Toxicity

The toxicity of the tetraalkyl pyrophosphates was determined by intraperitoneal injection on male white mice. The toxicity data are summarized in Table I.

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Summary

1. Tetraethyl pyrophosphate has been prepared by the controlled hydrolysis of diethyl chlorophosphate. Removal of hydrogen chloride was effected either under reduced pressure or by chemical treatment with bases such as pyridine or sodium hydrogen carbonate.

2. The method of controlled hydrolysis of dialkyl chlorophosphates in the presence of pyridine has been employed for the preparation of the methyl, *n*-propyl, isopropyl and *n*-butyl pyrophosphates.

3. Diethyl phosphoric acid has been prepared in high purity from the initial hydrolysis product of tetraethyl pyrophosphate.

4. Comparative data are presented for the hydrolysis rates and the toxicities to white mice of a series of tetraalkyl pyrophosphates.

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Radioactive Diisopropyl Fluorophosphate

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Diisopropyl fluorophosphate has been shown to have a specific action on the enzyme cholinesterase.[1,2,3] As part of a program to investigate the mode of action in the body more completely by means of radioactive tracer techniques, we have synthesized, on a millimole scale, diisopropyl fluorophosphate containing the radioisotope P32 as part of the molecule.

The synthesis of diisopropyl fluorophosphate has been previously described.[2,3,4] Radioactive phosphorus trichloride, a necessary intermediate, was synthesized from radioactive potassium dihydrogen phosphate or radioactive phosphoric acid, in which case potassium monohydrogen phosphate was added as a carrier.

Experimental

1. **Preparation of Calcium Phosphate—Pyrophosphate Mixture.**—A mixture of 1 g. of radioactive potassium dihydrogen phosphate and 0.412 g. of potassium hydroxide was dissolved in 3.5 ml. of water. The solution was added, with stirring, to a 15-ml. centrifuge tube containing 2.2 g. of calcium chloride dissolved in 7 ml. of water. Dicalcium phosphate and some calcium phosphate precipitated.[5] The mixture was centrifuged, and washed three times with 3 ml. of water per washing to remove the excess calcium chloride. The tube containing the moist dicalcium and calcium phosphates was heated at 500° for three hours in an electric furnace to convert the dicalcium phosphate to calcium pyrophosphate.[6]

2. **Conversion to Phosphorus Pentachloride and Oxychloride Mixture.**—A quartz tube, 15 mm. i.d. × 60 cm., was ground to fit a 25-ml. collecting flask with a side-arm to which was attached a drying tube containing Drierite.

The calcium phosphate–pyrophosphate mixture (0.93 g.) was intimately mixed with 2.5 g. of 30 to 60 mesh charcoal. The mixture was introduced into the quartz tube. The tube, which was held in a horizontal position during the run, was heated by an electric furnace at 700°. While the mixture was heated, a stream of chlorine at a flow rate of 20–25 ml. per minute was passed through the tube. The collecting flask was cooled with a Dry Ice-bath during the run. After six hours, the exposed part of the tube was heated with free flame of a Meker burner to volatilize and collect any product which remained in the tube. The yield of product, which consisted of a mixture of phosphorus pentachloride and oxychloride, was 1.5 g.[7,8]

3. **Phosphorus Trichloride.**—The collecting flask was connected to a quartz tube, 4 mm. i.d. × 19 cm., into which was introduced 0.35 g. of 30- to 60-mesh charcoal. The quartz tube, which was held in a horizontal position, was attached to a 5-ml. two-necked receiver. A stirrer, consisting of a piece of soft iron completely sealed in glass was placed into the receiver. Stirring was accomplished by rotating a magnet beneath the flask. The receiver was connected to a water condenser, which terminated in a drying tube containing Drierite. The quartz tube was heated at 650° in an electric furnace.

The collecting flask was cooled with Dry Ice and 0.9 g. of powdered antimony (20-mesh and finer) was rapidly sprayed into it from an eye dropper in such a manner as to cover completely the surface of the phosphorus pentachloride–oxychloride mixture. The Dry Ice-bath was removed, and the flask was cautiously heated with a free flame until the exothermic reaction between antimony and the pentachloride set in. The antimony reduced the pentachloride to the trichloride.[9] In some runs it was even necessary to moderate the reaction with an ice-bath. When the vigorous reaction had subsided, the mixture was heated by an oil-bath. The bath temperature was gradually raised over a period of ninety minutes to 150°. Phosphorus oxychloride was reduced to the trichloride as the vapors passed over the carbon in the hot tube.[8,10]

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[1] McCombie and Saunders, *Nature*, **157**, 776 (1946).

[2] McCombie, Saunders and Stacey, *J. Chem. Soc.*, 380 (1945).

[3] Saunders and Stacey, *J. Chem. Soc.*, 695 (1948).

[4] T. P. Dawson, TDMR 832, Diisopropyl Fluorophosphate. Available only from Office of Technical Services, Dept. of Commerce, Washington 25, D. C.

[5] Berzelius, *Ann. chim. phys.*, [1] **11**, 114 (1819); Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. III.

[6] Baer, *Pogg. Ann.*, **75**, 152 (1847); Mellor, *loc. cit.*, Vol. III.

[7] Gay-Lussac and Thénard, *Recherches Physico-Chemiques*, Paris, **2**, 176 (1811); Mellor, *loc. cit.*, Vol. III.

[8] Rozhdestvenskii, *Trans. State Inst. Applied Chem.* (*U. S. S. R.*), **20**, 47 (1934); *C. A.*, **29**, 2311 (1935).

