by bubbling anhydrous ammonia through it for ten minutes. The precipitated $(NH_4)_2SO_4$ was filtered and the solvent removed by evaporation under reduced pressure. The solid thus obtained was sublimed to yield 5.8 g. (90%) of $CF_2BrCFBrCONH_2$, m.p. 60.6–61.0°.

Anal. Calcd. for $C_3H_2Br_2F_3NO$: N, 4.92. Found: N, 4.86.

$CF_2{=}CFCONH_2$.—Two grams (0.03 mole) of zinc dust, a trace of zinc chloride and 100 ml. of dry acetone were mixed, heated to reflux, and 2 g. (0.007 mole) of $CF_2BrCFBrCONH_2$ dissolved in 20 ml. of acetone added slowly to this mixture. Reflux was maintained for two hours after the addition was completed. The solid obtained by solvent evaporation was sublimed to yield 0.55 g. (65%) of $CF_2{=}CFCONH_2$, m.p. 121.4–121.9°.

Anal. Calcd. for $C_3H_2F_3NO$: C, 28.81; N, 11.20. Found: C, 28.58; N, 11.18.

$CF_2BrCFBrCO_2H$.—This reaction was carried out in a manner similar to the reaction described by Wiley, *et al.*[14]; 4.4 g. (0.039 mole) of 85% sulfuric acid was heated to 150° and 10 g. (0.039 mole) of $CF_2BrCFBrCN$ added through the reflux condenser over a period of four hours. The temperature was kept at 150° for two hours after the addition of the nitrile. The bath was then allowed to cool to 90° and 10 ml. of water was added. The temperature was kept at 90° for about 15 hours. After the reaction mixture had cooled to room temperature, it was extracted with five 10-ml. portions of ether. The ether extracts were combined and neutralized with a solution of sodium carbonate. After separation of the water layer, the ether layer was extracted with two 10-ml. portions of water. The combined water layers were evaporated, yielding 6 g. of crude $CF_2BrCFBrCO_2Na$. Evaporation of the ether solution yielded 5 g. of $CF_2BrCFBrCONH_2$. The crude $CF_2BrCFBrCO_2Na$ was finely ground and suspended in 15 ml. of dry ether. Anhydrous HCl was then passed through the ether suspension of the salt for six hours. The reaction mixture was filtered and the ether then removed by distillation at atmospheric pressure. Distillation of the crude acid gave 3.8 g. of $CF_2BrCFBrCO_2H$, b.p. 72–73° at 2.5 mm. pressure, $n^{20}D$ 1.4458, d^{20}_4 2.191, neutral equivalent 287 (calculated 286).

Anal. Calcd. for $C_3HBr_2F_3O_2$: C, 12.60; Br, 55.91. Found: C, 13.99; Br, 54.20.

$CF_2BrCFBrCO_2C_2H_5$.—Forty grams (0.15 mole) of $CF_2BrCFBrCN$, 29 g. of 90% H_2SO_4 and 35 g. of C_2H_5OH were heated at reflux for nine hours. Ether extraction of the product followed by fractionation gave 28 g. (60% yield) of $CF_2BrCFBrCO_2C_2H_5$, b.p. 81–84° at 18–19 cm., $n^{25}D$ 1.426.

Anal. Calcd. for $C_5F_3Br_2O_2H_5$: C, 19.1; Br, 50.95. Found: C, 19.1; Br, 50.97.

Preparation of $CF_2{=}CFCO_2C_2H_5$.—About 23.6 g. (0.075 mole) of $CF_2BrCFBrCO_2C_2H_5$ was added to 8.0 g. (0.12 mole) of zinc dust and anhydrous alcohol-free ether with stirring. After the initial exothermic reaction ceased, the ether was refluxed for five hours. Isolation of the product and fractionation yielded 6.0 g. (52% yield) of $CF_2{=}CFCO_2C_2H_5$, b.p. 100.0–100.5° at 750 mm., $n^{25}D$ 1.3615–1.3619.

Anal. Calcd. for $C_5H_5F_3O_2$: C, 39.0; F, 37.1. Found: C, 39.0; F, 37.0.

Preparation of $CF_2BrCFBrCO_2CH_2C_3F_7$.—This ester was prepared *via* the same method used to make the saturated ethyl ester. Fractionation of the crude product gave a 67% yield of $CF_2BrCFBrCO_2CH_2C_3F_7$, b.p. 72–73° at 10 mm., $n^{25}D$ 1.3676–1.3680.

