Jap.*, 37 (1964) 37.
- 9 I. Hosokawa and F. Oshima, *Wat. Res.*, 7 (1973) 283.
- 10 F. Lucena-Conde and L. Prat, *Anal. Chim. Acta*, 16 (1957) 473.
- 11 Y. Ueno, N. Yoza and S. Ohashi, *J. Chromatogr.*, 52 (1970) 469, 481.
- 12 N. Yoza, *J. Chromatogr.*, 86 (1973) 325.