# NOTES

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

By W. H. Baldwin and C. E. Higgins

Received January 26, 1952

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.

Oak Ridge National Laboratory
Oak Ridge, Tenn.

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

By R. M. Anker[2a] and J. W. Boehne, III[2b]

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-

(1) This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

(2) (a) Department of Medicine, University of Colorado, Denver, Colorado; (b) Lederle Research Fellow in Pharmacology.

(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]: $6.75 \times 10^3$ at 390 mμ, $2.09 \times 10^3$ at 345 mμ, $0.61 \times 10^3$ at 300 mμ, $17.3 \times 10^3$ at 255 mμ, and $4.12 \times 10^3$ 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).

DEPARTMENT OF PHARMACOLOGY
SCHOOL OF MEDICINE
WESTERN RESERVE UNIVERSITY
CLEVELAND, OHIO

RECEIVED AUGUST 16, 1951

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

BY LEONARD L. BENNETT, JR.

RECEIVED DECEMBER 14, 1951

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.

(2) S. H. Zbarsky and I. Fischer, *Can. J. Research*, **27B**, 81 (1949).

(3) N. H. Marsh, L. C. Lane and D. J. Salley in M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. F. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 158.

(4) A. Murray, III, and A. R. Ronzio, THIS JOURNAL, **71**, 2245 (1949).

(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.

(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).

ORGANIC AND BIOCHEMISTRY DIVISION
SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALABAMA

## 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.

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY
UNIVERSITY OF CHICAGO
CHICAGO 37, ILLINOIS
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
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA
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.

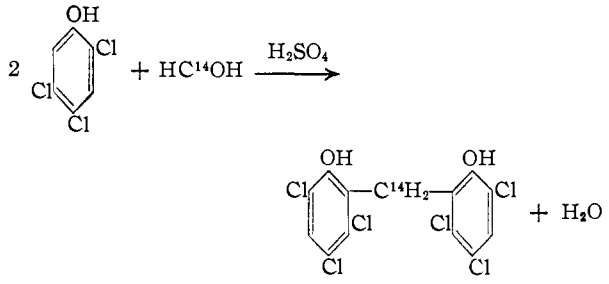
(3) A. Hölzer, *Ber.*, **16**, 2955 (1883).

(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).



Hexachlorophene-$C^{14}$ (m.p. 166–167°, cor.) was isolated in a 59.6% over-all chemical yield as two crops, in the second of which 12.5% was recovered with carrier; specific activity (of undiluted product): $3.58 \times 10^8$ (±4.6%) cts./min./mM.[3]; specific activity of starting formaldehyde-$C^{14}$: $3.52 \times 10^8$ (±4.4%) cts./min./mM.[4]; radiochemical yield: 60.6 ± 3.9%.

(3) Counted as a film (<0.01 mg./cm.²) in a proportional flow counter. A geometry of ~55% was estimated for this counter from the data of W. E. Graf, *et al.*, *Nucleonics*, **9**, No. 4, 22 (1951). The per cent. error shown includes errors of counting and pipetting.

(4) Counted as a film (<0.01 mg./cm.²) of the dimedone derivative.

RADIOISOTOPE LABORATORY
U. S. TESTING COMPANY, INC.
HOBOKEN, N. J., AND THE
RESEARCH LABORATORIES OF THE
GIVAUDAN CORPORATION
DELAWANNA, N. J.

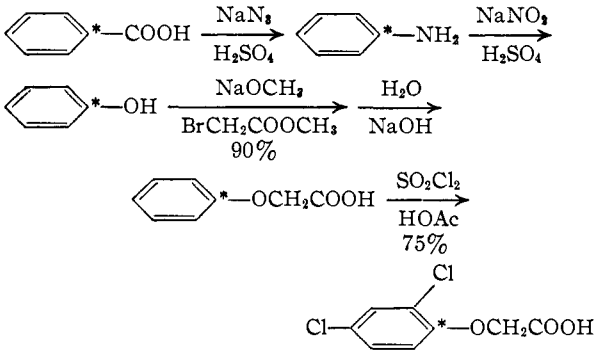
## Synthesis of 2,4-Dichlorophenoxyacetic Acid Labeled with Isotopic Carbon[1]

BY MELVIN FIELDS, SEYMOUR ROTHCHILD AND MORRIS A. LEAFFER

Investigation of the synthesis of carbon-14 labeled 2,4-dichlorophenoxyacetic acid, 2,4-D, was undertaken with the purposes of preparing samples of this plant growth regulator labeled at each of the carbon atoms of the acetic acid chain and in the aromatic ring, and of developing procedures suited to the ultimate preparation of material with high specific activity. After our work had been completed there was reported the synthesis of carboxyl labeled 2,4-D from potassium acetate in yields of 36–72%.[2] By the procedure described in this communication the conversion of sodium acetate to side chain-labeled 2,4-D was achieved in yields of 75–80% and the transformation of ring labeled benzoic acid to 2,4-D was accomplished in yields of 25–45%.

The conversion of 1 millimole of tagged sodium acetate to carboxyl or methylene labeled 2,4-D was achieved by bromination of the acetate with a mixture of red phosphorus and bromine containing 0.1 millimole of freshly distilled acetyl chloride and 1 millimole of anhydrous hydrogen chloride, followed by treatment of the bromoacid with an excess of 2,4-dichlorophenol in alkaline solution.

The preparation of 2,4-dichlorophenoxy-1-$C^{14}$ acetic acid from benzoic acid-1-$C^{14}$[3] was accomplished by the following sequence of reactions



The concentration of the sulfuric acid employed in the Schmidt reaction with benzoic acid plays a significant role in determining the yield of aniline. In Table I are summarized the results observed with 96–100% sulfuric acid; under comparable conditions with 75% sulfuric acid, the yield of aniline is reported to be only 15%.[4]

TABLE I

EFFECT OF CONCENTRATION OF SULFURIC ACID IN SCHMIDT REACTION

| Concn. sulfuric acid | Yield, % Aniline hydrochloride (uncorrected) | Benzoic acid recovered | Aniline hydrochloride (corrected) |
|---|---|---|---|
| 96 | 60–65 | 20–25 | 75–87 |
| 98 | 71 | 13 | 89 |
| 100 | 91 | 0 | 91 |

An increase in the amount of sodium azide or of the quantity of 96% sulfuric acid used appeared to have no appreciable effect on the extent of the conversion. Although 100% sulfuric acid has been employed in the Schmidt reaction with hindered aromatic acids,[5] the advantage of its use with unhindered acids does not appear previously to have been noted.

Several procedures for the chlorination of phenoxyacetic acid were investigated. With excess sulfuryl chloride in glacial acetic acid solution at 75°, 2,4-D was obtained in 74% yield. With this reagent in the absence of a solvent, phenoxyacetic acid was converted to the *p*-chloro derivative while in boiling carbon tetrachloride no reaction was observed. As reported by Haskelberg[6] reaction with chlorine gas in acetic acid solution afforded 2,4-D in 75% yield; on a small scale, however, regulation of the quantity of chlorine is troublesome, and an excess of the reagent leads to formation of the 2,4,6-trichloro derivative.[6] Chlorination of phenoxyacetic acid with alkaline hypochlorite gave 2,4-D in at best 59% yield as compared with a reported yield of 75%.[7]

The specific activities of the 2,4-dichlorophenoxyacetic acid-1-$C^{14}$ and -2-$C^{14}$ were $1.1 \times 10^4$ and

(1) This paper is based upon work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Maryland, under Contract No. DA-18-064-CML-10 with Tracerlab, Inc.

(2) H. R. Mahler, R. J. Speer and A. Roberts, *Science*, **110**, 562 (1949).

(3) M. Fields, M. A. Leaffer and J. Rohan, *ibid.*, **109**, 35 (1949).

(4) L. H. Briggs, G. C. DeAth and S. R. Ellis, *J. Chem. Soc.*, 61 (1942).

(5) M. S. Newman and H. C. Gildenhorn, THIS JOURNAL, **70**, 317 (1948).

(6) L. Haskelberg, *J. Org. Chem.*, **12**, 426 (1947).

(7) C. Y. Hopkins and M. J. Chisholm, *Can. J. Research*, **24**, 208 (1946).

$1.2 \times 10^4$ c./sec./mg., respectively; that of the ring-labeled compound was 235 c./sec./mg.[8]

### Experimental

**2,4-Dichlorophenoxyacetic Acid** from Bromoacetic Acid.—From the reaction of 139 mg. of bromoacetic acid with a fifteen-fold excess of 2,4-dichlorophenol in sodium hydroxide solution, 210 mg. (95%) of 2,4-D, m.p. 138.5–139.5°, was isolated.

**Conversion of Sodium Acetate to 2,4-D.**—Thirty-seven and one-half milligrams (1 millimole) of anhydrous hydrogen chloride, 7.9 mg. (0.1 mM.) of freshly distilled acetyl chloride and 192 mg. (1.2 mM.) of bromine were distilled under high vacuum into a 25-ml. r.b. flask chilled in liquid nitrogen containing 82 mg. of sodium acetate and 1.5 mg. of red phosphorus. The flask, sealed in vacuum, was immersed in a boiling water-bath until the bromine color had nearly disappeared (1 to 5 hours). The product was dissolved in 1 cc. of water and converted to 2,4-D as already described. Consistent yields of 75–80% from sodium acetate were obtained. Omission of the hydrogen chloride and acetyl chloride from the bromination mixture lowered the over-all yield to 40–63%.

**Aniline.**—Ten grams of sodium azide was added over a period of 50 minutes to a rapidly stirred mixture of 12.2 g. of benzoic acid, 80 ml. of chloroform and 40 ml. of 100% sulfuric acid maintained at 40°. When the evolution of nitrogen had ceased, the reaction mixture was diluted with water and made alkaline; extraction with ether and treatment with hydrogen chloride afforded 11.8 (91%) of aniline hydrochloride; m.p. 194.5°.

