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]

(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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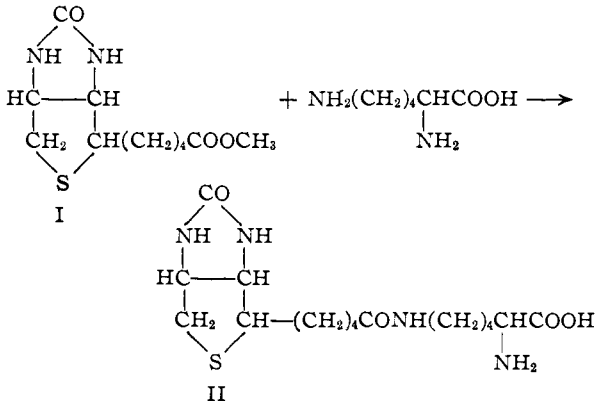
Biocytin, the biotin conjugate obtained from controlled autolysates of yeast,[1] was found to be ε-N-biotinyl-L-lysine (II).[2] Its synthesis was accomplished by two different methods[3] consisting of (a) reaction between the copper complex of L-lysine and biotin acid chloride, and (b) reaction between 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°, $[\alpha]^{25}D$ +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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