A Convenient Transformation of Radioactive Carbon Dioxide into Radioactive Cyanide[1]

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Six methods for the preparation of C-14 sodium cyanide from C-14 carbon dioxide have been published.[2–7] Our experience in this field agrees with the conclusions reached by others regarding the questionable reliability and scale of operation of these various methods. The present paper reports a procedure which compares favorably with that of Belleau and Heard[5] with respect to yield and simplicity of manipulation and which has the added advantage of being appreciably more rapid.

The chief advantage of this method over that of Belleau and Heard is the conversion of the acid to the nitrile in one step,[8] while a secondary advantage is found in the simplicity of the Grignard reaction compared with the preparation and carbonation of sodium triphenylmethide.

The carbonation of the Grignard reagent differs from the usual procedure only in the relatively low temperature (−50°) employed to minimize secondary alkylations leading to α,α'-diphenylacetone and tribenzylcarbinol. The preparation of phenylacetonitrile from phenylacetic acid is a one-step reaction and it is comparatively rapid, especially when the 50-hr. refluxing of triphenylacetamide in toluene over phosphorus pentoxide to give triphenylacetonitrile[5] is considered. The apparatus is not complicated, and the procedure is that described by Reid[8] whereby the phenylacetic acid is added through a heated dropping funnel to a tube filled with silica gel heated to 490° through which a stream of anhydrous ammonia is passing. Conversion of half a mole of acid to the nitrile requires but 5 hr. For conversion to sodium cyanide it is not necessary to purify the nitrile obtained in this manner.

The reduction of phenylacetonitrile has been reported by Walter and McElvain,[9] and the present procedure is an adaptation of their method to give somewhat better yields. The over-all yield obtainable for the conversion of barium carbonate to sodium cyanide by the method described is 67–85%. The runs herein reported are typical, but do not represent the best yields obtained; and while the process is described for 0.5–1.0 *M* scale, our experience warrants the statement that the entire operation can either be scaled up or down to any desired size run, excepting actual micro operations.

Since we were interested in converting the carbon dioxide ultimately to cuprous cyanide the operation is reported below.

Experimental

Phenylacetic Acid-1-C^{14}.—Benzylmagnesium chloride was prepared by a standard method,[10] taking the usual precautions, from 63.3 g. (0.50 mole) of benzyl chloride in 150 ml. of anhydrous ether and 12.1 g. (0.448 mole) of magnesium turnings in 350 ml. of anhydrous ether.

The carbon dioxide generated by the reaction of 59.2 g. (0.30 mole) of radioactive barium carbonate and 100 g. (72%, 0.716 mole) of perchloric acid diluted to 120 ml. was swept by a continuous stream of dry, deoxygenated nitrogen into the above Grignard solution which had been previously cooled to approximately −55°[11] in a Dry Ice–chloroform-bath. The reaction mixture was stirred continuously during the carbonation of the Grignard reagent which required approximately 5–6 hours to complete. Stirring was interrupted after about 4 hours due to the caking of the reaction mixture. The generator flask was heated to 100° for approximately 10 minutes after the evolution of carbon dioxide had ceased, and the train swept out with nitrogen for an additional 30 minutes.

A mixture of 70 ml. of concentrated hydrochloric acid (sp. gr. 1.18) and 70 ml. of water was added with stirring and cooling (ice-bath) over a period of 30 minutes. The ether layer was separated and the aqueous layer extracted three times with 80-ml. portions of ether. The combined ether extracts were added to the original ether layer and dried over anhydrous sodium sulfate.

The ether was distilled off and the oily residue placed in a 600-ml. beaker. One hundred ml. of water was added and approximately 19 g. of anhydrous sodium carbonate was introduced in small portions until the aqueous layer was definitely basic (*p*H 8). The top layer of toluene which separated was siphoned off, and the lower layer was made acidic to congo red paper with concentrated hydrochloric acid. The mixture was cooled in an ice-bath, filtered and dried in air, m.p. 76.5–78.0°, 38.4 g. of phenylacetic acid which titrated 95.6% pure with standard base: thus the yield is 89.8%.[12]

Phenylacetonitrile-1-C^{14}.—This procedure followed that described by Reid.[8] Seventy grams (0.515 mole) of phenylacetic acid-1-C^{14} was added through a heated dropping funnel over a period of 5 hours into a stream of anhydrous ammonia (100 ml./minute). The mixture passed over preconditioned silica gel[13] at a temperature of 490°. The crude nitrile (55.3 g., 92%) was separated from the aqueous layer and without further purification was used to make labeled sodium cyanide.