**Phenol.**—Diazotization of 13.00 g. of aniline hydrochloride followed by hydrolysis of the diazonium salt gave 7.04 g. (75%) phenol, b.p. 95° (25 mm.).

**Phenoxyacetic Acid.**—Methyl phenoxyacetate, prepared from sodium phenoxide and methyl bromoacetate, was hydrolyzed in dilute sodium hydroxide solution, which on acidification yielded phenoxyacetic acid, m.p. 97–98°[9] in 90% yield.

**2,4-Dichlorophenoxyacetic Acid.**—The temperature of a mixture of 1.74 g. of phenoxyacetic acid, 0.39 g. of sulfuryl chloride and 7 ml. of glacial acetic acid was slowly raised from 0 to 75° and then maintained at the latter temperature until gas evolution ceased. Dilution of the reaction mixture with water and crystallization of the precipitate from benzene afforded 1.87 g. (74%) 2,4-D, m.p. 137–138°.

(8) All samples were converted to barium carbonate and counted using the upper shelf of a Tracerlab lead pig and a Tracerlab TGC-2 Geiger–Müller tube with a 1.0 mg./cm.$^2$ mica window.

(9) R. Fusco and F. Mazzucchi, *Gazz. chim. ital.*, **71**, 406 (1941); *C. A.*, **37**, 121 (1943).

BOSTON 10, MASS. RECEIVED JULY 18, 1951

## Synthesis of Radioactive Iodine$^{131}$ Analog of DDT[1]

BY JENS A. JENSEN AND GEORGE W. PEARCE

The iodine$^{131}$ analog, 1,1,1-trichloro-2,2-bis-(*p*-iodo$^{131}$-phenyl)-ethane, of DDT has been prepared by the steps

$$C_6H_5NH_2 + HNO_2 \xrightarrow[NaNO_2]{HCl} C_6H_5N_2Cl \quad (1)$$

$$C_6H_5N_2Cl + NaI^{131} \longrightarrow C_6H_5I^{131} \quad (2)$$

$$2C_6H_5I^{131} + CCl_3CHO \xrightarrow{ClSO_3H} (I^{131}C_6H_4)_2CHCCl_3 \quad (3)$$

The method of Lucas and Kennedy[2] was used for the first two steps. Twenty-two millimoles of aniline was converted to 15 millimoles of iodobenzene, using 22 millimoles of NaI in which 25 millicuries of NaI$^{131}$ was incorporated. The steam distilled product plus 6 millimoles of inactive iodobenzene was condensed with 8 millimoles of chloral using chlorosulfonic acid as condensing agent. Recrystallization of the crude product from 1 to 1 acetone–alcohol gave 1.42 g. (2.6 millimoles) of DI*DT, m.p. 173–174°, 25% yield based on iodobenzene. Activity recovery was 22.4% corrected for decay, but not for inactive iodobenzene added. Specific activity was 2.5 microcuries per milligram. The synthesis can be completed in 2–3 days.[3]

(3) For a detailed description order Document 3488 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 $1.20 for photocopies (6 × 8 inches) readable without optical aid.

TECHNICAL DEVELOPMENT BRANCH
SAVANNAH, GEORGIA RECEIVED AUGUST 16, 1951

## A Preparation of C$^{14}$ Labeled Isopropyl N-Phenylcarbamate[1,2]

BY ALBERT V. LOGAN AND JOSEPH MURRAY[2]

The preparation of C$^{14}$ labeled isopropyl N-phenylcarbamate (IPC) was undertaken as the initial phase of a plan to study the mode of action of the compound upon many weedy annual grasses. The experimental procedures described here were adopted as the most economical and best suited for the preparation of the IPC. Plant studies are underway at the present time utilizing the radioactive compound.[3]

### Experimental

Acetic acid labeled with C$^{14}$ in the carbonyl group was prepared by a modification of the Grignard method used by Van Bruggen.[4] The yields on two separate runs were 69.1 and 77.3% based on the $BaC^{14}O_3$ used. The radioactive barium acetate (254 mg., 0.995 millimole) obtained from the neutralization of the acetic acid was placed in a combustion tube backed by an equal amount of inactive barium acetate in a separate boat. Pyrolysis was carried out under vacuum at 500°.[5] The resulting carbonyl labeled acetone was collected in a liquid nitrogen cooled trap. The acetone was redistilled and reduced by the action of lithium aluminum hydride[6] in ether solution. The labeled isopropyl alcohol was dried over recalcined calcium oxide and vacuum transferred to a dry reaction vessel.

The IPC was prepared by heating 0.3 ml. of phenyl isocyanate with the isopropyl alcohol at 80° for three hours. The product was crystallized from boiling heptane; 160 mg. of IPC, m.p. 84–85°, was obtained. A second crop of crystals 164 mg., m.p. 79–80°, was obtained from the mother liquor. The yield of pure compound was 38% based upon $BaC^{14}O_3$ used and barium acetate added. The over-all

(1) Published with the approval of the Monograph Publications Committee, Oregon State College, as Research Paper No. 191, School of Science, Department of Chemistry.

(2) This note is based on a thesis submitted by Joseph Murray in partial fulfillment of the requirements for the degree of Master of Science at Oregon State College, June, 1950.

(3) For detailed experimental description order Document 3564 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.

(4) J. T. Van Bruggen, C. K. Claycomb and T. T. Hutchens, *Nucleonics*, **7**, 45 (1950).

(5) A. V. Grosse and S. Weinhouse, *Science*, **104**, 402 (1946).

(6) R. F. Nystrom and W. G. Brown, THIS JOURNAL, **69**, 1197 (1947).

(1) From the Technical Development Branch, Communicable Disease Center, U. S. Public Health Service, Federal Security Agency, Savannah, Georgia.

(2) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 351.

yield, including the less pure compound, was approximately 77% calculated on the same basis.

Both samples of IPC were counted as $BaCO_3$, corrected for self absorption. The first sample (m.p. 84–85°) showed an activity of $7.78 \times 10^5$ counts/min./mg. The second sample (m.p. 79–80°) showed an activity of $6.69 \times 10^5$ counts/min./mg.

**Acknowledgment.**—Funds for the purchase of the isotopes used were provided by the General Research Council, Oregon State College.

DEPARTMENT OF CHEMISTRY
OREGON STATE COLLEGE
CORVALLIS, OREGON RECEIVED JANUARY 28, 1952

## Synthesis of Carbon-14 Labeled Urea[1,2]

BY ALBERT L. MYERSON[3]

Carbon-14 labeled urea was conveniently synthesized in small quantities through the direct combination of carbon dioxide and ammonia at room temperature, to form ammonium carbamate. The latter compound was sealed in a capillary and heated to 135°, to form urea. The first reaction is quantitative, while the second reaches equilibrium at 40% conversion.

This synthesis constitutes one of the simplest operations by which radioactive carbon dioxide can be incorporated into an organic compound on a micro scale. The preparation of urea from carbon dioxide and ammonia was originally reported[4] using 10 to 20 g. quantities, where maximum conversion was obtained by heating 16 g. of carbamate in a volume of 37 cc. In the present work, two radioactive syntheses were carried out employing 30 and 300 mg. of barium carbonate, respectively, the total activity in each case being 0.35 mc. The m.p. of the white crystals of urea was 131.5° without recrystallization, compared to reported values of 132 to 133°.

(1) For experimental details of this synthesis order Document 3493 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) Reported at a symposium "Isotopes and Medicine," at the University of Wisconsin, Madison, Wis., in September, 1948.

(3) The Franklin Institute, Philadelphia, Pa.

(4) F. Fichter and B. Becker, *Ber.*, **44**, 3473 (1911).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WIS. RECEIVED DECEMBER 22, 1951

## A Synthesis of Formaldehyde-$C^{14}$ [1]

BY A. R. JONES AND W. J. SKRABA

Methanol-$C^{14}$ has been converted to formaldehyde-$C^{14}$ by the chlorination of methyl-$C^{14}$ acetate followed by hydrolysis of the chlorinated product. The reactions, first studied by Henry[2] and Michael[3] gave a 60% yield of product when isolated with the aid of inert formaldehyde.[4]

(1) This document is based upon work performed under Contract Number W-7405 eng. 26 for the Atomic Energy Commission at the Oak Ridge National Laboratory.

(2) L. Henry, *Ber.*, **6**, 739 (1873).

(3) A. Michael, *Am. Chem. J.*, **1**, 418 (1879).

(4) Since the preliminary report of this procedure (Jones and Skraba, *Science*, **110**, 332 (1949)), another synthesis has been proposed by A. Murray and A. R. Ronzio, AECU-991; LADC-778.

Since the hydrolysis of carefully purified chloromethyl acetate[5] gave a quantitative yield of formaldehyde, efforts to improve the over-all yield of formaldehyde from methanol were confined to the acetylation and chlorination steps. The over-all yield was not improved by conducting the acetylation at atmospheric pressure under reflux, and was considerably decreased when a mole proportion of pyridine was added before acetylation.

The chlorination yield was not affected by ultraviolet irradiation of the reaction mixture, nor by varying the reaction temperature from 20–60°. The over-all yields of formaldehyde were best when a slightly less than molar proportion of chlorine was used.

