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.

(10) C. K. Cain, M. F. Mallette and E. C. Taylor, Jr., *ibid.*, **68**, 1996 (1946).

(11) W. Traube, *Ber.*, **33**, 1371 (1900).

(12) L. F. Cavalieri, A. Bendich, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3875 (1948).

(13) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 703 (1948).

(14) M. F. Mallette, E. C. Taylor, Jr., and C. K. Cain, THIS JOURNAL, **69**, 1814 (1947).

(15) A. Bendich, J. F. Tinker and G. B. Brown, *ibid.*, **70**, 3109 (1948).

(16) G. B. Elion, W. S. Ide and G. H. Hitchings, *ibid.*, **68**, 2137 (1946).

(17) F. F. Heyroth and J. R. Loofbourow, *ibid.*, **56**, 1728 (1934).

(18) M. M. Stimson, *ibid.*, **71**, 1470 (1949).

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

BY J. S. INGRAHAM[3]

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

(1) Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial fund of the University of Chicago and by a research grant from the Eli Lilly Company, Indianapolis, Indiana.

(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.

(4) D. Pressman, H. N. Eisen, M. Siegel, P. J. Fitzgerald and A. Silverstein, *J. Immunol.*, **65**, 559 (1950).

$H_2S^{35}O_4$[5] and then converting to sulfanilic acid using a small scale modification of the baking method of Huber.[6] The yields were about 95% for 50 mg. and 60 to 70% for 2 to 5 mg. batches. The product contained less than 0.2% of ortho or meta isomers and two recrystallizations from carrier sulfate reduced the free $S^{35}O_4^{=}$ to less than 0.2% of the total S^{35}. Not more than 0.6% of the sulfanilic acid sulfur exchanged with free sulfate in acid, basic, or neutral solution in 55 days at 80°.

Full experimental details of this preparation are available on microfilm.[7]

(5) Obtained from the Isotopes Division of the U. S. Atomic Energy Commission, Oak Ridge, Tennessee.

(6) W. Huber, *Helv. Chim. Acta*, **15**, 1372 (1932).

(7) For full experimental details of this preparation order Document 3489 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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RECEIVED DECEMBER 15, 1951

Small Scale Synthesis of Several Carbon-14 Labeled α-Hydroxy Acids[1]

BY D. M. HUGHES, R. OSTWALD[2] AND B. M. TOLBERT

For a series of biological studies the preparation of the two singly labeled glycolates and the three singly labeled lactates was undertaken as follows[3-5]

$$RCO_2Na + HCl\,(g) \longrightarrow RCO_2H\,(anhyd.) + NaCl$$

$$CH_3CO_2H + Cl_2 \xrightarrow[CH_3COCl]{P_4,\ I_2} CH_2ClCO_2H + HCl$$

$$CH_2ClCO_2H + CaCO_3 \longrightarrow (CH_2OHCO_2)_2Ca + CaCl_2 + CO_2$$

$$CH_3CH_2CO_2H + Br_2 \xrightarrow[CH_3CH_2COCl]{P_4,\ I_2} CH_3CH_2BrCO_2H + HBr$$

$$2CH_3CH_2BrCO_2H + 4OH^- + Zn^{++} \longrightarrow (CH_3CH_2OHCO_2)_2Zn + 2Br^-$$

The purity of the calcium glycolate was checked by three methods. In the first, the salt was recrystallized from water and the specific activity remeasured. It was found that the activity did not change, thus confirming the gross purity of the product. A C and H analysis of the product agreed well with the calculated values, although it was noted that sometimes the dihydrated salt crystallized out and sometimes the anhydrous calcium glycolate was obtained. A two-dimensional paper chromatographic separation (butanol–propionic acid–water in the first direction; phenol–water in the second) and radioautographs of the resulting paper showed only one radioactive spot, thus confirming the radioactive purity of the salt.[6]

The purity of the zinc lactate was similarly checked by elementary analysis and paper chromatography. Attempts to recrystallize the crude zinc lactate from distilled water failed to give pure products because of partial hydrolysis of the zinc lactate. This was corrected by crystallizing the product from a 0.1 *M* zinc chloride solution.

In order to produce a stable zinc lactate of uniform hydration, the product was first dried *in vacuo* and then hydrated in the laboratory (relative humidity ~ 50%). Evidence for exactly three waters of hydration was obtained not only from the analytical work (C, H, ash) but also from the weight ratio of the anhydrous to the hydrated material.

For biological experiments these salts can be easily and quantitatively converted to an aqueous solution of the free acid by mixing a solution of the salt with excess Dowex-50 ion exchange resin in the acid form. When the resin is filtered off, a zinc or calcium-free solution of the organic acid is left.

Yields, specific activities and scale of the several reactions are summarized in Table I.

TABLE I

Compound	Scale of reaction, mmoles	Sp. act. of prod., μc/mg.	Yield based on fatty acid, %
Calcium glycolate-1-C^{14}	13.3	0.24	65.0
Calcium glycolate-2-C^{14}	13.7	0.30	60.5
Zinc lactate-1-C^{14}	6.3	3.85	81.8
Zinc lactate-2-C^{14}	13.5	6.90	84.0
Zinc lactate-3-C^{14}	12.7	3.09	76.3

Acknowledgment.—The authors would like to thank Prof. M. Calvin for his continued help and encouragement in this work.

(6) A. A. Benson, *et al.*, THIS JOURNAL, **72**, 1710 (1950).

RADIATION LABORATORY AND
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RECEIVED JANUARY 31, 1952

The Synthesis of Bis-(2-hydroxy-3,5,6,-trichlorophenyl)-methane-C^{14} (Hexachlorophene)[1]

BY HERBERT M. ISIKOW AND WILLIAM S. GUMP

RECEIVED JANUARY 31, 1952

In the course of a study of the uptake of bis-(2-hydroxy-3,5,6-trichlorophenyl)-methane[2] (hexachlorophene) from soap solutions by the skin of experimental animals, it was necessary to prepare this compound labeled with carbon-14. Its preparation was readily effected by condensing 2,4,5-trichlorophenol with formaldehyde-C^{14} in the presence of sulfuric acid. Carbon-14 was thus incorporated into the methylene bridge of hexachlorophene as shown by the equation

(1) Details of the chemical procedure are available on microfilm. Order Document 3567 from the American Documentation Institute, 1719 N Street, N. W., Washington 16, D. C., remitting $1.00 for microfilm or $1.20 for photocopies readable without optical aid.

(2) Supported by a grant to Prof. D. M. Greenberg, University of California, from the American Cancer Society, Committee on Growth of the National Research Council. The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

(3) A. Hölzer, *Ber.*, **16**, 2955 (1883).

(4) A. Kekulé, *Ann.*, **130**, 18 (1864).

(5) E. Fischer and G. Zemplén, *Ber.*, **42**, 4891 (1909).

(1) For detailed paper order Document 3568 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.

(2) W. S. Gump, U. S. Patent 2,250,480 (July 29, 1941).