A CONVENIENT SYNTHESIS OF CRYSTALLINE POTASSIUM PHOSPHATE-$^{18}O_4$ (MONOBASIC) OF HIGH ISOTOPIC PURITY

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Received January 26, 1978
Revised March 15, 1978

SUMMARY

The synthesis of crystalline potassium phosphate-$^{18}O_4$ (monobasic) of high (∿ 90%) isotopic purity starting with PCl_5 and 99% water-^{18}O is described. The yield of the salt free from contamination by metaphosphates and pyrophosphate is 85%.

Key Words: Orthophosphate, Potassium dihydrogen phosphate, Oxygen-18.

INTRODUCTION

The preparation of ^{18}O-labelled phosphate is an important prelude to studies of isotope exchange, position of bond cleavage, the further syntheses of labeled compounds, etc., including especially many biochemical examples of such problems. Present methods for the determination of isotope content involve mass spectral measurements on the volatile trimethyl phosphate (1,2) or tris-trimethylsilyl phosphate (3) derivatives. These together with nuclear magnetic resonance techniques (4-6) may be expected to supplant previously employed methods (7-9). Accurate data analysis particularly with the new methods is considerably aided by the use of phosphate of relatively high isotopic purity, as has been pointed out by Kenyon et al. (3). Four methods for the preparation of phosphate-^{18}O are described in the literature (1,3,8-10) while three techniques for the isolation of the product are employed (1,8,10). Of the four preparations and three isolation techniques no single one is totally satisfactory with respect to ease of preparation, isolation, chemical and isotopic purity, stability of product and

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0362-4803/78/0015-0533$01.00

ease of storage. Methods based on ^{18}O-exchange of KH_2PO_4 (3,6,9) are very inconvenient and require a substantial excess of ^{18}O-water in order to achieve high isotopic purity in the product. Formation of labelled phosphate by methods starting with $POCl_3$ either limit the ultimate isotopic purity or require the use of ^{18}O-$POCl_3$ together with ^{18}O-water (1). We describe here a method based on the reaction of PCl_5 with H_2O-^{18}O (10) and isolation of the product as the crystalline monopotassium salt. The method should be equally useful in the synthesis of phosphate-^{17}O.

EXPERIMENTAL

The synthesis of ^{18}O-labelled phosphate is conveniently carried out in a test tube no smaller than 18 x 150 mm contained in a Dewar flask. The test tube is closed with a one-hole rubber stopper into which is inserted a glass Y, one arm of which is connected to a $CaCl_2$ drying tube and the other arm of which is connected to a water aspirator. Water-^{18}O (99 atom % excess, normalized, Norsk Hydro, Oslo, Norway) (1.0 ml; a larger scale is not recommended) is transferred to the test tube with exclusion of atmospheric moisture and frozen with the aid of Dry Ice in the Dewar flask. Phosphorus pentachloride (2.90 g) is transferred quickly to the frozen water and the drying tube replaced. Hydrogen chloride from the resulting exothermic reaction is removed by the water aspirator. (It was occasionally necessary to initiate the reaction by partially melting the water-^{18}O but after this the reaction proceeded smoothly.) The reaction vessel is brought to room temperature and then warmed to 80°C in a water bath over 90 min to remove most of the HCl. After cooling to room temperature 2 M KOH is added to adjust the pH to 4.66.

Two alternative procedures for the isolation of the product are available. Precipitation of the pure salt may be achieved by adding four times the volume of 66% ethanol and refrigerating 24 hours at 4°C. A second crop of crystals (averaging 4% of the total yield) may be isolated by addition of 100% ethanol to the mother liquor to the saturation point and refrigeration for an additional 24 hours. A second, faster method for precipitation is by the addition of two

volumes of 100% ethanol. Precipitation occurs immediately and the product is collected after a settling time of 15 to 30 minutes. Although this method results in slightly higher yield of the ^{18}O-labelled phosphate salt it is also contaminated with a slight amount of KCl.

The crystals are collected on a fine-porosity fritted-disc funnel, washed with two volumes of 100% ethanol and one volume of anhydrous ethyl ether, and dried in a 100°C oven for one hour. The stable, easily handled white crystals are stored in a dark bottle in a desiccator.

A Varian 100XL-nuclear magnetic spectrometer was used to obtain ^{31}P-NMR spectra. ^{31}P was observed at 40.546 MHz in a 23487 Gauss field using solvent D_2O as an internal lock. The sample was at a concentration of 0.49 M and 85% H_3PO_4 was used as the internal standard.