To avoid the competing chlorination of the methyl group of the acetate moiety, the methyl esters of chlorocarbonic, oxalic, chloroacetic, trichloroacetic, bromoacetic, benzoic and *p*-toluenesulfonic acids were chlorinated. Poor yields were obtained in all cases. A mixture of methyl bromoacetate and bromine was decolorized after two days at room temperature, but hydrolysis of the product yielded only a small amount of formaldehyde.[6]

### Experimental

**Acetylation.**—Methanol-$C^{14}$, 332 mg., 10.4 mmoles, 21.43 microcuries (2.06 microcuries/mmole) and acetyl chloride, 816 mg., 10.4 mmoles, were consecutively high-vacuum distilled[7] into the liquid nitrogen cooled nipple of a one-liter bulb. The reaction vessel was isolated from the manifold and the frozen reagents were warmed to 40–50° for 45 minutes with a heat lamp. The contents were then frozen into the nipple by immersing the latter in liquid nitrogen. To remove a part of the hydrogen chloride, the nipple was warmed to −80° (Dry Ice and trichloroethylene) and the bulb was evacuated to $10^{-4}$ mm.

**Chlorination.**—The Dry Ice–trichloroethylene-bath was replaced by liquid nitrogen and 1400 ml. (27° and 12.9 cm. pressure), 9.6 mmoles, of commercial chlorine gas, from which impurities non-condensable with liquid nitrogen had been removed, was distilled into the reaction bulb. The pressure of chlorine was determined with a manometer in which the mercury was protected by a layer of sulfuric acid. The bulb was isolated from the manifold and the contents were allowed to warm to room temperature in subdued light. Loss of the chlorine color began while the reactants were still quite cold. The contents of the bulb were recondensed and allowed to return to room temperature several times to ensure thorough mixing. When all trace of chlorine color had disappeared, one to two hours, part of the hydrogen chloride was removed as described above.[8]

**Hydrolysis.**—A 25-ml. hydrolysis bulb containing 3 ml. of distilled water and equipped with a spring-loaded 4-mm. straight-bore stopcock was attached to the manifold, im-

(5) M. Descude, *Compt. rend.*, **132**, 1567 (1901).

(6) Radioactive paraformaldehyde was prepared by treating chloromethyl-$C^{14}$ acetate with sufficient commercial formalin solution to furnish the water for hydrolysis. This procedure produced a paste which left a residue of dry polymeric formaldehyde when the volatile material was removed under high vacuum.

In attempts to prepare an isolable solid derivative from which formaldehyde would be easily recoverable, formaldehyde oxime, hexamethylenetetramine and the methylol derivatives of saccharin and phthalimide were investigated. None gave sufficiently high yields of derivative from reaction with aqueous formaldehyde.

Direct oxidation of dilute methanol to formaldehyde with potassium persulfate (P. D. Bartlett and J. D. Cotman, THIS JOURNAL, **71**, 1419 (1949)) was attempted as a preparative method, but was not found feasible because of the difficulty of recovering formaldehyde from the dilute aqueous solution necessary for reaction to take place.

(7) All joints and stopcocks were greased with Dow-Corning silicone vacuum grease.

(8) After a number of runs the reaction bulb contained a trace of white non-volatile material and the over-all yields of formaldehyde decreased. Replacement of the bulb corrected the matter.

mersed in liquid nitrogen, and evacuated. After the crude chloromethyl-$C^{14}$ acetate was transferred to the hydrolysis bulb, the stopcock was closed and the vessel was removed from the vacuum line, and immersed to the stopcock in boiling water for thirty minutes to allow hydrolysis to take place.[9] The flask was cooled to room temperature and with the aid of a file mark on the stem, the stopcock was removed and the contents were transferred to a 20-ml. pear-shaped flask. Five milliliters of 37% commercial formalin solution containing approximately 60 mmoles of formaldehyde was used to rinse the hydrolysis bulb and complete the transfer. The mixture was made slightly basic with potassium hydroxide pellets, and then barely acidified to phenolphthalein with acetic acid. A neutral formalin solution which weighed 9.027 g. was obtained by distillation to dryness at atmospheric pressure into the ice-cooled receiver of the aliquoter (Fig. 1).

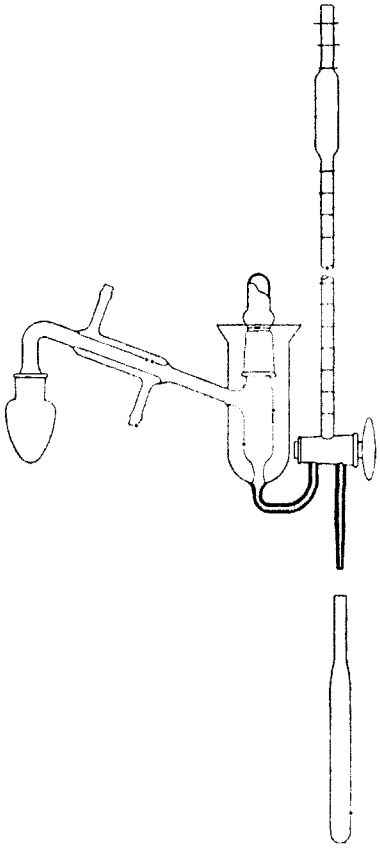


Fig. 1.—Distillation and aliquoting apparatus.

**Analysis.**—A 75-microliter aliquot (78 mg.) of this solution was added to a solution of 0.40 g. of dimedon reagent in 100 ml. of water. After standing for 24 hours at room temperature, the dimedon derivative of formaldehyde was filtered off, washed with water and dried. In this way, 159 mg. of dimedon-formaldehyde was obtained.

The dry combustion of a 22.6-mg. sample of the derivative gave 181 ml. of carbon dioxide (28.5° and 13.7 cm. pressure) which produced an ion current of $6.80 \times 10^{-14}$ amperes when the radioactivity assay was made with a dynamic condenser electrometer. The factors $1.17 \times 10^{-16}$ ampere per disintegration per second and $3.7 \times 10^{4}$ disintegrations per second per microcurie were used to convert the ion current to microcuries. The total activity of the formaldehyde in the neutral distillate was calculated to be 12.9 microcuries, a radiochemical yield of 60.5%.

To show that no isotopic dilution had occurred, a run was made starting with 258 mg. of methanol-$C^{14}$, 8.06 mmoles, 96.75 microcuries (sp. act. 12.00 microcuries per millimole). A 0.295-g. aliquot of the acid hydrolysis solution (3.755 g. total weight) gave 100 mg. of formaldehyde-dimedon derivative, 0.342 mmole, 4.17 microcuries (sp. act. 12.18 microcuries per millimole). From these figures both the radiochemical yield (51%) and the chemical yield (59%) can be calculated.

For the analysis of production runs, where the isotopic ratio was much greater, a small aliquot of the neutral distillate was diluted with carrier formaldehyde solution and aliquots of this mixture were analyzed radiochemically by the method given above.

OAK RIDGE, TENN. RECEIVED NOVEMBER 2, 1951

(9) There has been no failure of either bulb or stopcock observed in more than fifty hydrolyses.

## Adrenergic Blocking Agents. V. Synthesis of N - Benzyl - N - (1 - phenoxyisopropyl) - β - chloroethylamine Hydrochloride Labeled with $C^{14}$

BY EDWARD J. NIKAWITZ, WILLIAM S. GUMP, JAMES F. KERWIN AND GLENN E. ULLYOT

RECEIVED JULY 18, 1951

Since the discovery[1] of the remarkable adrenergic-blocking ability of N,N-dibenzyl-β-chloroethylamine hydrochloride following intravenous administration, our attention has been directed toward the development of a compound effective at a tolerated dosage level with the view that such an agent might find practical therapeutic application. Progress toward this goal has been achieved recently in the synthesis of N-benzyl-N-(1-phenoxyisopropyl)-β-chloroethylamine hydrochloride.[2] In order that further studies regarding the absorption, distribution, fate, site of action and mechanism of action of an adrenergic blocking drug of this type might be undertaken, it was deemed desirable to prepare a quantity of this compound labeled with $C^{14}$. Because of the availability of labeled benzyl chloride and because of the desire to label a group which might be expected to remain with the nitrogen containing moiety of a possible breakdown product we chose to prepare the compound labeled at the methylene of the benzyl group (see I).

$$C_6H_5OCH_2CHCH_3$$
$$C_6H_5\overset{*}{C}H_2—N—CH_2CH_2Cl\cdot HCl \qquad (I)$$

The synthetic procedure was that previously employed but adapted to a suitable scale.

### Experimental[3]

(1) **$C^{14}$-Labeled N-Benzyl-N-(1-phenoxyisopropyl)-2-aminoethanol.**—Benzyl chloride (0.684 g.) labeled with $C^{14}$ in the side chain,[4] N-(1-phenoxyisopropyl)-2-aminoethanol (1.09 g.), anhydrous sodium carbonate (0.29 g.) and 7 ml. of absolute alcohol were heated under reflux at 85–90° for 10 hours. The alcohol was then removed by sucking the vapors away by means of an inverted glass funnel and vacuum. The remaining salt and oil were mixed with small amounts of water and ether. The ether solution was separated, dried, concentrated to a small volume and transferred into bulb 1 of a distilling apparatus having 3 bulbs (Fig. 1). A small portion of additional ether was used to wash the flask. The ether was then removed by heating bulb 1 in a water-bath at 40–50°.

(1) M. Nickerson and L. S. Goodman, *Federation Proc.*, **5**, 194 (1946); *J. Pharm. Expt. Therap.*, **89**, 167 (1947); Nickerson and Gump, *ibid.*, **97**, 25 (1949).

(2) J. F. Kerwin, G. C. Hall, F. J. Milnes, I. H. Witt, R. A. McLean, E. Macko, E. J. Fellows and G. E. Ullyot, THIS JOURNAL, **73**, 4162 (1951).

(3) The synthesis with the tagged material was carried out in the laboratories of U. S. Testing Co., Inc., Hoboken, N. J.

(4) Obtained from Tracerlab, Inc., Boston, Mass., with a specific activity of 1.3 mc./mM.