Labeled Potassium Cyanide.—This procedure is partially based on one described by McElvain.[9]

In a two-liter, 3-neck, round-bottom flask equipped with a mechanical stirrer, reflux condenser and a dropping funnel were placed 300 ml. of toluene and 65 g. (2.8 mole) of sodium metal. The toluene was heated to boiling, and the mixture stirred vigorously until the sodium was finely divided. To this was added, just rapidly enough to keep the mixture refluxing vigorously, 55.3 g. (0.472 mole) of crude phenylacetonitrile-1-C^{14} dissolved in 65 g. of absolute ethanol (addition time approximately 45 minutes). After this addition, another 65 g. of absolute ethanol was added over a period of 20 minutes at the same rate. Sodium ethoxide precipitated during the addition. The mixture was allowed to cool and 200 ml. of water was added cautiously. The mixture was stirred until all the excess sodium reacted and two clear layers separated. The upper toluene layer was extracted three times with 100-ml. portions of water.

The above aqueous layer containing sodium cyanide was added to a mixture of 200 ml. of concentrated sulfuric acid (sp. gr. 1.84) and 200 ml. of water. The hydrocyanic acid evolved was trapped in a solution of 35 g. of potassium hydroxide (min. assay 85%) and 250 ml. of water. The acidic mixture was distilled for two hours until approximately 100 ml. of distillate was collected. An aliquot portion (5

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(1) Abstracted from a portion of the Ph.D. Dissertation of Donald I. McCane, University of Michigan, 1953.

(2) R. D. Cramer with G. B. Kistiakowsky, *J. Biol. Chem.*, **137**, 547 (1941); *cf.* R. B. Loftfield, *Nucleonics*, **1**, 54 (1947).

(3) A. W. Adamson, This Journal, **69**, 2564 (1947).

(4) R. Abrams, *ibid.*, **71**, 3835 (1949).

(5) B. Belleau and R. D. H. Heard, *ibid.*, **72**, 4268 (1950).

(6) J. A. McCarter, *ibid.*, **73**, 483 (1951).

(7) A. G. MacDiarmid and N. F. Hall, *ibid.*, **75**, 4850 (1953).

(8) E. E. Reid, *ibid.*, **53**, 321 (1931).

(9) L. A. Walter and S. M. McElvain, *ibid.*, **56**, 1614 (1934).

(10) H. Gilman and R. McCracken, *ibid.*, **45**, 2462 (1923).

(11) The Grignard reagent precipitates at this temperature to form a slurry. Higher temperatures, *e.g.*, 0°, give rise to lower yields of acid and higher yields of undesirable side products.

(12) More acid may be recovered from the aqueous filtrate by extracting it with ether and repeating the precipitation procedure keeping the volumes of reagents as small as possible. Two identical runs, with the working up of filtrates, gave an average yield of 93.4% of phenylacetic acid-1-C^{14}.

(13) Commercial silica gel (6–16 mesh, Grade A) was conditioned by passing a slow stream of air over it for 4–5 hours at a temperature of 220°. The temperature was then gradually raised to the operating temperature (490°).

ml.) of the solution was titrated for cyanide ion with standard silver nitrate solution (0.1 *N*) according to the method of Volhard (81.2% yield based on crude nitrile; however, yields well in excess of 90% are possible when pure nitrile is employed).

The above solution of potassium cyanide may be evaporated to dryness to obtain solid potassium cyanide; however, this step was eliminated in the preparation of labeled cuprous cyanide.

Labeled Cuprous Cyanide.[14]—One-hundred and ten grams (0.44 mole) of cupric sulfate pentahydrate was dissolved in 500 ml. of water and acidified to congo red paper with sulfuric acid. The mixture was heated to 40–50° with stirring while a solution of 29 g. (0.28 mole) of sodium bisulfite in a 100 ml. of water was added dropwise over a period of 10 minutes. The mixture of labeled potassium cyanide and potassium hydroxide obtained from the previous preparation was added immediately thereafter through a dropping funnel over a period of 15 minutes. The mixture was heated and stirred for an additional 15 minutes, filtered hot and washed with two liters of hot (80°) water and then with ethanol. The cuprous cyanide was dried at 60°; 32.0 g. (93% yield based on previous cyanide titration).

Activity of Samples.—The barium carbonate used was prepared by dilution of 10.3% C^{14}-enriched (one millicurie) barium carbonate by a standard procedure.[15]

The specific activity of the samples used 215 ± 1 c./min./mg.[16]

The cuprous cyanide was "plated" and counted under identical conditions in the same manner as the barium carbonate to give 461 ± 7 c./min./mg.[16]

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[14] The length of time required for the preparation of cuprous cyanide may be shortened considerably by using the sodium cyanide solution obtained directly from the cleavage of the nitrile, thus eliminating the previous step of distilling out hydrocyanic acid to get potassium cyanide. The yields of cuprous cyanide are the same.

[15] *Cf.* M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert and P. E. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N. Y., 1949, pp. 96–98.

[16] The authors are indebted to Mr. Charles Ver Nooy of this Laboratory for an independent check of these specific activities.