Anal. Calcd. for $C_7F_{10}Br_2H_2O_2$: Br, 34.2; C, 17.9; F, 40.6. Found: Br, 34.3; C, 18.1; F, 40.7.

Preparation of $CF_2{=}CFCO_2CH_2C_3F_7$.—A 71% yield of $CF_2{=}CFCO_2CH_2C_3F_7$, b.p. 61.0–61.5° at 50 mm., $n^{25}D$ 1.3189, was obtained from the debromination reaction.

Anal. Calcd. for $C_7F_{10}O_2H_2$: C, 27.24; F, 61.68. Found: C, 27.3; F, 61.0.

ST. PAUL, MINNESOTA

(14) P. F. Wiley and G. A. Nesty, "Process for Producing Esters of Acrylic Acid," U. S. Patent 2,526,310 (1950).

[CONTRIBUTION FROM THE MEDICAL BIOLOGICAL LABORATORY OF THE NATIONAL DEFENCE RESEARCH COUNCIL T.N.O.]

Synthesis of P^{32} Labeled Diisopropylphosphorofluoridate

BY R. A. OOSTERBAAN AND J. VAN ROTTERDAM

RECEIVED APRIL 25, 1956

A method is described for the preparation of P^{32} labeled diisopropylphosphorofluoridate in water or oil solution starting from radioactive phosphoric acid. The specific radioactivity amounts to 200 microcuries/mg.

Introduction

The synthesis of P^{32} labeled diisopropylphosphorofluoridate (DFP) of high specific activity (200 microcuries/mg.) has proved to be valuable in medical work; it has been used for the study of the metabolic fate of DFP and for the determination of plasma protein turnover and the life span of erythrocytes and thrombocytes.[1–3] Moreover the material has been used for studies with the purpose of collecting information on the chemical groups of esterases which are responsible for the enzymatic and DFP binding properties of these proteins.[4–6]

A number of methods for the synthesis of DFP[7–10] and DFP^{32} [11,12] have been published. These methods could not be used unmodified for our purpose. Either starting material of sufficient specific activity cannot be obtained readily or the methods are not designed for the desired micro-scale operation.

The present method is based on the conversion of phosphoric acid, which is obtainable in carrier-free form, into P^{32} and consequently into $P^{32}Cl_3$. The further synthesis is a micro-scale adaptation of Saunders' method,[11] according to the reaction scheme

$$3ROH + PCl_3 \longrightarrow (RO)_2POH + RCl + 2HCl$$
$$(RO)_2POH + Cl_2 \longrightarrow (RO)_2P(O)Cl + HCl$$
$$(RO)_2P(O)Cl + NH_4F \longrightarrow (RO)_2P(O)F + NH_4Cl$$

(1) J. A. Cohen and M. G. P. J. Warringa, *J. Clin. Invest.*, **33**, 459 (1954).

(2) C. H. W. Leeksma and J. A. Cohen, *Nature*, **175**, 552 (1955).

(3) C. H. W. Leeksma and J. A. Cohen, *J. Clin. Invest.*, **35**, in press (1956).

(4) R. A. Oosterbaan, P. Kunst and J. A. Cohen, *Biochim. et Biophys. Acta*, **16**, 229 (1955).

(5) J. A. Cohen, R. A. Oosterbaan, M. G. P. J. Warringa and H. S. Jansz, *Discussions Faraday Soc.*, **20**, 114 (1956).

(6) R. A. Oosterbaan, H. S. Jansz and J. A. Cohen, *Biochim. et Biophys, Acta*, **20**, 402 (1956).

(7) H. McCombie, B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 380 (1945).

(8) B. C. Saunders and G. J. Stacey, *ibid.*, 695 (1948).

(9) H. Goldwhite and B. C. Saunders, *ibid.*, 2041 (1955).

(10) C. Monard and H. Jean, *Bull. soc. chim. France*, 544 (1952)

(11) B. C. Saunders and T. S. Worthy, *J. Chem. Soc.*, 1320 (1950).

(12) B. Witten and J. I. Miller, THIS JOURNAL, **70**, 3886 (1948).