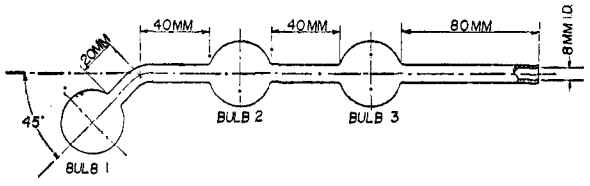



Fig. 1.—Inside diameter of all bulbs about 37 mm.

The residual aminoalcohol was distilled in high vacuum by first putting bulbs 1 and 2 of the distilling apparatus into a small metal box-shaped oven, fitted with a mica window on one side, a removable cover, a slit on one end and a thermometer, and heated with a bunsen burner. During the distillation, the slit in the box was covered with a piece of thick asbestos paper and wet cloth was wrapped around the bulbs remaining outside of the box.

A temperature of 142° was maintained inside of the oven for 15 minutes, allowing the forerun to distil at 0.15 mm. into bulb 3. After that period, the distilling apparatus was moved so that only bulb 1 remained in the box. Small amounts of the forerun condensed in bulb 2 were driven into bulb 3 by careful heating with a bunsen burner. The desired aminoalcohol was then distilled into bulb 2 as a colorless oil at 0.15 mm. and an oven temperature of 160–178°, the operation taking about 25 minutes.

Bulb 2 was then separated by cutting the connections to the other bulbs and the aminoalcohol (1.1205 g.) poured into a Pyrex tube of 100 mm. length and 22 mm. inside diameter. Three ml. of chloroform was used to wash the bottle.

(2) **$C^{14}$-Labeled N-Benzyl-N-(1-phenoxyisopropyl)-β-chloroethylamine Hydrochloride.**—The tube containing the chloroform solution of the aminoalcohol was cooled in an ice-bath and the procedure given in the literature[2] was followed in preparing the desired compound. Shiny white crystals (1.0414 g.) of the m.p. 137.5–140° (lit.[2] 137.5–140°) were obtained.

RESEARCH LABORATORIES OF GIVAUDAN-DELAWANNA, INC., AND
SMITH, KLINE AND FRENCH LABORATORIES
PHILADELPHIA 1, PENNA.

## A Convenient Synthesis of Uracil 2-$C^{14}$ from Urea[1]

BY H. GEORGE MANDEL AND CURTIS L. BROWN

Uracil, a normal constituent of pentose nucleic acid, has been shown by many authors to act as a growth factor for various organisms.[2-6] It therefore became desirable to prepare this compound labeled with $C^{14}$ in order to study its physiological disposition in several species. Since $C^{14}$ urea is commercially available,[7] it was desirable to devise a synthesis with this substance as the limiting reagent. Non-radioactive uracil has been prepared in a 25% yield, based on urea, by Davidson and Baudisch.[8] The yield was improved slightly by temperature modifications introduced by Chi and Chen.[9] Hilbert[10] has observed that the amount of sulfur trioxide in the fuming sulfuric acid, the temperature of heating and the order of addition of the reagents influence the success of related condensation reactions.

The procedure outlined below permits the preparation of uracil in a better than 60% yield based on $C^{14}$-urea.

### Experimental

Twenty ml. of fuming sulfuric acid (18% of $SO_3$) was placed in a three-necked 50-ml. flask equipped with a mercury seal stirrer, a thermometer and a funnel-shaped inlet tube. After cooling the solution in a Dry Ice–alcohol-bath to −5°, 4.4 g. (0.033 mole) of finely pulverized malic acid was added with stirring, keeping the temperature below 0°. When the material had been finely dispersed, 1.7 g. (0.028 mole) of urea, previously pulverized and desiccated, and containing 1 mc. of $C^{14}$-urea was introduced in small portions over a period of ten minutes, keeping the temperature below 5°, and stirring vigorously. The mixture was then slowly warmed to 80°, whereupon all solid material dissolved. The solution was stirred at 80–85° for one hour, cooled and poured over 60 g. of crushed ice. After 48 hours in the ice-box, the uracil had separated. It was centrifuged, resuspended repeatedly in ice water, filtered off and dried. Recrystallization from hot water, carried out with non-radioactive uracil, showed that this step was unnecessary.

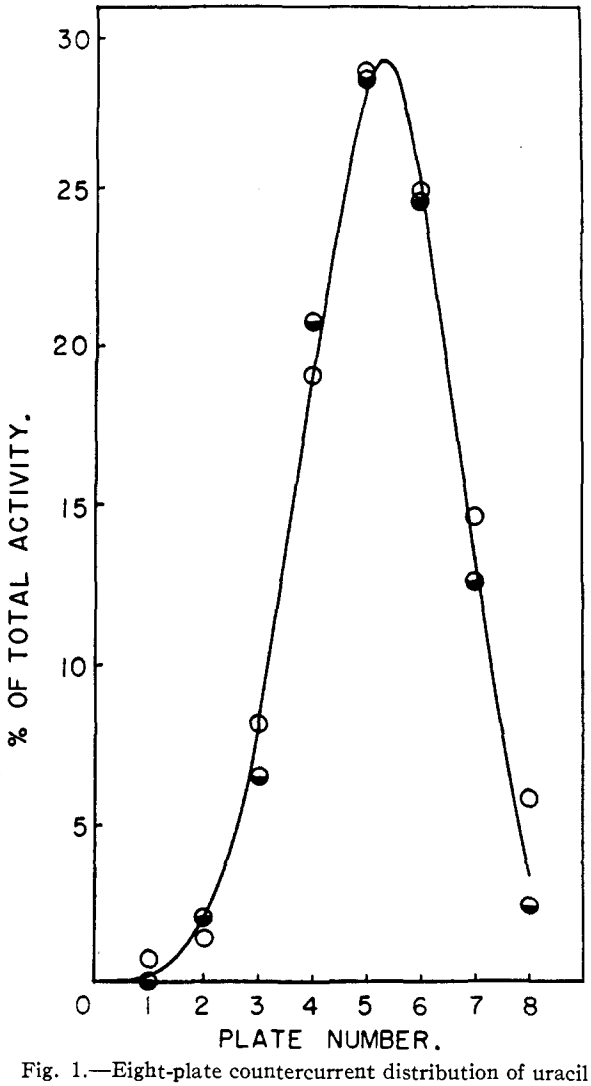



Fig. 1.—Eight-plate countercurrent distribution of uracil 2-$C^{14}$. Theoretical curve $K$ = 1.85 system 1 $M$ phosphate buffer $p$H 6.8, $n$-butanol and $t$-butanol; O, optical density at 260 mμ; ◒, radio assay.

(1) Aided by grants from the National Cancer Institute, of the National Institutes of Health, Public Health Service, and the Damon Runyon Fund.

(2) R. D. Housewright and S. A. Koser, *J. Infectious Diseases*, **75**, 113 (1944).

(3) S. H. Hunter, *Arch. Biochem.*, **4**, 119 (1944).

(4) R. E. Feeney, J. H. Mueller and P. A. Miller, *J. Bact.*, **46**, 559 (1943).

(5) E. Diczfalusy and H. v. Euler, *Arkiv Kemi, Mineral. Geol.*, **24A**, No. 38 (1947).

(6) G. W. Kidder, *Ann. N. Y. Acad. Sci.*, **49**, 99 (1947).

(7) Purchased from U. S. Atomic Energy Commission, Los Alamos Scientific Laboratory.

(8) D. Davidson and O. Baudisch, THIS JOURNAL, **48**, 2382 (1926).

(9) Y. F. Chi and Y.-H. Chen, *Trans. Science Soc. China*, **8**, 83 (1934).

(10) G. Hilbert, THIS JOURNAL, **54**, 2081 (1932).

A yield of 1.95 g. of uracil (62% based on urea) was obtained with a specific activity of 0.33 μc./mg.

The substance exhibited an ultraviolet absorption spectrum and an extinction coefficient identical with those reported in the literature.[11,12] A descending chromatogram of the material in a mixture of *t*-butanol–glacial acetic acid–water (65:25:10 v./v.), using Whatman No. 1 paper, showed a single radioactive component having an $R_f$ value of 0.60. The product was subjected to an 8-plate countercurrent distribution in a system of 1 *M* potassium phosphate buffer at *p*H 6.8 and a mixture of equal volumes of *n*-butanol and *t*-butanol. The optical density at 260 mμ for the aqueous and organic layer of each plate was measured, and the sum of the values for the two phases of the various plates is plotted in Fig. 1. The relative radioactivity of each plate was determined by the addition of a constant volume of methanol and water to each plate to make the two layers mutually soluble. Aliquots of these solutions were then plated in plastic cups, dried and assayed for radioactivity in a gas-flow proportional counter. It was found that in addition to the background a correction for naturally occurring $K^{40}$ of the buffer was necessary. The resulting values are plotted in Fig. 1. The close agreement, within the accuracy of the technique, with the calculated curve for authentic uracil having a distribution of 1.85 in such a system, indicated that the substance was of high purity.

(11) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

(12) F. F. Heyroth and J. R. Loofbourow, THIS JOURNAL, **56**, 1728 (1934).

DEPARTMENT OF PHARMACOLOGY
THE GEORGE WASHINGTON UNIVERSITY
SCHOOL OF MEDICINE
WASHINGTON 5, D. C.

RECEIVED JULY 18, 1951

## Absence of Rapid Exchange of Sulfur Atoms between Sulfate and Persulfate Ions

BY P. C. RIESEBOS AND A. H. W. ATEN, JR.

In aqueous solutions a rapid exchange between $Hg^{++}$ and $Hg^{2++}$-ions has been reported.[1] This observation suggests that an investigation of exchange reactions between ion pairs of the same type might be a matter of some interest. As the sulfate–persulfate combination is fairly easy to handle, we have performed some experiments with this system. It may be pointed out that in this exchange process an oxygen–oxygen bond is affected, whereas in the sulfur exchanges studied earlier, like the $SO_4^{=}$–$HS^-$, $SO_4$–$SO_3^{=}$, $S_2O_3^{=}$–$HS^-$ and $S_2O_3^{=}$–$SO_3^{=}$ reactions,[2] bonds between a sulfur and an oxygen atom or between two sulfur atoms were attached.

