NOTES

The Preparation of Tri-N-butyl Phosphate[1]

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Tri-*n*-butyl phosphate containing radioactive phosphorus (P^{32}) has been prepared in 60% yield by refluxing a mixture of radioactive silver phosphate and excess *n*-butyl bromide for a total of eight hours. The equation representing this reaction is $Ag_3PO_4 + 3C_4H_9Br = (C_4H_9O)_3PO + 3AgBr$. The silver orthophosphate was prepared by mixing phosphoric acid (containing some P^{32}) and aqueous silver nitrate. Complete experimental details are available on microfilm.[2]

(1) This document is based on work performed for the Atomic Energy Commission at the Oak Ridge National Laboratory.

(2) For detailed paper order Document 3563 from American Documentation Institute, 1719 N Street, N. W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35-mm. motion picture film) or $1.00 for photocopies (6 × 8 inches) readable without optical aid.

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Preparation of Xanthopterin-6,7-C^{14} [1]

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Carbon-14 can be introduced most conveniently into positions six and seven of the xanthopterin molecule by using C^{14}-oxalic acid as an intermediate. This was prepared from C^{14}-formic acid by the method of Leslie and Carpenter,[3] the formic acid being obtained by the reduction of $C^{14}O_2$.[4] The oxalic acid was condensed with 2,5,6-triamino-4-hydroxypyrimidine[5] to give leucopterin. The latter was reduced to xanthopterin after partial purification, and the final product was separated from impurities on a column of "Dowex-1" anion exchanger. The over-all yield of xanthopterin from CO_2 was 5 per cent., the specific activity of the product being 33 μc. per millimole. Purrmann's synthesis of xanthopterin[6] had to be modified considerably in order to avoid the use of *excess* C^{14}-oxalic acid. However, this modification resulted in the formation of impurities which appear to originate from the self-condensation of the aminopyrimidine and from the condensation of one molecule of oxalic acid with two molecules of pyrimidine. The reduction of the reaction mixture containing leucopterin produced relatively large amounts of an impurity, probably identical with "red precipitate" described by Elion, *et al.*[7] The reaction conditions and quantities of reagents described below are the result of many trials, and they are believed to be optimal for the conversion of oxalic acid into xanthopterin on a scale of approximately 50 mg. With these quantities the methods of Totter[8] and of Elion, *et al.*,[7] proved to be unsatisfactory.

Experimental

C^{14}-Oxalic Acid.—The method of Leslie and Carpenter[3] was used for the conversion of C^{14}-sodium formate (0.009 mole) into sodium oxalate, except that the oxalic acid was recovered from the reaction mixture in the form of its silver salt. This was washed with hot water and decomposed with hydrogen sulfide. Pure oxalic acid resulted in 51% yield on evaporation of the filtrates from the silver sulfide.

Leucopterin-6,7-C^{14}.—C^{14}-Oxalic acid (0.002 mole, 183 mg.) and 2,5,6-triamino-4-hydroxypyrimidine (0.005 mole, 645 mg.) were mixed in a 10 × 75 mm. Pyrex ignition tube which had been constricted near the open end.[9] After driving off the water vapor at 130°, the tube was sealed and the temperature raised gradually to 250° over a period of 90 minutes. The tube was allowed to cool, the internal pressure was released carefully[10] and the product dissolved in hot sodium hydroxide (0.5 *N*, 30 ml.). A brown impurity could be removed by boiling with charcoal, and, following filtration, the solution was poured into boiling hydrochloric acid (1 *N*, 30 ml.). After refrigeration overnight, the product was collected, washed with water and dried; yield 234 mg. of a pale yellow solid contaminated by a red substance.

Xanthopterin-6,7-C^{14}.—For reduction the impure leucopterin (223 mg.) was divided into portions of approximately 50 mg. Each lot was covered with anhydrous[11] ethylene glycol (2 ml.) in a 10 × 75 mm. ignition tube fitted with a reflux condenser. Three portions of 5% sodium amalgam (0.5 g. each) were added initially, and after 30 and 60 minutes, respectively. The total heating time was 90 minutes in a bath at 200°. The tube was cooled rapidly, anhydrous acetone (5 ml.) was added, followed by a solution of hydrogen chloride in anhydrous methanol (15%), which was added dropwise until the acetone and glycol layers became miscible. The solution remained strongly alkaline at this point. Excess acid produced a precipitate of free dihydroxanthopterin, which gave inferior yields on oxidation by atmospheric oxygen. The alkaline solution was transferred to a flask with anhydrous acetone, leaving the mercury behind; the final volume was adjusted to 100 ml. After refrigeration overnight, the precipitate was collected on a sintered glass filter, washed with acetone and dried. The solid was dissolved in ammonium hydroxide (0.5 *N*) by allowing small portions (7 ml.) of the solvent to percolate slowly through the filter without any attempt to exclude air. During this process the sodium dihydroxanthopterin was oxidized to xanthopterin. This method of oxidation was superior to the use of any of the numerous oxidizing agents that have been tried. Pure xanthopterin could be recovered from such percolates by repeated applications of the usual methods of purification. However, ap-

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(3) E. H. Leslie and C. D. Carpenter, *Chem. Met. Eng.*, **22**, 1195 (1920).

(4) D. B. Melville, J. R. Rachele and E. B. Keller, *J. Biol. Chem.*, **169**, 419 (1947).

(5) Generously supplied by the American Cyanamid Company, through the courtesy of Dr. James M. Smith, Jr.

(6) R. Purrmann, *Ann.*, **544**, 182 (1940).

(7) G. B. Elion, A. E. Light and G. H. Hitchings, This Journal, **71**, 741 (1949).

(8) J. R. Totter, *J. Biol. Chem.*, **154**, 105 (1944).

(9) The volume of the sealed tube must be as small as possible to minimize sublimation of the oxalic acid from the reaction mixture.

(10) Considerable pressure was developed during the reaction. This effect was enhanced by increasing the proportion of the pyrimidine component. The composition of the reaction mixture as given represents the maximum percentage of pyrimidine compatible with the safety of the procedure.

(11) Use of anhydrous solvents is necessary owing to the apparent hydrolysis of sodium dihydroxanthopterin.