

NOTE

A SIMPLE PROCEDURE FOR PRODUCING OXYGEN-LABELLED  
INORGANIC PHOSPHATE FROM ISOTOPICALLY  
LABELLED WATER THAT OPTIMIZES  
YIELD BASED ON THE ISOTOPE

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**Summary**

Phosphorus pentachloride can be treated with stoichiometric amounts of oxygen-labelled water to produce 80-90 percent yields of labelled inorganic phosphate when the reaction is conducted in dioxane. Any phosphorus-chlorine bonds remaining at the end of the reaction are eliminated by reaction with diethylamine prior to adding water.

Key Words: [ $^{18}\text{O}$ ]-labelled; [ $^{17}\text{O}$ ]-labelled; inorganic phosphate.

**Introduction**

The Risley-Van Etten procedure for producing [ $^{18}\text{O}$ ] $_4\text{PO}_4$  by reaction of  $\text{PCl}_5$  with excess [ $^{18}\text{O}$ ] $\text{H}_2\text{O}$  provides excellent yields of product, based on  $\text{PCl}_5$ , but is inefficient from the standpoint of utilizing the isotopic label. While this may be tolerable when supplies of [ $^{18}\text{O}$ ] water are abundant, it clearly is unacceptable for [ $^{17}\text{O}$ ] $\text{H}_2\text{O}$ . In an attempt to maximize incorporation of the label, we studied the reaction of  $\text{PCl}_5$  with stoichiometric amounts of water in sealed evacuated tubes. However, the reaction produced polyphosphates and the yield of [ $^{18}\text{O}$ ] $_4\text{PO}_4$  was low. However, when the reaction was conducted in dioxane, 80-90% yields based on [ $^{18}\text{O}$ ] $\text{H}_2\text{O}$  were obtained. This communication briefly describes our procedure.

**Experimental Section**

Excess  $\text{PCl}_5$  was weighed into one arm of a Y-tube assembly, placed under high vacuum and partially sublimed into a second, detachable arm that previously had been weighed. After admitting dry air into the system, the detachable arm was removed, closed and reweighed. Approximately 0.3 ml of

Dioxane (Spectrograde, dried over  $\text{CaH}_2$ ) was added per 100  $\mu\text{moles}$  of  $\text{PCl}_5$ . After the  $\text{PCl}_5$  dissolved, four equivalents of  $[\text{}^{18}\text{O}]\text{H}_2\text{O}$  in a similar volume of dioxane was added and the mixture allowed to stand 30 min at room temperature. Excess dry diethylamine was added directly and the mixture allowed to stand for a second 30-min period. The reaction mixture was diluted 10-fold with water and absorbed on a column of Dowex-1-8%,  $\text{HCO}_3^-$  form. Inorganic phosphate was separated via gradient elution with 0-0.5 M  $(\text{Et}_3\text{NH})(\text{HCO}_3)$ . After eliminating most of the salt by lyophilization, the remaining salt plus the  $\text{Et}_3\text{NH}^+$  counterion was eliminated by dissolving the residue in water, adding freshly washed Dowex-50- $\text{H}^+$  until the pH remained below 5 on subsequently swirling under vacuum, and passing the solution through a short column of the same resin. The pH of the effluent was adjusted to 7 with NaOH: yield, 80-90% based on  $[\text{}^{18}\text{O}]\text{H}_2\text{O}$  used.

#### Results/Discussion

Our initial modification of the Risley-Van Etten procedure involved the use of  $\text{PCl}_5$  sublimed under vacuum in order to avoid problems that could arise from manipulating small quantities of  $\text{PCl}_5$  in air that is less than scrupulously dry. In this way as little as 21 mg (100  $\mu\text{moles}$ ) of  $\text{PCl}_5$  could be used conveniently without working in a dry box. Even smaller quantities likely could be employed.

A number of studies were conducted where sublimed  $\text{PCl}_5$  and 4 equivalents of ice, frozen in a small container, was added to the  $\text{PCl}_5$  at liquid nitrogen temperature and allowed to react on thawing under vacuum. Not only was the yield of inorganic phosphate quite low due to formation of polyphosphates, it was even lower when the product of the reaction was treated with ammonia before ion exchange chromatography. This observation suggests that P-Cl bonds remained at the end of the above reaction.

However, the yield of polymeric phosphates was substantially reduced by using dioxane as a solvent for the reaction. The subsequent addition of diethylamine eliminated any remaining P-Cl bonds. Subsequent chromatographic separation of the products produced an  $85 \pm 5\%$  conversion of  $[\text{}^{18}\text{O}]\text{H}_2\text{O}$  into

$[^{18}\text{O}]_4\text{PO}_4$ . High conversions are particularly desirable when working with  $[^{17}\text{O}]\text{H}_2\text{O}$  or even with  $[^{18}\text{O}]\text{H}_2\text{O}$ , given the current scarcity of this isotope.

**References**

1. Risley, J. M., and Van Etten, R. L.-J. Label. Compound. Radiopharm. 15:533(1978).