Solutions containing radioactive potassium sulfate, labeled with $S^{35}$ (about 0.001 or 0.002 molar) and inactive potassium persulfate (about 0.0005 or 0.001 molar) were kept at room temperature for a week. The sulfate fraction was precipitated as barium sulfate after which the persulfate was decomposed by boiling with hydrochloric acid. Experiments were performed at *p*H values of about 1, about 7 and about 10. In all cases the average value of the specific activity of the sulfur in the persulfate amounted to less than 2% of the specific activity of the sulfate sulfur. (Large differences between figures obtained in duplicate experiments suggest, however, that most or all of the small activity found in the persulfate fraction may well be due to incomplete separation of the two fractions.) Under these circumstances the half-time of exchange amounts to about half a year at least.

Another series of exchange experiments was performed at *p*H about 10 in which the solutions were boiled for 5 minutes. This resulted in the decomposition of about 1/3 of the persulfate, after which the average of the radioactivity in this fraction still did not amount to more than 1.5% of the total activity in the system. (Here again the wide variation of the results suggests that this limit may be far too high.)

We are pleased to express our gratitude to the "Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek" and to the "Stichting voor Fundamenteel Onderzoek der Materie" for their support.

(1) S. Ruben, G. T. Seaborg and J. W. Kennedy, *J. Appl. Phys.*, **12**, 308 (1941).

(2) H. Voge, THIS JOURNAL, **61**, 1032 (1939); D. Ames, in A. C. Wahl and N. A. Bonner, "Radioactivity Applied to Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1951, p. 347.

INSTITUTE FOR NUCLEAR RESEARCH
AMSTERDAM, NETHERLANDS

RECEIVED JULY 18, 1951

## Synthesis of Histamine-2-$C^{14}$-Imidazole[1]

BY RICHARD W. SCHAYER[2]

Certain bacteria possess an enzyme which converts L-histidine into histamine and carbon dioxide.[3] Rodwell[4] has isolated unspecified strains of *Lactobacilli* possessing a very high histidine decarboxylase activity. Using an acetone powder preparation of these bacteria,[5] radioactive L-histidine has been decarboxylated and the radioactive histamine isolated as the dipicrate.

### Experimental

**Thiol-L-histidine-2-$C^{14}$-imidazole.**—Radioactive sodium cyanide (approximately 3 mc.) was prepared from $C^{14}$-barium carbonate without dilution of the isotope by the method of Belleau and Heard.[6] The sodium cyanide was converted to sodium thiocyanate by the method of Castiglioni[7] as adapted by Borsook, *et al.*[8] After dilution with carrier equal to 1.5 times the estimated weight of the isotopic material, the sodium thiocyanate was treated with α,δ-diamino-γ-ketovaleric acid[9] (γ-ketoörnithine) producing 155 mg. of crystalline thiol-L-histidine which failed to melt up to 300°, as reported by Ashley and Harington.[9] Additional radioactive thiolhistidine was crystallized from the mother liquor after addition of carrier.

**L-Histidine-2-$C^{14}$-imidazole.**—One hundred and fifty mg. of thiolhistidine was oxidized with ferric sulfate to histidine[8,

(1) Supported in part by research grants from the U. S. Public Health Service and the Chicago Heart Association. Radioactive barium carbonate allocated by the Isotopes Division, U. S. Atomic Energy Commission.

(2) The author is indebted to Rosa L. Smiley for assistance.

(3) E. F. Gale, "Advances in Enzymol." Vol. 6, Interscience Publishers, Inc., New York, N. Y., 1946.

(4) A. W. Rodwell, private communication to Dr. Hutton Slade of this Institute.

(5) The author is greatly indebted to Dr. A. W. Rodwell, Research Officer, Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, for his generosity in supplying the acetone powder of the *Lactobacilli*.

(6) B. Belleau and R. D. H. Heard, THIS JOURNAL, **72**, 4268 (1950).

(7) A. Castiglioni, *Gazz. chim. ital.*, **63**, 171 (1933).

(8) H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, *J. Biol. Chem.*, **187**, 839 (1950).

(9) J. N. Ashley and C. R. Harington, *J. Chem. Soc.*, 2586 (1930).

which was isolated from the reaction mixture as the diflavianate yielding 370 mg. of L-histidine diflavianate, m.p. 245–247°. From the diflavianate was obtained 59.6 mg. of L-histidine, m.p. 294°. Ashley and Harington report 295°. For a 2% aqueous solution of a non-radioactive sample prepared by the same method $[\alpha]^{20}D$ −35.5°. Ashley and Harington found $[\alpha]^{20}D$ −36.0°.

**Histamine-2-C$^{14}$-imidazole.**—Histidine decarboxylase was prepared by incubating 50 mg. of acetone powder of the *Lactobacilli* with 5 ml. of McIlvaine buffer at *p*H 4.8 for 6 hours. After removing the cells by centrifugation, the enzyme preparation was added to 18.4 mg. of radioactive L-histidine in a Warburg flask and incubated at 30° for 65 minutes at which time carbon dioxide evolution was complete. The solution was transferred to a small separatory funnel, made strongly alkaline, and extracted four times with *n*-amyl alcohol. At this point 95% of the radioactivity was in the alcohol fraction. After one additional extraction the alcohol fractions were dried and evaporated to dryness *in vacuo*. The residue was dissolved in 3 ml. of water, a hot solution of 60 mg. of picric acid in 4 ml. of water added, the mixture heated to boiling and filtered; 44.3 mg. of histamine dipicrate, m.p. 238–242°, was obtained, a 65% yield from L-histidine. Pyman[10] reported m.p. 238–242°. Additional isotopic histamine dipicrate was crystallized from the mother liquor after the addition of carrier.

The activity using an internal counter was 9.3 × $10^6$ c.p.m. per mg. of histamine base.

*Anal.* (for a non-radioactive sample synthesized in the same manner) Calcd. for $C_5H_9N_3(C_6H_3O_7N_3)_2$: C, 35.8; H, 2.66; N, 22.2. Found[11]: C, 35.7; H, 2.79; N, 22.3.

A paper chromatogram of the histamine (as the dihydrochloride) in butanol–ammonia showed a single sharp radioactive peak at $R_F$ 0.80; under identical conditions the radioactive L-histidine produced a single sharp peak at $R_F$ 0.15. Thus the histamine is free of demonstrable contamination by histidine. Chromatograms of the histamine in other solvents showed single peaks suggesting absence of significant amounts of other radioactive impurities.

Before use in animal experiments the radioactive histamine dipicrate was recrystallized from water, a sample dissolved in 0.15 *N* hydrochloric acid, the picric acid extracted with ether, and the solution of histamine dihydrochloride neutralized with sodium bicarbonate just before use. In a test for pharmacological activity[12] a very dilute solution of the radioactive histamine dihydrochloride produced the same contraction of guinea pig uterus as did the same amount of commercial histamine dihydrochloride.

(10) F. L. Pyman, *J. Chem. Soc.*, **49**, 668 (1911).

(11) Analysis by Micro-Tech Laboratories.

(12) Kindly performed by Dr. Georges Ungar of this Institute.

RHEUMATIC FEVER RESEARCH INSTITUTE
NORTHWESTERN UNIVERSITY MEDICAL SCHOOL
CHICAGO, ILLINOIS RECEIVED AUGUST 22, 1951

## Synthesis of *dl*-Adrenalin-β-C$^{14}$ and *dl*-Adrenochrome-β-C$^{14}$

BY RICHARD W. SCHAYER[1]

The syntheses of radioactive adrenalin and adrenochrome were accomplished by known procedures modified for small-scale use and for conserving isotopic materials.

### Experimental

**Chloroacetic Acid-carboxyl-C$^{14}$.**—Barium carbonate-C$^{14}$ (3.0 millicuries)[2] was diluted to 4.92 g. and converted by the Grignard reaction to 1.74 g. (85% yield) of carboxyl-labeled sodium acetate.[3] Chloroacetic acid was synthesized by the method of Ostwald.[4] After recrystallization from ligroin, 2.01 g., m.p. 58°, a yield of 56% from sodium acetate after allowing for 400 mg. carrier, was obtained.

**Chloroacetylcatechol.**—Chloroacetic acid, 1.95 g., was heated on a steam-bath with 1.95 g. of catechol and 2.0 ml. of freshly distilled phosphorus oxychloride in an atmosphere of sulfur dioxide.[5] When the reaction was complete (about 45 minutes) the mixture was dissolved in 30 ml. of hot water, filtered and the residue washed. Crude chloroacetylcatechol, 1.50 g., m.p. 169–170°, was obtained. After recrystallization from hot water containing traces of hydrochloric acid and sodium bisulfite, 1.13 g. (29% yield from chloroacetic acid) was obtained having the reported melting point of 173°.

***dl*-Adrenalone Hydrochloride** (4-Methylaminoacetylcatechol Hydrochloride).—Chloroacetyl catechol, 1.00 g., was mixed with 5.0 ml. of 25% methylamine and allowed to stand at room temperature for 20 hours with frequent shaking.[6] Alcohol, 9 ml., was added and after standing 90 minutes in the cold, the brown precipitate was filtered, washed with 50% alcohol, absolute alcohol and finally ether. The crude adrenalone was dissolved in a minimum of dilute hydrochloric acid, diluted to about 20 ml. with water, and reprecipitated by addition of ammonia producing 0.52 g. of adrenalone (54% yield). Adrenalone, 0.52 g., was dissolved in a minimum of 3 *N* hydrochloric acid, filtered, absolute alcohol and finally ether added. Adrenalone hydrochloride, 0.50 g., crystallized, an 81% yield from adrenalone.

