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THE SYNTHESIS OF SARCOSINE AND BETAINE WITH C^{14} IN THE METHYL GROUP[1]

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In synthesizing C^{14}-labeled sarcosine and betaine for use in metabolic studies it was necessary to devise special methods with particular attention being paid to the problem of recovery of the radioactive by-products. The preparation of methylamine hydrochloride was based on the procedure of Naegeli, Grüntuch, and Lendorff (1). The C^{14}-labeled methylamine was reacted with bromomalonic acid as described by Knoop and Oesterlin (2). Since a great excess of methylamine had to be employed in this reaction efficient recovery of the unreacted methylamine was worked out. We have been successful in changing the above methods so that they give a more satisfactory yield of products than the other methods given in the literature.

The following scheme of synthesis was adopted:

$$C^{14}H_3C^{14}OONa \longrightarrow C^{14}H_3C^{14}OCl \xrightarrow{NaN_3} C^{14}H_3NC^{14}O \xrightarrow[-C^{14}O_2]{HCl} C^{14}H_3NH_2 \cdot HCl \quad (a)$$

$$C^{14}H_3NH_2 + BrCH(COOH)_2 \rightarrow C^{14}H_3NHCH(COOH)_2 \xrightarrow[-CO_2]{} C^{14}H_3NHCH_2COOH \quad (b)$$

Step (a) led to a 72–76% yield based on the acetyl chloride used, whereas, step (b) gave 65–68% of sarcosine as calculated from the bromomalonic acid employed. Direct methylation of sarcosine with methyl sulfate led to betaine chloride in 80% yield. This method was a change from the procedure of Novak (3) inasmuch as we used sarcosine as the starting material. On the basis of the activities found, it was safe to assume that the exchange between the labeled methyl group of the sarcosine and the methyl group of the methyl sulphate may have amounted to 18%.

EXPERIMENTAL

Preparation of $C^{14}H_3NH_2 \cdot HCl$. To 3.5 ml. (3.87 g., 49.3 mM) of acetyl chloride, prepared from doubly labeled sodium acetate,[3] kept cold in a Dry Ice ethyl-alcohol bath was added 12 ml. of anhydrous benzene. After 3.85 g. of sodium azide (59.2 mM) was added, the flask was fitted with a double reflux condenser (the lower half cooled with running water and the upper half with Dry Ice and alcohol) and allowed to come to room temperature overnight with the upper condenser in operation and the system closed. The apparatus was equipped with a carbon dioxide trap containing 400 ml. of a solution of 16 g. of barium hydroxide (octahydrate) and protected from carbon dioxide. With the reaction flask in an ice-bath, 12 ml. of concentrated hydrochloric acid was added quickly from the dropping-funnel to the benzene solution by applying a very slight vacuum to the soda-lime as the

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stopcock was opened, and then the stopcock was immediately closed. The flask was slowly brought to 75°, kept at this temperature for 6 hours, and left to stand overnight at room temperature with the trap closed off. The recovered $C^{14}O_2$ yielded 6.63 g. (33.7mM) of barium carbonate with an activity of 450 μc/g.

The reaction products were transferred with water to a 250-ml. round-bottom flask and evaporated to dryness under a vacuum on a steam-bath. The residue was extracted three times with 25-ml. portions of ethyl alcohol and the alcohol was removed under a vacuum on a water-bath leaving the dry salt residue behind. This residue was extracted four times with 50-ml. portions of isopropyl alcohol. The extracts were pooled and concentrated to a small volume under a vacuum on a water-bath. Then 250 ml. of anhydrous ethyl ether was added and the precipitate was collected by centrifuging and placed in a desiccator. Yield of $C^{14}H_3NH_2 \cdot HCl$, 2.58 g. (76%) (38.2 mM); m.p. 226–227°.

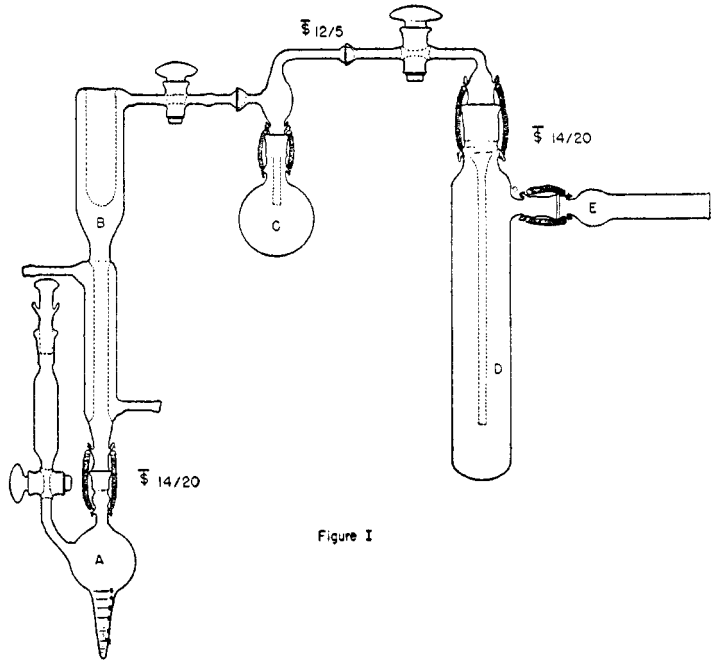

Figure I

FIGURE 1. A, 50 ml. reaction vessel; B, double cold-finger condenser; C, 100 ml. trap; D, 500 ml. carbon dioxide trap; E, absorption tube filled with Drierite.

