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.

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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

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(5) E. Dyer and T. B. Johnson, *ibid.*, **56**, 222 (1934).

(6) C. W. Bills and A. R. Ronzio, *ibid.*, **72**, 5510 (1950).

(7) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908).

(8) S. M. McElvain and R. L. Clarke, THIS JOURNAL, **69**, 2657 (1947).

(9) N. C. Deno, *ibid.*, **69**, 2233 (1947).

filtered and the filtrate evaporated to dryness in a tared flask to be used in the next step. The guanidine hydrochloride weighed 0.96 g. (76.3% from barium carbonate). If the hydrochloride was yellow, indicating contamination with picrate, it was decolorized by passing the aqueous solution through a short column (*ca.* 1 cm. × 1 cm.[2]) of Dowex-1 anion exchange resin (200–400 mesh). From the combined picrate filtrate and washings, 1.8 mc. (6% of the initial activity) of guanidine picrate was recovered by washing out with inactive guanidine picrate.

The guanidine hydrochloride was used without further purification for the synthesis of guanine and diaminopurine.

2,4,5-Triamino-6-hydroxypyrimidine-2-C^{14} and Guanine-2-C^{14}.—The first of these was prepared essentially by the procedure of Cain, Mallette and Taylor.[10] The yield was not lowered when the reduction was carried out without isolation of 2,4-diamino-5-nitroso-6-hydroxypyrimidine. The final product was isolated in 67–74% yield as the sulfate ($C_4H_7N_5O \cdot H_2SO_4 \cdot H_2O$). This was used without further purification for the synthesis of guanine by the method of Traube[11] using 98–100%, instead of 90% formic acid. The product was isolated as the sulfate ($(C_5H_5N_5O)_2 \cdot H_2SO_4 \cdot 2H_2O$); yield 85% after one crystallization. The product was usually pure at this stage, but could be recrystallized from 2 *N* sulfuric acid with 85–90% recovery.

The product of an inactive run was analyzed after being dried over phosphorus pentoxide *in vacuo* (1 mm.) at 140–160°.

Anal. Calcd. for $(C_5H_5N_5O)_2 \cdot H_2SO_4$: C, 30.0; H, 3.02. Found: C, 29.6, 29.7; H, 3.06, 3.22.

The ultraviolet absorption spectrum of the radioactive sample at *p*H 6.5 had maxima at 246 mμ (ε 12,050), and at 275 mμ (ε 9,330) in essential agreement with that reported by Cavalieri, *et al.*[12] An ascending filter paper chromatogram, run on Whatman No. 1 paper in a medium consisting of *n*-butanol (4 parts), diethylene glycol (1 part), and water (1 part) in an ammonia atmosphere[13] showed only one spot when scanned in ultraviolet light; the R_f value was 0.21, the same as that of an authentic sample of guanine run concurrently. An autoradiogram of the filter paper strip showed only one radioactive-spot, coinciding with the spot visible in ultraviolet light.

2,4,5,6-Tetraminopyrimidine-2-C^{14} and 2,6-Diaminopurine-2-C^{14}.—Tetraminopyrimidine, prepared from guanidine-C^{14} and malononitrile,[14] was isolated as the sulfate (30–45% yield from guanidine), which was converted to diaminopurine sulfate by the procedure of Bendich, Tinker and Brown[15]; yield 65–85% after two crystallizations from 2 *N* sulfuric acid.

Anal. Calcd. for $(C_5H_6N_6)_2 \cdot H_2SO_4 \cdot H_2O$: C, 28.8; H, 3.87. Found: C, 29.3; H, 3.67.

The ultraviolet absorption spectrum at *p*H 6.5 had maxima at 247 mμ (ε 10,000) and at 280 mμ (ε 11,500), in essential agreement with that reported by Cavalieri, *et al.*[12] A filter paper chromatogram, made as described for guanine, gave only one spot, visible in ultraviolet light, which had the same R_f value (0.33) as an authentic diaminopurine sample. However, the autoradiogram of the filter paper strip showed a second spot with an R_f value the same as that of guanine; the second spot was faint and was observed only with a sample of high specific activity (11 μc./mg.).