***dl*-Adrenalin-β-C$^{14}$** (Methylaminomethyl-(3,4-dihydroxyphenyl)-carbinol).—Adrenalone hydrochloride, 0.24 g., was dissolved in 10 ml. of water, 0.20 g. of catalyst (5% palladium-on-aluminum oxide) added and the mixture hydrogenated at ordinary pressure and temperature for two hours.[7] After filtering off the catalyst and adding excess ammonia 150 mg. of *dl*-adrenalin-β-C$^{14}$ (74% yield from adrenalone hydrochloride) was obtained. The over-all yield from barium carbonate to adrenalin was 4.4%.

*Anal.* (for a non-radioactive sample synthesized by the same method) Calcd. for $C_9H_{13}O_3N$: C, 59.00; H, 7.27; N, 7.65. Found[8]: C, 59.11; H, 7.36; N, 7.52.

The activity measured with an internal counter was 2.72 × $10^5$ c.p.m. per mg. The compound had the same effect on the blood pressure of a dog as did commercial synthetic epinephrine. A paper chromatogram of the adrenalin in butanol–acetic acid produced a single peak at $R_F$ 0.45.

***dl*-Adrenochrome-β-C$^{14}$.**[9]—*dl*-Adrenalin-β-C$^{14}$, 40 mg., plus non-isotopic adrenalin, 60 mg., were dissolved in 3.0 ml. of absolute methanol containing 0.06 ml. of 99% formic acid. After warming to 35°, 0.7 g. of silver oxide was added, the mixture shaken and maintained at 35° for exactly one minute, filtered through a rapid filter and washed with 1 ml. of methanol. Crystals started forming immediately. After storing at −15° for 30 minutes the adrenochrome was filtered and washed successively with 1:1 methanol–ether, 1:3 methanol–ether, and ether. A first crop of 22 mg. red-brown crystals was obtained. By careful addition of ether to the mother liquor an additional 18 mg. of adrenochrome crystallized giving a total yield of 40%.

*Anal.* (for a non-radioactive sample synthesized by the same method, after correction for 2.12% ash) Calcd. for $C_9H_9O_3N$: C, 60.3; H, 5.06; N, 7.82. Found[8]: C, 59.2; H, 5.30; N, 7.69.

The activity measured with an internal counter was 1.06 × $10^5$ c.p.m. per mg. Biological tests indicated that there was no observable contamination by adrenalin.

CONTRIBUTION FROM THE
RHEUMATIC FEVER RESEARCH INSTITUTE,
NORTHWESTERN UNIVERSITY MEDICAL SCHOOL
CHICAGO, ILLINOIS RECEIVED AUGUST 22, 1951

(1) Supported in part by a research grant from the United States Public Health Service. With the assistance of Rosa L. Smiley.

(2) Supplied by the Monsanto Chemical Company, on allocation from the United States Atomic Energy Commission.

(3) W. Sakami, W. E. Evans and S. Gurin, THIS JOURNAL, **69**, 1110 (1947).

(4) R. Ostwald, *J. Biol. Chem.*, **173**, 207 (1948).

(5) H. Hoberman, THIS JOURNAL, **57**, 1382 (1935).

(6) H. D. Dakin, *Proc. Roy. Soc.* (*London*), **B76**, 491 (1905).

(7) S. Sergievskaya and N. Fedotova, *Org. Chem. Ind., U. S. S. R.*, **2**, 96 (1936).

(8) Analysis by Micro-Tech Laboratories.

(9) C. L. MacCarthy, *Chim. et Ind.*, **55**, 435 (1946).

## The Isotope Effect. II. Pyrolysis of Lithium Acetate-1-$C^{14}$

BY ARTHUR ROE AND J. B. FINLAY

RECEIVED JULY 18, 1951

Several recent investigations have established the fact that an appreciable isotope effect may occur in reactions involving carbon-14. Yankwich and Calvin[1] reported a 12% effect in the decarboxylation of malonic-1-$C^{14}$ acid; this work was repeated by Roe and Hellmann[2] who found a 6% effect in the same reaction. Stevens and Attree[3] found a 16% effect in the hydrolysis of carboxy-labeled ethyl benzoate, and Stranks and Harris[4] reported an 11% effect in the absorption of $CO_2$–$C^{14}$ by a cobaltamine complex. Ropp, Weinberger and Neville[5] found an 8.6% effect at 25° in the dehydration of labeled formic acid. Theoretical discussions of the isotope effect have recently appeared.[6,7]

The pyrolysis of an acid salt yielding a ketone and carbon dioxide (or a carbonate) has been used frequently as a degradative procedure in organic tracer work as a means of determining the amount of isotopic carbon in the carboxyl group of the acid; each of the two products was assumed to contain the same molar concentration of isotopic carbon that was originally present in the carboxyl group. This assumption has been shown to be valid enough under certain conditions for most purposes[8]; however, except for a study of barium adipate in which no fractionation of ordinary carbon containing 1.06% $C^{13}$ was found,[9] no report appeared to be sufficiently exact to preclude the possibility that an over-all isotope effect might be taking place in the reaction where carbon-14 was involved.

A careful study of the pyrolysis of lithium acetate-1-$C^{14}$ was therefore undertaken to determine if an isotope effect did occur; no isotope effect was found. Lithium acetate was chosen because it is reported[10] that pyrolysis of it gave an excellent yield of acetone. The reactions which take place are indicated in the accompanying equations.

$$\left.\begin{matrix}CH_3C^{14}OOLi\\ CH_3C^{12}OOLi\end{matrix}\right\} \xrightarrow{490^\circ} \left\{\begin{matrix}CH_3{-}C^{14}O{-}CH_3 + Li_2C^{12}O_3 & (1)\\ CH_3{-}C^{12}O{-}CH_3 + Li_2C^{14}O_3 & (2)\end{matrix}\right.$$

$$2CH_3C^{14}OOLi \xrightarrow{490^\circ} CH_3{-}C^{14}O{-}CH_3 + Li_2C^{14}O_3 \quad (3)$$

$$2CH_3C^{12}OOLi \xrightarrow{490^\circ} CH_3{-}C^{12}O{-}CH_3 + Li_2C^{12}O_3 \quad (4)$$

If equations 1 and 2 proceed at different rates, then the molar concentrations of carbon-14 in the acetone and carbonate will differ from each other and from that of the lithium acetate. Reaction 3 is unimportant because of the relatively small amount of tracer material present.

The results of the work are summarized in Table I in which the activities (expressed in millivolts per millimole per second) are compared. Since the activities of the acetone and acetate are identical within limits of experimental error, it is evident that there is no over-all isotope effect. The low value for the activity of the lithium carbonate is attributed to dilution resulting from contamination with $C^{12}O_2$ arising from a certain amount of decomposition which always takes place during the reaction. This was expected in view of the reports[8a,8b] that a certain amount of activity was found in barium carbonate from pyrolysis of *methyl*-labeled barium acetate. (In this Laboratory, Mr. E. L. Albenesius has found that on pyrolysis of methyl-labeled lithium acetate, approximately 2% of the activity was detected in the lithium carbonate formed.)

TABLE I

ACTIVITIES OF REACTANT AND PRODUCTS IN THE PYROLYSIS OF LITHIUM ACETATE-1-$C^{14}$

| Compound | Activity, mv./sec./mmole | Av. deviation from mean |
|---|---|---|
| Lithium acetate | 49.1 | ±0.1 |
| Acetone | 49.0 | ± .1 |
| Lithium carbonate | 48.2 | ± .1 |

It has been pointed out[9] that whether or not an isotope effect will be observed in disproportionation reactions depends on whether or not the labeled atom is involved in the bond rupture (or formation) when the symmetry of the molecule is destroyed. The fact that no isotope effect was found in the present pyrolysis indicates that neither the cleavage nor the formation of a $C^{12}$–$C^{14}$ bond is involved in a step in which the symmetry of the molecule is altered.

Further work on the isotope effect is in progress in this Laboratory.

**Acknowledgment.**—This work was supported in part by the Atomic Energy Commission, Contract AT-(40-1-)270. Some of the apparatus used was purchased by a grant from the Carnegie Research Fund.

### Experimental

**Preparation of Lithium Acetate-1-$C^{14}$.**—Acetic acid-1-$C^{14}$ was prepared by carbonation of methylmagnesium iodide with $C^{14}O_2$ at −70° in a manner and in an apparatus somewhat like that previously described.[11] After steam distillation, the acetic acid was neutralized with carbonate-free lithium hydroxide solution, after which a drop of acetic acid in excess was added, and the solution evaporated to dryness. The lithium acetate was recrystallized from ethanol and dried at 110°. Activity measurements taken from time to time over a period of eight months showed no change. Approximately 6 g. of acid having an activity of 150 mv./sec./mmole was prepared; this was diluted 2–3 times with inactive material for the reactions.