Preparation of $C^{14}H_3NHCH_2COOH$. The methylamine hydrochloride was combined with 5.62 g. of sodium methylate ($NaOCH_3 \cdot 2CH_3OH$) (47.6mM) in a 50-ml. round-bottom flask equipped with a dropping-funnel. Meanwhile 1.3 g. of bromomalonic acid (7.1 mM) were dissolved in 5 ml. of methyl alcohol in a 60-ml. glass bomb equipped with a pressure stopcock. The flask and bomb were connected to the high vacuum isotope line and frozen with liquid nitrogen and then evacuated. Next 10 ml. of methyl alcohol was added to the flask from the dropping-funnel while the flask was cold from the liquid nitrogen. (The connection between the funnel and the flask must not be so cold as to freeze the alcohol). The C^{14}-methylamine and methyl alcohol were distilled into the bomb still immersed in liquid nitrogen. The flask was warmed to room temperature so that there was no excessive bumping and the last traces of C^{14}-methylamine and methyl alcohol were driven off by warming the flask on a water-bath to about 35°. At the conclusion of the distillation the bomb was closed off and allowed to stand in an ice-box.

After three days, crystallization of the N-methylaminomalonic acid on the walls of the bomb was complete. The excess C^{14}-methylamine and methyl alcohol was distilled on the high vacuum line into a flask containing an excess of frozen 1 *N* hydrochloric acid. Since

the methyl alcohol had a tendency to bump, a Kjeldahl trap was used to connect the bomb to the vacuum line. After thawing the distillate, it was evaporated to dryness on a steam-bath, yielding 1.10 g. of $C^{14}H_3NH_2 \cdot HCl$ with an activity of 1.0 μc./mg.

The residue in the bomb and the trap was washed twice with 5-ml. portions of methyl alcohol. The crystals in the bomb were then dissolved in water and the solution washed from the bomb and trap into a centrifuge tube, using about 20 ml. of water in all. Lead acetate (trihydrate) (2.68 g.) was dissolved in 5 ml. of water and added to the above solution and the centrifuge tube was placed in the ice-box. After two-days standing and occasional scratching the walls of the tube were coated with crystals of the lead salt of N-methylaminomalonic acid. These were centrifuged down and the supernatant liquid was returned to the ice-box to allow further crystallization. The crystals were suspended in 20 ml. of water, saturated with hydrogen sulfide to remove lead, and the residue was centrifuged and washed; the supernatant liquid and wash waters were pooled and removed under a vacuum at 35–40°. The white solid remaining, which was N-methylaminomalonic acid, was slowly heated in an oil-bath to 160° in order to complete the decarboxylation. The flask was then protected from moisture and was left to cool. The resultant white powder was pure sarcosine. The yield was 350.6 mg. (3.93 mM) with an activity of 0.78 μc/mg. (69.4 μc/mM). A second crop was recovered from the mother liquor increasing the total yield to 430.6 mg. (76%).

Preparation of $[(C^{14}H_3)_3N^+CH_2COOH] \cdot Cl^-$. Methyl-labeled sarcosine (270 mg.) prepared by the method described above was dissolved in a minimum of water and neutralized with potassium hydroxide solution. Then 560 mg. of potassium hydroxide in 5 ml. of water and 1.0 ml. of methyl sulfate were added alternately in small portions (with due caution to keep the solution alkaline throughout the methylation). The solution was boiled 15 minutes, cooled, carefully neutralized with about 2.2 ml. of 5% sulfuric acid, concentrated to a syrup under a vacuum at 60°, and extracted three times with 15-ml. portions of 95% ethyl alcohol. At this point 45 ml. of water was added and the mixture, as above, was again concentrated under a vacuum to a light-yellow colored syrup which was then dissolved in a minimum of hot water. A small portion of 1 *N* hydrochloric acid was added and the solution was boiled for half an hour. A 20% solution of barium chloride was then added until no more precipitate formed. The mixture was centrifuged and, after the supernatant clear solution was filtered off, the sediment was washed with distilled water and centrifuged again. The supernatant solutions were pooled and concentrated by heating on a steam-bath for three hours with the addition of water from time to time to keep the solution from going to dryness. The concentrate was tested for sulfate with a drop of barium chloride solution. It was evaporated to dryness under a vacuum and extracted twice with 95% ethyl alcohol. The alcohol was removed under a vacuum and the residue recrystallized from hot absolute alcohol. On cooling, white crystals of betaine hydrochloride separated. A second crop was obtained by precipitating betaine hydrochloride with absolute ethyl ether from its alcoholic mother liquor. Total yield, 375 mg. (80% based on the sarcosine used). Activity, 0.37 μc/mg. (56.8 μc/mM).

Anal. Calc'd for $C_5H_{12}ClNO_2$: N, 9.12; Cl, 23.13.
Found: N, 9.03; Cl, 22.92.

SUMMARY

The synthesis of C^{14}-methyl-labeled sarcosine and betaine hydrochloride is described. The methods employed, adapted to radioactive work, led to more satisfactory yields than other methods given in the literature.

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