[9] Baudrimont, *Ann. chim. phys.*, [4] **2**, 12 (1864); Jacobson, "Encyclopedia of Chemical Reactions," Vol. I, p. 205.

[10] Riban, *Compt. rend.*, **95**, 1160 (1882); *Bull. soc. chim.*, [2] **39**, 14 (1883); Mellor, *loc. cit.*, Vol. III.

The phosphorus trichloride (0.81 g., 80%), which distilled into the 5-ml. receiver, had a boiling point of 76–78°.

A run was made in which the phosphorus pentachloride–oxychloride mixture was treated with antimony but the charcoal reaction was omitted. The product boiled at 90–100°, indicating the presence of phosphorus oxychloride.

4. **Diisopropyl Hydrogen Phosphite.**—The synthesis of phosphorus trichloride was carried out as described above except that into the 5-ml. receiver was introduced 1.37 g. of anhydrous isopropyl alcohol. An ice-bath, which surrounded the receiver, rested above a rotating magnet. As phosphorus trichloride condensed into the receiver, it reacted with the isopropyl alcohol at a temperature of 0 to 10°. The mixture was stirred vigorously during the reaction. After all of the trichloride had been introduced, the temperature was maintained at 0 to 10° for twenty minutes. The ice-bath was then removed, and the temperature was allowed to rise to 25° (approximately thirty minutes).

5. **Diisopropyl Chlorophosphate.**—A gas inlet tube was introduced into the flask containing the hydrogen phosphite. The flask was cooled to 0°. The system was evacuated to 450 mm., and a stream of dry chlorine precooled to −25° was introduced into the flask at a flow rate of about 200–400 ml. per minute. The chlorine was bubbled through the reaction mixture until the colorless solution became light green and this color persisted for at least five minutes. Further chlorination resulted in a decrease in yield. The chlorination was carried out in a slight vacuum to remove hydrogen chloride as rapidly as it formed, since its presence also resulted in a lower yield. The chlorine was replaced by a slow stream of nitrogen, and the pressure was reduced to 50 mm. The temperature was maintained at 0° for thirty minutes and at 5° for thirty minutes. The pressure was then reduced to 35 mm., and the temperature was kept at 60° for thirty minutes. In this way, the last traces of hydrogen chloride were removed. The flask was connected to the micro still (Fig. 1) which was a modification of that reported by Craig,[11] and the fraction which came over at a bath temperature of 85° and a column temperature of 90° at 6 mm. was collected. The yield of product was 0.6 g. As a collecting flask in this distillation, a 5-ml. two-necked flask was used.

6. **Diisopropyl Fluorophosphate.**—One gram of anhydrous sodium fluoride and 1 ml. of dry carbon tetrachloride were added to the two-necked flask containing the chlorophosphate. One arm of the flask was stoppered. A condenser and drying tube were attached to the other arm. The mixture was refluxed for five hours with vigorous agitation. The reaction flask was connected to the micro still. Carbon tetrachloride was removed at 200 mm. pressure, and diisopropyl fluorophosphate was distilled directly from the solid. The product came over at a bath temperature of 80° and a column temperature of 85° at 8 mm. The yield of product was 0.45 g. (33%, based on potassium dihydrogen phosphate). It had a specific activity of 20 mc./g.

Anal. Calcd. for $C_6H_{14}O_3FP$: P, 16.82; F, 10.32. Found: P, 17.10; F, 10.55.

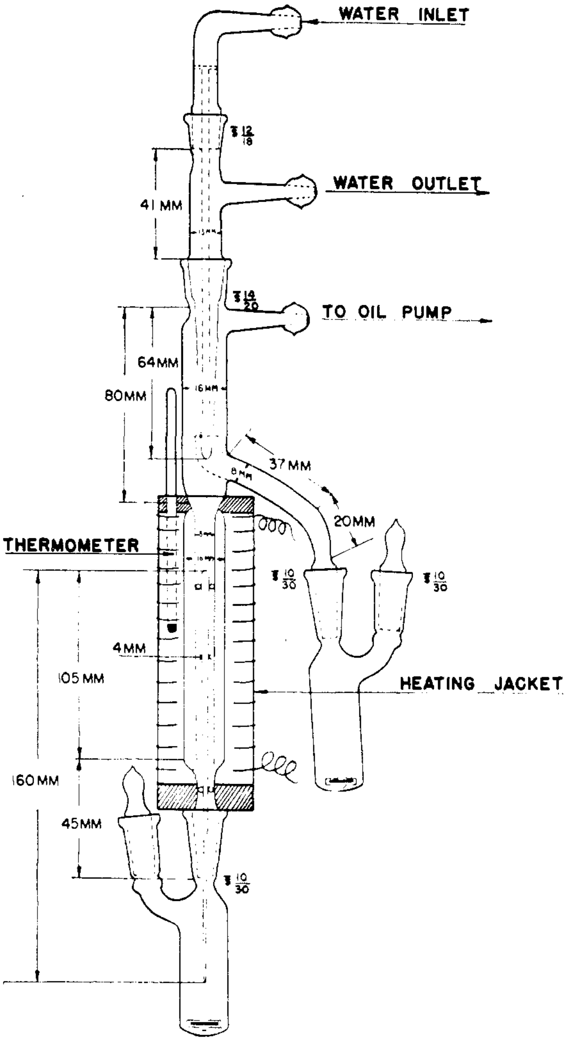

Fig. 1.

Summary

Radioactive diisopropyl fluorophosphate was synthesized on a millimole scale, from radioactive potassium dihydrogen phosphate in 33% yield. The specific activity was 20 mc./g.

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[11] Craig, *Ind. Eng. Chem., Anal. Ed.*, **9**, 441 (1937).