**Pyrolysis of Lithium Acetate-1-$C^{14}$.**—Ten pyrolyses were carried out as follows: approximately 2.5 mmoles of lithium acetate was weighed into a platinum boat five inches long. The boat was inserted into a platinum sleeve of equal length and the assembly inserted into a Pyrex combustion tube maintained at 490° with an electric furnace. The acetone produced was swept out by means of a nitrogen stream and

(1) Yankwich and Calvin, *J. Chem. Phys.*, **17**, 109 (1949).
(2) A. Roe and M. Hellmann, *ibid.*, **19**, 660 (1951).
(3) W. Stevens and R. Attree, *Can. J. Research*, **B27**, 807 (1949).
(4) D. R. Stranks and G. M. Harris, *J. Chem. Phys.*, **19**, 257 (1951).
(5) G. A. Ropp, A. J. Weinberger and O. K. Neville, Cleveland Meeting of the Am. Chem. Soc., before the Division of Physical Chemistry, THIS JOURNAL, **73**, 5573 (1951).
(6) J. Bigeleisen, *J. Chem. Phys.*, **17**, 425 (1949).
(7) K. Pitzer, *ibid.*, **17**, 1341 (1949).
(8) (a) H. G. Wood, *et al.*, THIS JOURNAL, **63**, 2140 (1941); (b) M. Calvin, *et al.*, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 248; (c) S. Aronoff, V. A. Haas and B. Fries, *Science*, **110**, 476 (1948).
(9) A. A. Bothner-By, L. Friedman and J. Bigeleisen, Brookhaven Conference Report, BNL 44 (C-10), pp. 39–49.
(10) W. Kronig, *Z. angew. Chem.*, **37**, 667 (1924).
(11) Reference 8b, p. 175, and following.

was collected in one of three ways: (1) in a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid, (2) in Dry Ice traps, (3) in liquid air traps. The yield of acetone by any of these procedures was in the neighborhood of 80% as determined by precipitation of the 2,4-dinitrophenylhydrazone.

**Methods of Activity Measurement.**—All samples were converted to carbon dioxide by combustion; the carbon dioxide was collected in an ionization chamber and the activity measured using a Vibrating Reed Electrometer (Model 30, Applied Physics Corporation, Pasadena). Activities are expressed in terms of millivolts per second per millimole. One microcurie of activity corresponds to approximately 350 mv./sec./mmole. An ionization chamber containing a small piece of radioactive polystyrene was used as a standard to eliminate small daily fluctuations of the Reed.

The lithium acetate was assayed by wet combustion of weighed samples with Van Slyke–Folch[12] oxidizing mixture; the carbon dioxide produced was either led directly into the ionization chamber, or collected in carbonate-free base, precipitated as barium carbonate, and the barium carbonate acidified, the liberated carbon dioxide then being led to an ionization chamber as before. The two methods gave results agreeing closely. The activity in Table I is the average of twenty determinations.

The acetone was best assayed by collecting in Dry Ice or liquid air traps, and oxidizing with Van Slyke mixture; attempts to assay the 2,4-dinitrophenylhydrazone gave less satisfactory results. The activity in Table I is the average of nine determinations.

The lithium carbonate was decomposed by acid and converted to barium carbonate which was in turn acidified and the carbon dioxide measured as before. The activity value in Table I is the average of sixteen determinations.

(12) D. D. Van Slyke, J. Folch and J. Plazin, *J. Biol. Chem.*, **136**, 509 (1940).

DEPARTMENT OF CHEMISTRY AND
RADIOISOTOPE LABORATORY
UNIVERSITY OF NORTH CAROLINA
CHAPEL HILL, N. C.

## Friedel–Crafts Synthesis of Functionally Labeled Ketones

BY ROBERT J. SPEER AND J. K. JEANES

This Laboratory, under the auspices of Atomic Energy Contract AT-(40-1)-274, has undertaken to develop suitable methods for the synthesis of functionally labeled ketones from carboxylic acids and their derivatives. Shantz and Rittenberg[1] have reported the preparation of acetophenone-carbonyl-$C^{14}$ from sodium acetate through the intermediate acetic anhydride. Brown and Neville[2] have secured this same product directly from acetic acid. In addition, benzophenone-carbonyl-$C^{14}$ was obtained as an intermediate in a synthesis reported by Fleming and Rieveschl.[3] Despite these investigations, information is unavailable relative to the generality of the methods employed, and in many cases experimental details are lacking. This study had as its primary purpose an evaluation of the generality of the Friedel–Crafts method for production of functionally labeled ketones from carboxylic acids. It has proven feasible to extend this synthetic method to include alkyl–aryl, diaryl and alicyclic ketones. As specific examples, acetophenone-$C^{14}$, propiophenone-$C^{14}$, stearophenone-$C^{14}$, *p*-methylbenzophenone-$C^{14}$, benzophenone-$C^{14}$, *p*-methoxybenzophenone-$C^{14}$, *p*-chlorobenzophenone-$C^{14}$, acenaphthenone-$C^{14}$ and 1-indanone-$C^{14}$ have been prepared in yields ranging from 71 to 89% of theory. In many cases, existing procedures have been simplified and isotopic conversion efficiencies improved.[4]

In the course of this study, acetophenone and propiophenone have been prepared directly from the corresponding potassium salts of acetic and propionic acid. Eliminating as it does, the necessity for isolation of the free anhydrous acids or the preparation of the volatile acid chlorides and anhydrides, this innovation constitutes a very practical advantage in the handling of isotopic materials.

(1) E. M. Shantz and D. Rittenberg, THIS JOURNAL, **68**, 2109 (1946).

(2) W. G. Brown and O. K. Neville, Atomic Energy Commission, MDDC-1168.

(3) R. W. Fleming and G. Rieveschl, Jr., Abstract of paper presented before American Chemical Society, New York, September, 1947.

(4) For full experimental details order Document 3501 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.

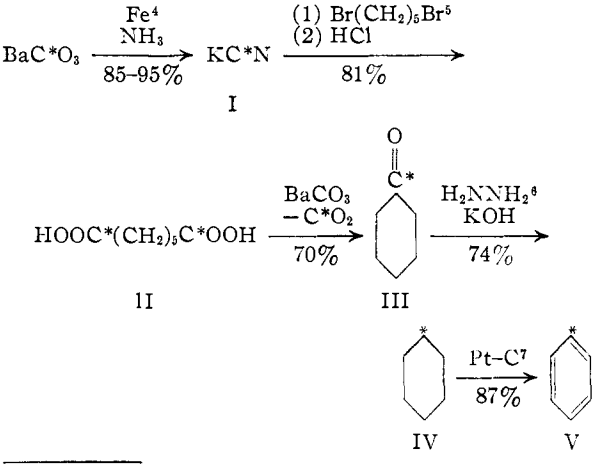
RADIOCHEMICAL DIVISION
TEXAS RESEARCH FOUNDATION
RENNER, TEXAS

RECEIVED NOVEMBER 30, 1951

## A Method for the Synthesis of High Specific Activity Benzene-$C^{14}$ [1,2]

BY ROBERT J. SPEER, MARY L. HUMPHRIES AND AMMARETTE ROBERTS

A semimicro method for the synthesis of high specific activity benzene-$C^{14}$ has been developed. Potassium cyanide-$^{14}$, pimelic acid-1,7-$C^{14}$, cyclohexanone-$C^{14}$ and cyclohexane-$C^{14}$ were employed as intermediates in the sequence of reactions[3] as follows:




(1) This work was done under Atomic Energy Commission Contract AT-(40-1)-274.

(2) Presented at Southwest Regional Meeting of the American Chemical Society, Austin, Texas, December, 1951.

(3) For full experimental details order Document 3500 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.

(4) J. A. McCarter, THIS JOURNAL, **73**, 483 (1951).

(5) J. Cason, L. Wallcave and C. N. Whiteside, *J. Org. Chem.*, **14**, 37 (1949).

(6) Huang-Minlon, THIS JOURNAL, **68**, 2487 (1946).

(7) R. P. Linstead and S. L. S. Thomas (with K. A. O. Michaelis), *J. Chem. Soc.*, 1127 (1940).

An over-all isotopic conversion efficiency of 22% from $BaC^{14}O_3$ was achieved.

RADIOCHEMICAL DIVISION
TEXAS RESEARCH FOUNDATION
RENNER, TEXAS RECEIVED JANUARY 23, 1952

## Synthesis for Carbon-14 Labeled *dl*-Glutamic Acid

BY ROBERT J. SPEER, AMMARETTE ROBERTS, MARGARET MALONEY AND HENRY R. MAHLER

Under the auspices of the Atomic Energy Commission, Contract AT-(40-1)-274, synthetic methods for *dl*-glutamic acid-5-$C^{14}$ and *dl*-glutamic acid-1,2-$C^{14}$ have been developed. *dl*-Glutamic acid-5-$C^{14}$ has been successfully prepared by modification of the method of Marvel and Stoddard[1] through the sequence of reactions[2,3]

$$BaC^*O_3 \xrightarrow[85\text{–}95\%]{NH_3,\ Fe} KC^*N \xrightarrow[80\%]{ClCH_2CH_2OH[5]} \underset{I}{HOCH_2CH_2C^*N} \xrightarrow[92\%]{HBr[5]} \underset{II}{BrCH_2CH_2C^*OOH} \xrightarrow[88\%]{C_2H_5OH[1]} \underset{III}{BrCH_2CH_2C^*OOC_2H_5}$$

$$\underset{IV}{BrCH_2CH_2C^*OOC_2H_5} + [C_6H_4(CO)_2]NCH(COOC_2H_5)_2 \xrightarrow[70\text{–}75\%]{(1)\ NaOH[1]\ (2)\ HCl} \underset{V}{HOOCCH(NH_2)CH_2CH_2C^*OOH}$$

By this procedure, an over-all isotopic conversion efficiency of 47% on the basis of potassium cyanide was achieved.

