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.

preciable losses were encountered, and the following chromatographic purification was developed for small quantities of valuable material: the crude xanthopterin was digested with hot hydrochloric acid (1 *N*), and the bulk of the "red precipitate" was eliminated by filtration of the cooled digest. Partially purified xanthopterin (67 mg.) was recovered from the filtrate, dissolved in ammonium hydroxide (1 *N*, 50 ml.) and put on a 12 × 100 mm. column of "Dowex-1" anion exchanger (chloride-form, 300 mesh). The solvent was displaced with water and the column eluted with ammonium chloride (0.02 *N*). The xanthopterin appeared on the column as a yellow band which showed intense greenish-yellow fluorescence in ultraviolet light. Samples of the eluate were taken intermittently and adjusted to *p*H 11 with sodium hydroxide for measurements of absorption in the ultraviolet region. These were made at 390, 345[12] and 300 mμ and the eluate was collected when the ratio of the optical densities at 390 and 345 mμ was 3.0 and that at 390 and 300 mμ was at least 11. Approximately 3 liters of eluate, satisfying these criteria, yielded 41 mg. of xanthopterin. The homogeneity of this specimen was demonstrated by the appearance of a single yellow-fluorescing band on a paper chromatogram with an aqueous 2,4-lutidine solvent. The following values for the molecular extinction coefficients were calculated for anhydrous xanthopterin; they agree closely with those of O'Dell, *et al.*[13]: 6.75×10^3 at 390 mμ, 2.09×10^3 at 345 mμ, 0.61×10^3 at 300 mμ, 17.3×10^3 at 255 mμ, and 4.12×10^3 at 220 mμ.

(12) The "red precipitate" shows a maximum of light absorption at 345 mμ in sodium hydroxide solution at *p*H 11.

(13) B. L. O'Dell, J. M. Vandenbelt, E. S. Bloom and J. J. Pfiffner, THIS JOURNAL, **69**, 250 (1947).

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Synthesis of Some Purines and Pyrimidines Labeled in the 2-Position with C^{14} [1]

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Isotopically labeled purines and pyrimidines are currently of much interest in biological tracer studies. The present note is concerned with the synthesis of guanine, 2,6-diaminopurine, uracil and thymine, each labeled in the 2-position with C^{14}. The procedures employed were modifications of known syntheses, which in several instances resulted in somewhat improved methods.

The starting material for each synthesis was barium cyanamide, which was prepared from isotopic barium carbonate as described by Zbarsky and Fischer[2] and by Marsh, Lane and Salley.[3] Because of its simplicity and high yield, this method was preferred to the alternate method of Murray and Ronzio.[4] Guanidine hydrochloride, used for the synthesis of guanine and diaminopurine, was prepared from barium cyanamide[2,3] in 75–84% yield from barium carbonate, a yield considerably higher than those previously reported[2,3] for this method.

For the synthesis of thiouracil, ethyl β,β-diethoxypropionate[5] was condensed with isotopic thiourea (prepared from barium cyanamide[6]); in our hands this has given better results than the original procedure of Wheeler and Liddle[7] which involves the use of the sodium salt of ethyl formylacetate, since shown to be only about 40% pure.[8] Similarly ethyl α-methyl-β,β-diethoxypropionate[9] was found to give better results in the synthesis of thiothymine than the sodium salt of formylpropionate.

These procedures have been used for the synthesis of products of high specific activity. The purity of the final products was checked by ultraviolet absorption spectra and by filter paper chromatograms and autoradiograms of the filter paper strips. By these criteria, guanine, uracil and thymine were shown to be homogeneous. Diaminopurine contained a trace of guanine, which was detectable only on the autoradiogram of a sample of high specific activity.

The over-all yields from barium carbonate were guanine, 40–50%, 2,6-diaminopurine, 15–20%, uracil 32–40% and thymine 20–28%.

Experimental

Barium Cyanamide and Guanidine.[2,3]—These conversions, carried out essentially by the procedure of Marsh, Lane and Salley,[3] are described in detail to include certain observations on the reaction not hitherto recorded.

Barium carbonate (2.6 g., 0.013 mole, 30 mc.), in a fused silica boat, was placed in a Vycor combustion tube. To one end of the tube was attached a bubble counter and to the other, two gas washing bottles in series, containing 10% sodium hydroxide solution. A thermocouple well, extending to the boat, was attached to one end of the tube. Ammonia gas was passed through the tube while it was heated at 820 ± 15°. During the heating, water condensed in the cooler part of the tube and the contents of the boat contracted and hardened. A small amount of a radioactive gas, formed during the reaction and not absorbed by the alkali traps, was vented through the hood. After four hours, heating was discontinued and the tube was allowed to cool in a stream of ammonia. The change in weight of the boat and contents was almost the theoretical. Barium carbonate, precipitated from the alkali traps, accounted for 0.35 mc. (1.17% of the initial activity) of C^{14}. The contents of the boat were transferred to a 250-ml. centrifuge tube and ground together with 3.2 g. (0.04 mole) of ammonium nitrate. The tube was attached to a gas washing bottle containing dilute sodium hydroxide after which the mixture was heated at 165° for 20 minutes while the gases evolved were passed through the alkali trap (to remove any active carbon dioxide resulting from unchanged barium carbonate). The tube was removed from the heating bath and flushed with nitrogen through the alkali trap and finally reheated at 165° for 10 minutes while the melt was stirred with a glass rod. The reaction mixture was cooled and 200 ml. of warm 1.8% aqueous ammonium picrate was added, while the solution was stirred vigorously. The picrate, after being allowed to crystallize overnight, was washed by centrifugation twice with 20-ml. portions of 0.8% aqueous ammonium picrate and twice with 20-ml. portions of water and finally dried *in vacuo* over phosphorus pentoxide. The solid was then suspended in ether and dry hydrogen chloride was bubbled in while the suspension was agitated with a magnetic stirrer. When the ether was saturated with hydrogen chloride, the suspension was allowed to settle, after which the supernatant was removed with a filter stick. The precipitated guanidine hydrochloride was washed twice with ethereal hydrogen chloride and dissolved in water; the solution was

(1) This work was performed under Contract AT-(40-1)-278 with the Isotopes Division, United States Atomic Energy Commission.

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