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Improved Synthesis of Biocytin

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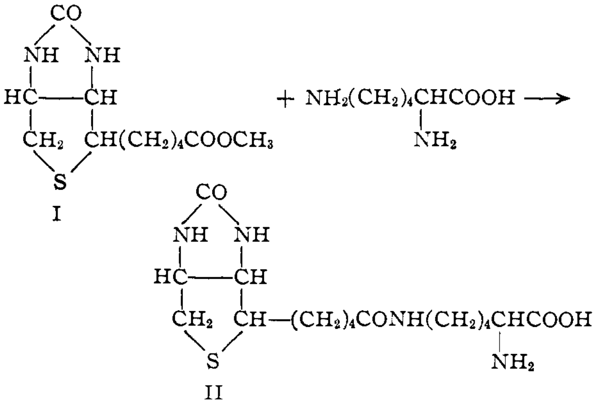
Biocytin, the biotin conjugate obtained from controlled autolysates of yeast,[1] was found to be ε-N-biotinyl-L-lysine (II).[2] Its synthesis was accomplished by two different methods[3] consisting of (a) reaction between the copper complex of L-lysine and biotin acid chloride, and (b) reaction between α-N-formyl-L-lysine and biotin acid chloride followed by selective hydrolysis. These two methods of synthesis of biocytin had as their primary value the confirmation of the structure of biocytin as determined by degradation. It was evident that these methods, as well as the technique of the isolation of biocytin from the reaction mixtures, were not satisfactory for preparative work on a much larger scale. Disadvantage of these two methods for larger scale preparations is probably due to the sensitivity of biotin acid chloride toward water. Consequently, a better method of synthesis was sought. Such a method was found in the reaction between biotin methyl ester (I) and L-lysine. The reaction was carried out in a mixture of toluene and 1,2,4-trichlorobenzene since the reactants showed appreciable solubility in this solvent mixture at elevated temperatures. The yield of biocytin was 68% based on the biotin methyl ester consumed.



Experimental Part

ε-N-Biotinyl-L-lysine.—In a one-liter flask was placed 13.3 g. (0.051 mole) of methyl ester of biotin[4] and 18 g. (0.12 mole) of lysine base followed by 400 cc. of toluene and 200 cc. of 1,2,4-trichlorobenzene, the mixture was heated to boiling, a few cc. of the solvent was distilled to remove traces of moisture, then the mixture was refluxed under a blanket of nitrogen for 24 hours with rapid stirring. The toluene was removed by distilling *in vacuo*, 500 cc. of anhydrous ether was added to the residual trichlorobenzene mixture, the solid was filtered and washed thoroughly with anhydrous ether. The solid (32 g.) was treated with 100 cc. of water, the unchanged biotin methyl ester was removed by filtration, washed with water and dried (3.3 g.). The combined aqueous filtrate and washings were adjusted to *p*H 6.5 with dilute hydrochloric acid and concentrated *in vacuo* to dryness. The residue was dissolved in 50 cc. of water (previously adjusted to *p*H 3 with hydrochloric acid) and subjected to a 10-plate countercurrent distribution between 50-cc. layers of water (*p*H 3) and *o*-cresol–chloroform mixture 1:1, the latter being the moving phase.[3] Plates 3 to 9 inclusive were poured into 7500 cc. of petroleum ether and agitated. The layers were separated and the organic layer was washed with three 200-cc. portions of water. The combined aqueous extracts were washed with ether, then concentrated *in vacuo* to dryness below 40°. The crude biocytin (14 g.) was dissolved in 75 cc. of water, the solution was treated with 1 g. of Darco, filtered and washed with 25 cc. of water. To the clear, straw-colored solution was added 1000 cc. of acetone, the precipitated product was collected on a sintered glass funnel, washed with acetone and dried *in vacuo;* yield 9.75 g. of pure biocytin (68%), m.p. 241–243°, $[\alpha]^{25}\text{D}$ +53° (*c* 1.00/100 cc. 0.1 *N* NaOH).

Anal. Calcd. for $C_{16}H_{28}O_4N_4S$: C, 51.59; H, 7.58; N, 15.04. Found: C, 51.52; H, 7.57; N, 15.22.

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[1] L. D. Wright, E. L. Cresson, H. R. Skeggs, T. R. Wood, R. L. Peck, D. E. Wolf and K. Folkers, THIS JOURNAL, **72**, 1048 (1950); **74**, 1096 (1953); L. D. Wright, E. L. Cresson, H. R. Skeggs, R. L. Peck, D. E. Wolf, T. R. Wood, J. Valiant and K. Folkers, *Science*, **114**, 635 (1951).

[2] R. L. Peck, D. E. Wolf and K. Folkers, THIS JOURNAL, **74**, 1099 (1953).

[3] D. E. Wolf, J. Valiant, R. L. Peck and K. Folkers, *ibid.*, **74**, 2002 (1953).

[4] D. Melville, K. Hofmann and V. du Vigneaud, *Science*, **94**, 308 (1941).