The synthesis of *dl*-glutamic acid-1,2-$C^{14}$, reported by Koegl, *et al.*,[2] during the course of this work, was achieved as follows

$$C^*O_2 \xrightarrow[80\%]{C_2H_5MgBr[6]} \underset{VI}{CH_3CH_2C^*OOH} \xrightarrow[80\%]{(1)\ KMnO_4[7,8]\ NaOH\ (2)\ Ca^{++}} \underset{VII}{(C^*OO)_2Ca} \xrightarrow[92\%]{(1)\ IR\text{-}100\text{-}H\ (2)\ Ag^+} \underset{VIII}{(C^*OOAg)_2} \xrightarrow[70\%]{CH_3I[2]} \underset{IX}{(C^*OOCH_3)_2}$$

$$\underset{IX}{(C^*OOCH_3)_2} \xrightarrow[60\text{–}70\%]{(1)\ (CH_2COOC_2H_5)_2[9]\ KOCH_3\ (2)\ HCl} \underset{X}{HOOCCH_2CH_2C^*OC^*OOH} \xrightarrow[64\%]{Pd\text{–}C[2]\ NH_3\text{–}H_2} \underset{XI}{HOOCCH_2CH_2C^*H(NH_2)C^*OOH}$$

Utilization of the techniques developed by these investigators, together with modifications from this Laboratory, served to attain an over-all isotopic yield of 20.4% from the starting propionic acid-1-$C^{14}$.

RADIOCHEMICAL DIVISION
TEXAS RESEARCH FOUNDATION
RENNER, TEXAS RECEIVED JANUARY 23, 1952

(1) C. S. Marvel and M. P. Stoddard, *J. Org. Chem.*, **3**, 198 (1938).

(2) F. Koegl, J. Halberstadt and T. J. Barendregt, *Rec. trav. chim.*, **68**, 387 (1949).

(3) For full experimental details order Document 3502 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.

(4) J. A. McCarter, THIS JOURNAL, **73**, 483 (1951).

(5) W. A. Jacobs and M. Heidelberger, *ibid.*, **39**, 1465 (1917).

(6) M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. F. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 171.

(7) P. Nahinsky and S. Ruben, THIS JOURNAL, **63**, 2275 (1941).

(8) P. Nahinsky, C. N. Rice, S. Ruben and M. D. Kamen, *ibid.*, **64**, 2299 (1942).

(9) *Org. Syntheses*, **26**, 42 (1946).

## Yeast Biosynthesis of Radioactive Sulfur Compounds

BY JOHN L. WOOD[1] AND JESSE D. PERKINSON, JR.

RECEIVED AUGUST 30, 1951

The possibility of synthesis of isotope labeled compounds by microörganisms is often dismissed by the organic chemist for lack of special equipment and because of the complexity of the mixtures of products obtained. These problems are minimized in sulfur labeling, due to the distribution of the isotope among relatively few compounds, and by the utilization of yeast culture in ordinary glassware. Moreover, the yeast itself is well established as a dietary supplement and source of protein. Radioactive yeast may be fed for introduction of labels into body sulfur compounds.

The production of yeast labeled with radioactive sulfur has been carried out by use of a synthetic medium[2] containing only the small amount of sulfur furnished by the impurities in C.P. chemicals.[3] Carrier-free $S^{35}$ sulfate, furnished by the Oak Ridge National Laboratory, was quantitatively incorporated by the yeast which was grown in 500-ml. erlenmeyers on a shaker. The labeled yeast was produced with a high specific radioactivity to permit dilution as desired before use.

Yeast prepared in separate runs of this procedure has been found to vary little in composition. It contained 6% nitrogen which was 50% non-protein. The protein fraction, however, contained 95% of the radioactivity labeled compounds. The biological availability of the sulfur was demonstrated by feeding the yeast as part of the diet of 3 rats. Radioactivity determinations done on blood, liver, kidney, muscle and urine showed an active metabolism of the sulfur compounds had occurred. The direct isolation of radioactive L-methionine and L-cystine from hydrolyzed yeast has been described.[4] Specific activities of the order of one microcurie per microgram of sulfur were obtained after a preliminary dilution of the product, with no indication that this was a limiting value. Analyses showed a moisture content of 5.3%, ash, 6%. Corrected percentage values

(1) Department of Chemistry, University of Tennessee, Memphis.

(2) A. S. Schultz and D. K. McManus, *Archiv. Biochem.*, **25**, 401 (1950).

(3) For complete experimental details order Document 3482 from American Documentation Institute, 1719 N St., N. W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35-mm. motion picture film) or $1.95 for photocopies (6 × 8 inches) readable without optical aid.

(4) J. L. Wood and G. C. Mills, THIS JOURNAL, **74**, 2445 (1952).

were phosphorus, 1.57; nitrogen, 6.0; non-protein N, 3.8. The sulfur content of non-radioactive samples was 0.25%.

MEDICAL DIVISION
OAK RIDGE INSTITUTE OF NUCLEAR STUDIES
OAK RIDGE, TENNESSEE

## Preparation of L-Methionine-S$^{35}$ and L-Cystine-S$^{35}$ from Radioactive Yeast[1]

BY JOHN L. WOOD AND GORDON C. MILLS

RECEIVED AUGUST 30, 1951

Radioactive L-cystine and L-methionine have been isolated from yeast labeled with radioactive sulfur.[2] The yeast was prepared by a method which ensured a high specific activity on the yeast sulfur.[3] This made a small scale operation possible and yielded L-methionine and L-cystine of high specific activity.

The yeast protein was separated from the carbohydrate by the procedure of Albanese, *et al.*,[4] and the protein was hydrolyzed with a hydrochloric acid–formic acid mixture. Dowex 50 was used to separate the sulfur amino acids.[5] Each amino acid was isolated from the proper ion-exchange fraction in a pure state after the addition of a small amount of the appropriate non-radioactive carrier.

A 4-g. sample of yeast ($1.5 \times 10^9$ counts/min.) yielded 161 mg. of L-methionine with a specific radioactivity of $1.6 \times 10^6$ counts per minute per mg. of methionine and 158 mg. of L-cystine with a specific activity of $3.7 \times 10^5$ counts per minute per mg. of cystine.

(1) This investigation was supported by research grants from the National Cancer Institute, of the National Institutes of Health, Public Health Service, and from the American Cancer Society.

(2) For complete experimental details order Document 3499 from American Documentation Institute, 1719 N St., N. W., Washington 6, D. C., remitting $1.00 for microfilm (images 1 inch high on standard 35-mm. motion picture film), or $1.05 for photo copies (6 × 8 inches) readable without optical aid.

(3) J. L. Wood and Jesse D. Perkinson, THIS JOURNAL, **74**, 2444 (1952).

(4) A. A. Albanese, D. L. Wagner, J. E. Frankston and V. Irby, *Anal. Chem.*, **16**, 609 (1944).

(5) W. H. Stein and S. Moore, *Symposia on Quant. Biol.*, **14**, 179 (1949).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF TENNESSEE
MEMPHIS, TENNESSEE

## The Synthesis of Thyroxine-1-C$^{14}$ [1]

BY S. C. WANG, J. P. HUMMEL AND T. WINNICK

RECEIVED JANUARY 7, 1952

Thyroxine labeled with radiocarbon on the carboxyl group has been synthesized by us on a semi-micro scale by a procedure based on the classical method of Harington and Barger.[2]

One hundred mg. of glycine-1-C$^{14}$ [3] representing 5.45 mc. was treated with benzoyl chloride to yield hippuric acid-1-C$^{14}$. The latter was condensed with 3,5-diiodo-4-(4′-methoxyphenoxy)-benzaldehyde. The resulting azlactone was converted to α-benzoylamino-3,5-diiodo-4-(4′-methoxyphenoxy)-cinnamic acid-1-C$^{14}$, and the latter in turn to 3,5-diiodothyronine-1-C$^{14}$. The iodination to thyroxine was conducted in ethylamine solution.[4] The yield of thyroxine-1-C$^{14}$ was 533 mg. or 53% based on the glycine-1-C$^{14}$. The product had a specific radioactivity of 530,000 counts per minute per mg., with a thin mica window counter.

The infrared spectra of thyroxine and diiodothyronine are given in Fig. 1. Our preparations were indistinguishable from commercial samples. Likewise thyroxine-1-C$^{14}$ had the same biological potency as commercial thyroxine, based on assays with thyroidectomized rats.[5] The position of the labeling was confirmed by the Van Slyke ninhydrin method. The thyroxine was decarboxylated at *p*H 2.5, and the evolved $C^{14}O_2$ accounted quantitatively for the radioactivity.

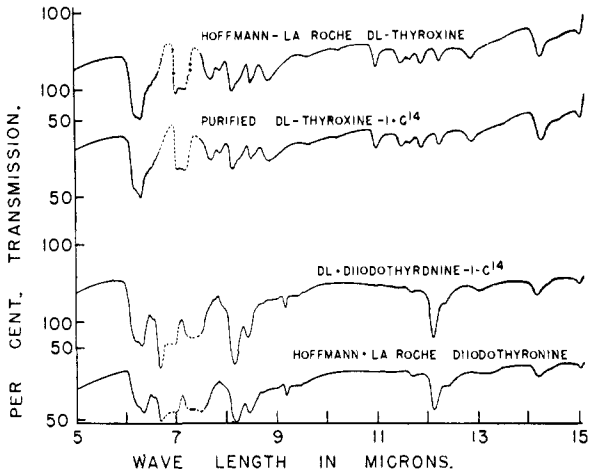



Fig. 1.—The Perkin-Elmer double beam spectrometer with NaCl optics was used; 20 mg. of sample per ml. of Nujol; dotted portions of records represent relatively opaque regions of the Nujol.

RADIATION RESEARCH LABORATORY AND
DEPARTMENT OF BIOCHEMISTRY, COLLEGE
OF MEDICINE, STATE UNIVERSITY
OF IOWA, IOWA CITY, IOWA

(1) For detailed descriptions order Document 3497 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 $1.65 for photocopies (6 × 8 inches) readable without optical aid.

(2) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

(3) Prepared by Tracerlab, Inc., Boston, Mass., from 10 mc. of $BaC^{14}O_3$ furnished by the U. S. Atomic Energy Commission.

(4) J. C. Clayton and B. A. Hems, *J. Chem. Soc.*, 840 (1950).

(5) Performed by Dr. S. B. Barker and Mr. H. B. Dirks of the Department of Physiology.