The analysis of the isotopic purity of the isolated KH_2PO_4 was accomplished following conversion to trimethyl phosphate (1,2) by gas chromatography-mass spectrometry on a Finnigan model number 4000 spectrometer using a 2 m column of 3% OV-3 on Gas Chrom Q, 100/120 mesh column with a helium flow of 20 ml/min, 225°C injector temperature, and a 100° isothermal program for the separation of solvent methylene chloride from trimethyl phosphate. A 70.0 V election-impact ionization was used; the mass range 135-155 was scanned at a rate of one second per scan in the mass spectrometer. The percentages of the individual peaks at m/e 148, 146, 144, 142 and 140 were determined.

RESULTS AND DISCUSSION

The choice of isolation technique is primarily determined by the eventual use of the labelled product. If chloride ion does not interfere with subsequent reactions then the choice would be the second isolation technique because of the higher yield. If the second method is used and later chloride-free phosphate-$^{18}O_4$ is desired, then recipitation with 66% ethanol gives results comparable to those of the first method. Table I gives the results for five syntheses where the first isolation technique was employed. The average yield was 85%.

Table I. $KH_2P^{18}O_4$ Yields from the Reaction of PCl_5 and $H_2{}^{18}O$

Expt #	PCl_5 (g)	$H_2{}^{18}O$ (ml)	$KH_2P^{18}O_4$ (g)		Yield (%)	$^{18}O_4$-phosphate (%)	total ^{18}O content (%)
			1st crop	2nd			
1	0.5841	0.200	0.290	0.0345	80.4	—	—
2	2.8981	1.000	1.633	0.0770	85.3	—	—
3	2.9000	1.002	1.809	0.0472	92.5	93.5	97.6
4	2.9672	1.020	1.631	0.0783	83.3	83.9	93.9
5	2.8827	1.004	1.547	0.1094	83.1	84.4	91.1

The parameters used to test the purity and ^{18}O-content of the salt were qualitative and quantitative tests, ^{31}P NMR, and mass spectroscopy of the trimethyl derivative. Because metaphosphoric acid is unstable in water and decomposes to form phosphoric acid, it is not formed in the reaction. Qualitative tests were used for the determination of pyrophosphates (11-13) and chloride ion (14,15). Orthophosphate does not react with $ZnSO_4$ at pH 3.8 or $CdCl_2$ in acetic acid, however pyrophosphate forms a white precipitate with both. In strong acid solution AgCl precipitates as a white solid while phosphoric acid does not react with the silver ion. (This reaction must be done in highly acidic solution to avoid the formation of a yellow precipitate resulting from reaction of silver ion with orthophosphate.) The results of these tests were negative for both pyrophosphate and chloride ion. The chloride ion test has a limit of detection of less than 0.01% chloride ion (15).

The quantitative test employed was the optical density measurement of a phosphomolybdate complex (16). Because the absolute weight difference between ^{16}O-phosphate and ^{18}O-phosphate is 5.55% the absorbance was calculated as a function of molar concentration. The plot [A_{820} vs. $[P^{18}O_4{}^{3-}]$, 11 pts, slope 5300 l mol^{-1} cm^{-1}, Y-intercept 0.004, $r = 0.999_{93}$] showed the two curves to be superimposable and the molar extinction coefficients were calculated to be 3700 for ^{16}O-phosphate and 3800 for ^{18}O-phosphate.

The ^{31}P NMR showed one peak centered at δ = -0.05. The mass spectrum of a derivatized sample of the ^{18}O-labelled phosphate showed peaks at m/e 148, 146, 144, 142 and 140 corresponding to 84.2%, 6.7%, 4.6%, 3.3% and 1.2%, respectively, and to a total isotopic purity of 92.3%. Other preparations gave comparable results.

Based on these results of purity and ^{18}O-content this synthesis has proved very useful for the easy preparation of chemically pure crystalline potassium dihydrogen phosphate of high isotopic purity.

ACKNOWLEDGEMENTS

Support for this research was obtained from U.S. Public Health Service grant CA 10585 of the National Cancer Institute. The authors thank Prof. Richard Caprioli and Ms. Karen Gooding for their kindness in supplying the mass spectral data and Dr. Robert Santini for help in obtaining nuclear magnetic resonance spectra.

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