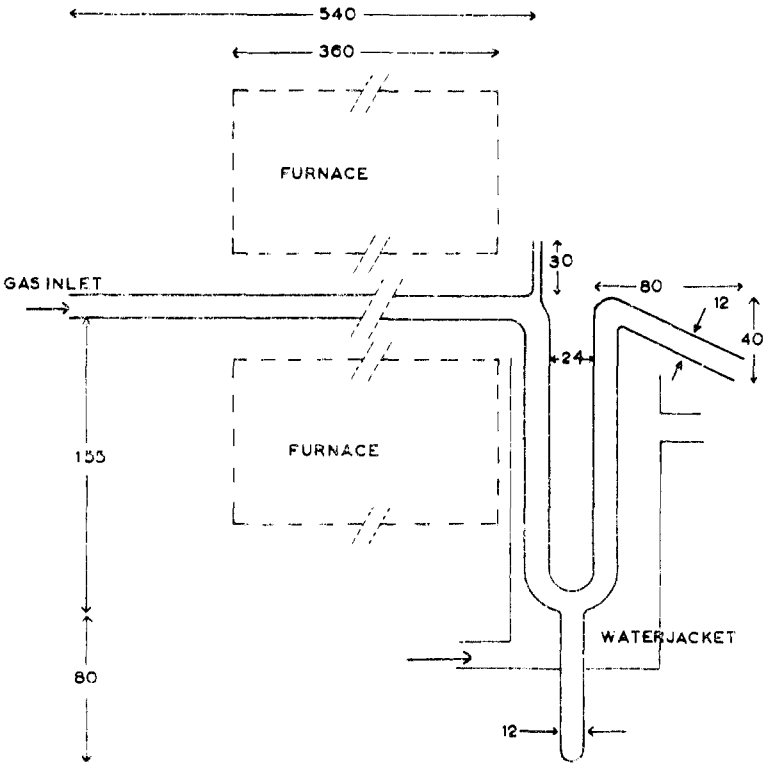

Fig. 1.—Diagram of the silica tube for the production of phosphorus and the conversion into PCl_3; measurements in mm.

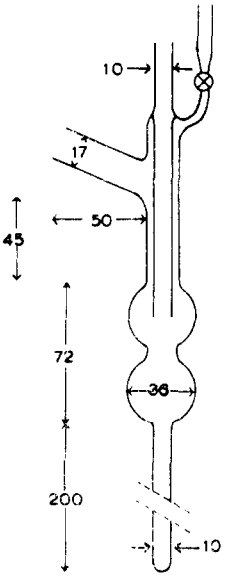

Fig. 2.—Diagram of the Pyrex reaction vessel; measurements in mm.

Only small quantities of DFP of a high specific radioactivity (100 to 200 microcuries/mg.) were needed. With the method described, yielding 70 to 100 mg. of DFP starting from 150 mg. of H_3PO_4, quantities of 150 millicuries can be handled safely. The procedures chosen were judged on the basis of their reliability and safety rather than on their resulting yields and have proved to give consistent and satisfactory results.

Experimental

Production of Phosphorus.—Three ml. of an aqueous solution containing 100 millicuries of carrier-free $H_3P^{32}O_4$ mixed with 150 mg. of H_3PO_4 is dropped on 1 g. of carbo adsorbens in 3 porcelain boats (WETA 3384). After each addition of three 0.3-ml. portions of the solution the material is dried by infrared heating. During this stage it is necessary to minimize the radiation hazard by using a small shielding box with a removable lid. The porcelain boats are placed in a silica tube (Fig. 1) and heated in an electric furnace to 400°; this temperature is maintained during 15 hours. During the preliminary drying the silica tube is flushed by dry and oxygen-free nitrogen gas (10 ml./min.). The temperature is now raised slowly to 900° within 4 hours, while the condensed water is driven out by local heating with a free flame. The removal of condensed water should be continued until the formation of phosphorus starts at 900°. When the phosphorus production starts the nitrogen stream is reduced to 2 ml./min., a trapping system is connected through a small silica gel tube and the curved part of the silica tube is placed in a Dewar vessel, filled with methylene dichloride cooled with Dry Ice (−75°).

The produced red phosphorus sublimes just beyond the hot zone, whereas the white phosphorus condenses in the cooled part of the silica tube. After an additional 8 to 9 hours at 950° the reduction of the phosphoric acid is completed; the electric furnace is cooled down to 200° by a strong draught and the Dewar vessel is removed (yield 60–70%).

