

An over-all isotopic conversion efficiency of 22% from BaC<sup>14</sup>O<sub>3</sub> was achieved.

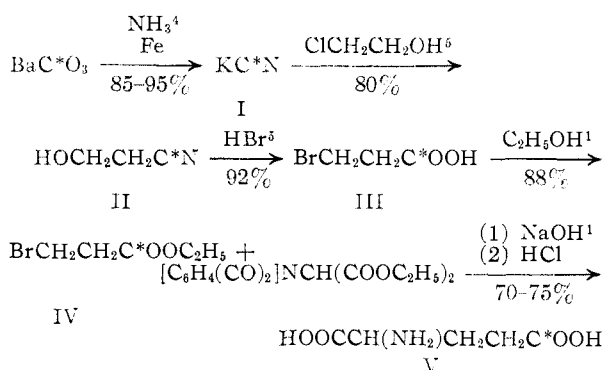
RADIOCHEMICAL DIVISION  
TEXAS RESEARCH FOUNDATION  
RENNER, TEXAS

RECEIVED JANUARY 23, 1952

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

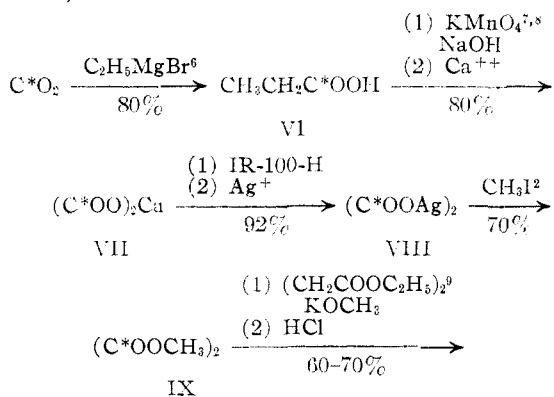
BY ROBERT J. SPEER, AMMARETTE ROBERTS, MARGARET MALONEY AND HENRY R. MAHLER

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



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<sup>14</sup>, reported by Koegl, *et al.*,<sup>2</sup> during the course of this work, was achieved as follows



(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).

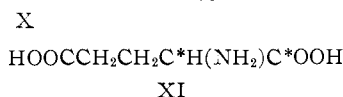
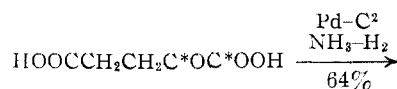
(5) W. A. Jacobs and M. Heidelberger, *ibid.*, **39**, 1465 (1917).

(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).



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<sup>14</sup>.

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### Yeast Biosynthesis of Radioactive Sulfur Compounds

BY JOHN L. WOOD<sup>1</sup> AND JESSE D. PERKINSON, JR.

RECEIVED AUGUST 30, 1951

The possibility of synthesis of isotope labeled compounds by microorganisms 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<sup>2</sup> containing only the small amount of sulfur furnished by the impurities in C.P. chemicals.<sup>3</sup> Carrier-free S<sup>35</sup> 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.<sup>4</sup> 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, 3.3. The sulfur content of non-radioactive samples was 0.25%.

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### Preparation of L-Methionine-S<sup>35</sup> and L-Cystine-S<sup>35</sup> from Radioactive Yeast<sup>1</sup>

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.<sup>2</sup> The yeast was prepared by a method which ensured a high specific activity on the yeast sulfur.<sup>3</sup> 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.*,<sup>4</sup> and the protein was hydrolyzed with a hydrochloric acid-formic acid mixture. Dowex 50 was used to separate the sulfur amino acids.<sup>5</sup> 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).

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### The Synthesis of Thyroxine-1-C<sup>14</sup><sup>1</sup>

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

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Thyroxine labeled with radiocarbon on the

(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.

carboxyl group has been synthesized by us on a semi-micro scale by a procedure based on the classical method of Harington and Barger.<sup>2</sup>

One hundred mg. of glycine-1-C<sup>14</sup><sup>3</sup> representing 5.45 mc. was treated with benzoyl chloride to yield hippuric acid-1-C<sup>14</sup>. The latter was condensed with 3,5-diiodo-4-(4'-methoxyphenoxy)-benzaldehyde. The resulting azlactone was converted to  $\alpha$ -benzoylamino-3,5-diiodo-4-(4'-methoxyphenoxy)-cinnamic acid-1-C<sup>14</sup>, and the latter in turn to 3,5-diiodothyronine-1-C<sup>14</sup>. The iodination to thyroxine was conducted in ethylamine solution.<sup>4</sup> The yield of thyroxine-1-C<sup>14</sup> was 533 mg. or 53% based on the glycine-1-C<sup>14</sup>. 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 indistinguish-

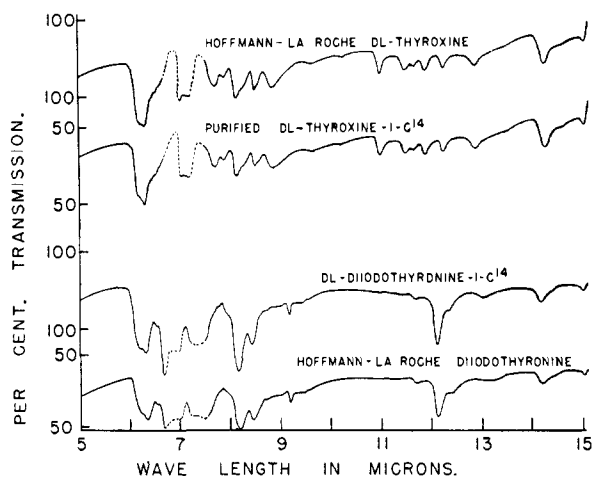


Fig. 1.—The Perkin-Elmer double beam spectrometer with NaCl optics was used; 20 mg. of sample per ml. of Nujol; dotted portions of records represent relatively opaque regions of the Nujol.

able from commercial samples. Likewise thyroxine-1-C<sup>14</sup> had the same biological potency as commercial thyroxine, based on assays with thyroidectomized rats.<sup>5</sup> The position of the labeling was confirmed by the Van Slyke ninhydrin method. The thyroxine was decarboxylated at pH 2.5, and the evolved C<sup>14</sup>O<sub>2</sub> accounted quantitatively for the radioactivity.

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

(3) Prepared by Tracerlab, Inc., Boston, Mass., from 10 mc. of BaC<sup>14</sup>O<sub>3</sub> 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.