Thiouracil-2-C^{14} and Uracil-2-C^{14}.—The methods described are modifications of the procedures of Wheeler and Liddle.[7] Thiourea-C^{14}[6] was used as the crude product (m.p. 160–165°).

A sodium ethylate solution, prepared from 0.7 g. of sodium and 35 ml. of dry alcohol, was added to a flask containing 1.35 g. of crude isotopic thiourea (about 85% pure) and 4.2 g. of ethyl β,β-diethoxypropionate. The solution was refluxed for four hours after which alcohol was removed in a stream of nitrogen. The residue was dissolved in cold water and thiouracil was precipitated by the addition of cold 50% acetic acid. The crude thiouracil weighed 1.1 g. (55% from barium carbonate; 40–55% yield on other runs). No difference in yield was noted when ethyl β,β-diethoxypropionate was prepared from ethyl bromoacetate and ethyl orthoformate, in which case it contains a considerable amount of ethyl β-ethoxyacrylate.[9]

For analysis the product of an active run was recrystallized twice from water and dried *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_4H_4N_2OS$: S, 25.0. Found: S, 24.6, 24.6.

The ultraviolet absorption spectrum at *p*H 6.5 had a maximum at 274 mμ (ε 13,500); at *p*H 11.0 the maxima were at 259 mμ (ε 10,200) and at 312 mμ (ε 7,160) in substantial agreement with the spectra reported by Elion, Ide and Hitchings.[16]

A sample of crude radioactive thiouracil was converted to uracil (73% yield) by the procedure of Wheeler and Liddle[7] and the product recrystallized once from water. The ultraviolet absorption spectrum at *p*H 6.2 had a maximum at 262 mμ (ε 8,320) in essential agreement with reported values.[17,18] A filter paper chromatogram and autoradiogram (made as described for guanine) showed only one spot; the R_f value was 0.54, the same as that of an authentic sample of uracil.

Thiothymine-2-C^{14} and Thymine-2-C^{14}.—These were prepared from thiourea-C^{14} and ethyl α-methyl-β,β-diethoxypropionate[9] by the same general procedures used for thiouracil and uracil; yields: crude thiothymine, 40–50%; thymine, 43–55% from crude thiothymine.

For analysis, products of inactive runs of thiothymine and thymine were recrystallized from water and dried over phosphorus pentoxide *in vacuo*.

Anal. Calcd. for $C_5H_7N_2OS$: S, 22.6. Found: S, 22.1, 22.5. Calcd. for $C_5H_7N_2O_2$: N, 22.2. Found: N, 22.1, 22.2.

The ultraviolet absorption spectrum of thiothymine at *p*H 6.5 had a maximum at 277 mμ (ε 15,200); that of thymine at *p*H 6.5 a maxima at 264 mμ (ε 7,640), in essential agreement with reported values.[16,18] A chromatogram and autoradiogram (made as described for guanine) of the active thymine sample showed only one spot with a R_f value of 0.74, the same as that of an authentic thymine sample run concurrently.

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Synthesis of S^{35}-Labeled Sulfanilic Acid[1,2]

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Sulfanilic acid labeled with S^{35} has been prepared by Pressman, *et al.*,[4] by heating in vacuum a mixture of $H_2S^{35}O_4$ with a large excess of aniline, but the yields were low and variable (20 to 40%) and the product contained 11% ortho and 4% meta isomers. Consistently high yields of pure sulfanilic acid have been obtained by allowing pure aniline acid sulfate to exchange with carrier-free

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(2) Taken in part from a dissertation submitted to the Division of Biological Sciences of the University of Chicago, August, 1950.

(3) Public Health Service Research Fellow of the National Heart Institute, March through August, 1950.

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