Phosphorus Pentachloride.—The phosphorus is converted to phosphorus pentachloride by a slow stream of chlorine gas. The PCl_5 produced is transported into the U part of the silica tube by a slow stream of nitrogen and careful local heating. Next the U part is cooled down to −20° and the excess of chlorine gas is driven out by 500 ml. of nitrogen gas. (During this process the electric furnace is cooled down further to eliminate the possibility of liberation of adsorbed chlorine by the carbo adsorbens.)

Phosphorus Trichloride.—The nitrogen stream is stopped, the Dewar vessel removed, the small silica gel tube on the outlet of the silica tube is replaced by another one and a cooling water jacket is fitted in position (Fig. 1). After the addition of 60 mg. of red phosphorus followed by 1.5 ml. of carbon tetrachloride the PCl_5 is converted to PCl_3 by refluxing during 2 hours. After the refluxing period the water jacket is detached and the reaction vessel (Fig. 2) is connected to the silica tube and cooled down to −75° with a mixture of methylene dichloride and Dry Ice. The PCl_3–carbon tetrachloride solution is transferred into the reaction vessel by boiling the solution in a slow stream of nitrogen gas. The last traces of PCl_3 are flushed over after the extra addition of 0.5 ml. of carbon tetrachloride. The cooling bath is removed and after thawing, the reaction vessel is detached and closed with a silica gel tube. The connection with the trapping system is moved to this silica gel tube. An aliquot is removed, hydrolyzed with alkali, and the radioactivity determined. The yield of PCl_3 (1.0 to 1.3 millimoles) is estimated on the assumption that the P^{32} is diluted by 60% due to the reaction $3PCl_5 + 2P \rightarrow 5PCl_3$.

Diisopropylphosphorochloridate.—The central tube of the reaction vessel (Fig. 2) is fitted with a gas inlet tube. The PCl_3 solution is cooled to 10° and the calculated amount of dry isopropyl alcohol (A.R.) allowing for a 20% excess is added through the small funnel (3.6 moles of alcohol per mole of PCl_3) and washed down with some drops of carbon tetrachloride. A slow stream of nitrogen mixes the solution and expels the produced HCl gas. After 5 minutes the cooling bath is removed. The stream of nitrogen is continued for 30 minutes (40 ml./min.). Next a slow stream of chlorine gas is passed in at 10° until the yellow color persists for at least 1 minute. The cooling bath is removed and

the HCl gas is expelled by a stream of nitrogen during 90 minutes (40 ml./min.). A minimum volume of about 0.75 ml. should be maintained by the addition of carbon tetrachloride.

Diisopropylphosphorofluoridate.—The fluorination is carried out by the method of Ford-Moore, *et al.*[13] The gas inlet tube is removed and a cooling water jacket is fitted in position; 500 mg. of pulverized ammonium fluoride and 2 ml. of acetone are added. The mixture is refluxed during 2 hours. Next the resulting DFP is purified by steam distillation. The solution is transported into the dropping funnel of a distillation apparatus by means o a syphoning tube fitted with a glass wool filter which is inserted into the reaction vessel. The distillation apparatus consists of a round flask of 750 ml. and a large surface condenser in vertical position. The residual precipitate in the reaction vessel is refluxed during one minute with an additional amount of acetone and also transported. The mixture is dropped carefully into the boiling water (7 ml. distillate/min.) and the distillate (40 ml.) is cooled with ice. The distillate is evacuated (20 min., 15 mm. at 20°) to expel acetone and carbon tetrachloride. To remove acidic impurities the solution is percolated through a column of Amberlite IR4 (10 × 1 cm.) pre-washed with NaOH and water and cooled down to 0° (3 ml./min.). The DFP content of the solution is assessed by essentially the same colorimetric method as has been described by Epstein, *et al.*,[14] for sarin. However in the case of DFP it is necessary to use a phosphate buffer at *p*H 11.1 instead of *p*H 8.8. The absorption of the unknown solution is compared with that of freshly prepared standard solutions containing 0.1 to 0.3 mg. of DFP/10 ml. The purity of the DFP^{32} end product is checked by the comparison of its DFP content according to the colorimetric analysis against that based on the radioactivity of the solution (the radioactivity of 1 mg. of P of the starting material corresponds to that of 9.89 mg. of DFP). The average yield amounts to 80 mg. of a purity of 95 ± 5% and a specific activity of 200 microcuries per mg. The DFP^{32} solution must be stored in Pyrex ampoules at low temperature to minimize hydrolysis.

DFP in Peanut Oil.—A filter apparatus consisting of a centrifuge tube fitted with a Zeiss asbestos filter and containing the required quantity of peanut oil is sterilized. A solution of DFP in water is sterilized by slowly pressing it through the asbestos filter. The centrifuge tube is detached and stoppered. After agitating during one minute and centrifuging during ten minutes the oil layer is recovered with a sterile syringe (the partition coefficient of DFP in oil–water amounts to 6–7). The DFP content of the oil is determined on the basis of its radioactivity. Intramuscular injections of DFP^{32} in oil in quantities up to 1 mg. of DFP have been given to a great number of patients and have never produced undesirable toxic effects.

Radiation Hazard.—The operator is protected by two large removable perspex shields (10 mm.).

Materials.—Red phosphorus (British Drug Houses, BDH). A lot of finely divided phosphorus is collected by decanting a suspension of red phosphorus in water. The collected material is boiled with water and filtered. The procedure is repeated until the supernatant is acid free. Finally the filtered material is washed with dry acetone and dried above P_2O_5 *in vacuo*. Carbon tetrachloride A.R. (BDH). The carbon tetrachloride is boiled with a small quantity of PCl_3, agitated with a Na_2CO_3 solution, washed with water, dried with P_2O_5 and distilled. Ammonium fluoride A.R. (BDH): this material is thoroughly dried by an azeotropic distillation with dry carbon tetrachloride.

RIJSWIJK (ZH), NETHERLANDS

(13) A. H. Ford-Moore, L. C. Lermit and C. Strattford, *J. Chem. Soc.*, 1776 (1953).

(14) J. E. Epstein, THIS JOURNAL, **78**, 341 (1956).

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, SOUTHWESTERN MEDICAL SCHOOL OF THE UNIVERSITY OF TEXAS]

Ethers of Phloroglucinol

BY JOSEPH C. TOUCHSTONE, JAMES ASHMORE AND MAX N. HUFFMAN[1]

RECEIVED APRIL 16, 1956

The syntheses of phloroglucinol mono-, di-, tri- and mixed-ethers are reported. These compounds represent a series of homologs prepared in conjunction with antitubercular studies.

In 1934, Woodward, Kingery and Williams[2] reported a study of the fungicidal activities of various phenols against *Monilia tropicalis*. The 3,5-dialkylphenols showed considerable fungicidal activity but because of their relative insolubility their medical usefulness was limited. In a search for more effective fungicidal agents, a series of 3,5-dialkyloxyphenols (phloroglucinol dialkyl ethers) have been prepared. Monoalkyl ethers were also formed in the preparation of our phloroglucinol diethers.

In a survey of the fungicidal activity of phloroglucinol monohexyl ether against a number of pathogenic fungi, Dr. Morris Moore[3] found that this compound had particularly high activity against various species of *Actinomyces*. Therefore, this series of phloroglucinol mono- and di-ethers was tested against *Mycobacterium tuberculosis* because of the generally held phylogenetic relationship between it and the *Actinomyces*. Against a 607-like strain it was found that phloroglucinol monohexyl ether was especially potent[4]; however, none of the phloroglucinol ethers were found to be highly active against a virulent strain of *Mycobacterium tuberculosis* (H37Rv).

Several investigators[5,6] have reported the preparation of ethyl and methyl ethers of phloroglucinol. Kaufler[7] and his co-workers reported the synthesis of the benzyl ethers of phloroglucinol. Herzig[8] reported the preparation of ethyl and methyl ethers of phloroglucinol by treating an absolute alcohol solution of the phenol with hydrogen chloride. Since these early reports, little work has been done on this type of derivative although as late as 1920, Freudenberg[9] prepared phloroglucinol dimethyl ether in 70–80% yields.

Attempted preparation of phloroglucinol ethers using the Williamson type synthesis gave negative

(1) Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma.

(2) G. J. Woodward, L. B. Kingery and R. J. Williams, *J. Lab. Clin. Med.*, **19**, 1216 (1934).

(3) Personal communication to M. N. H.

(4) This testing was performed by Dr. Andres Goth, who will report his results elsewhere.

(5) W. Will, *Ber.*, **17**, 2106 (1883).

(6) H. Weidel and J. Pollak, *Monatsh.*, **21**, 22 (1901).

(7) F. Kaufler, *ibid.*, **21**, 998 (1901).

(8) J. Herzig, *ibid.*, **15**, 701 (1895).

(9) K. Freudenberg, *Ber.*, **53**, 1425 (1920).