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Improvement of the method of synthesis of δ-Aminolevulinic acid-4-^{14}C Hydrochloride

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SUMMARY

δ-aminolevulinic acid-4-^{14}C was built up from phthalimide using K^{14}CN as the radioactive starting material. This method avoids the less practical synthesis via *glycine-^{14}C and its condensation with phthalic anhydride to afford phthalylglycine-1-^{14}C whose yield, based on K^{14}CN, is thus considerably improved.*

INTRODUCTION.

The advent of isotopic tracer techniques opened the modern era of research into porphyrin biosynthesis, and the origin of all the atoms of the tetrapyrroll haem system has been elucidated by using isotopically labelled precursors and subsequent degradation of the haem [1, 2]. ^{14}C proved to be particularly useful in the elucidation of the mechanism of porphyrin formation [3], and the role of δ-aminolevulinic acid in this biosynthesis was demonstrated employing the afore mentioned isotope [4, 5].

Shemin and coworkers have shown the usefulness of δ-aminolevulinic acid-4-^{14}C and 5-^{14}C in their studies on the formation and metabolism of porphyrins, and have proposed a method for the synthesis of the radioactive precursors [6, 7]. Pichat and Herbert [8] synthesized δ-aminolevulinic acid-4-^{14}C starting from glycine-l-^{14}C. The biological importance of this intermediate,

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the sensitivity and ease of determination of its metabolites, lead to the consideration of the possibility of improving its synthesis as regard to the yield.

For the preparation of the δ-aminolevulinic acid-4-^{14}C hydrochloride, K^{14}CN was chosen as the radioactive starting material. This salt has been used since 1945 in the synthesis of carboxyl- labelled compounds [9]. Phthalimide was first condensed with formaldehyde to yield the N-oxymethyl derivative. This product was then converted into the N-chloro-methyl compound, which was then subjected to a substitution reaction with K^{14}CN. The phthalyl-acetonitrile-1-^{14}C thus obtained was hydrolysed to the glycine derivative, which was then transformed into δ-aminolevulinic acid-4-^{14}C hydrochloride by the usual method.

The yield of phthalylglycine-l-^{14}C based on K^{14}CN, is appreciably better when the radioactive cyanide is reacted with N-chloromethyl phthalimide, (in the prior synthesis [8] glycine-l-^{14}C used as starting material, had to be previously synthesised and then condensed with phthalic anhydride); that improvement justifies the extra steps required by the new synthesis.

EXPERIMENTAL.

N-oxymethylphthalimide. 10 g phthalimide was heated 2 hr at 100° C with 25 ml 10% aqueous formaldehyde in a closed tube with occasional shaking until the phthalimide went into solution (about 30 min). After cooling and opening the tube the solvent was evaporated and the product recrystallized from ethanol-toluene (1 : 1) m.p. 146-148° C (lit. 141-142° C from toluene) [10].

N-chloromethylphthalimide. N-oxymethylphthalimide was reacted with excess thionyl chloride, for 2 hr at room temperature. The mixture was heated for 30 min at 90-100° C, then the excess reagent was evaporated. The residue was treated three times with anhydrous benzene, evaporating the solvent under reduced pressure each time. The product recrystallized from toluene melted at 133-134° C (lit. 132-134° C) from benzene [11].

Phthalylacetonitrile-1-^{14}C. 2,234 g N-chloromethylphthalimide dissolved in 5 ml hot dioxane was poured into a solution of 0.6517 g KCN containing 11.3 mg K^{14}CN (5 mCi) in 20 ml anhydrous methanol. The mixture was stirred 5 hr, then the precipitated KCl was centrifuged out and washed three times with small portions of hot dioxane which was then added to the solution. On evaporation of the solvent 1,769 g phthalylacetonitrile-1-^{14}C was obtained (95%). The product was recrystallized from water, m.p. 121-122° C (lit. 124-125° C, from ethanol) [12].

Phthalylglycine-1-^{14}C. 1,769 g crude phthalylacetonitrile-1-^{14}C was suspended in 30 ml acetic acid-HCl (3 : 1) and refluxed for 1 hr. The acid solution was evaporated under reduced pressure, the residue taken up with 5 ml water and evaporated to dryness, this treatment was repeated 2 or 3 times. The crystalline product was suspended in 5 ml of ice water afterwards, it was filtered

immediately and washed with another 5 ml ice water, and finally dried. 1,753 g phthalylglycine-1-^{14}C was obtained (90%, 85.5% based on K^{14}CN), m.p. 189-192° C [13].

Glycine-l-^{14}C was demonstrated to be present in the water washing by paper chromatography and Thin Layer Chromatography. This aqueous solution, which we colled (1) was worked as described below, for the purification of the final product.

Phthalylglycine-l-^{14}C chloride. 1,753 g phthalylglycine-1-^{14}C and 8 ml thionyl chloride were kept for 2 hr at 40-50° C and then heated to 80-90° C until all the solid had gone into solution (about 20 min). The excess reagent was evaporated under reduced pressure and eliminated completely by the addition of 3 portions of anhydrous benzene and subsequent evaporation. The slightly yellow crystalline product weighed 1,922 g (100%), m.p. 84-84.5° C (lit. 84-85° C) [14].

l-Diazo-3-phthalimidopropanone-2-^{14}C [8]. A solution of 1,922 g phthalylglycine 1-^{14}C chloride in 20 ml anhydrous ether was added drop by drop into a solution of 22 millimoles diazimethane in 60 ml ether with stirring, at —5° C throughout the addition. Immediately after the addition of some phthalylglycine-1-^{14}C chloride, a "flocculent" white precipitate, began to separate from the mixture, which was kept overnight in the refrigerator. Upon evaporation of the solvent, 1,878 g 1-diazo-3-phthalimidopropanone-2-^{14}C was obtained (95%, 82% based on K^{14}CN), m.p. 168° C decomp.

l-Bromo-3-phthalimidopropanone-2-^{14}C. 2.5 ml 48% HBr was slowly added, with stirring to a suspension of 1,878 g 1-diazo-3-phthalimidopropanone-2-^{14}C in 30 ml glacial acetic acid. The solid was observed to dissolve with evolution of nitrogen gas. Stirring was continued for 3 hr, after which the solvents were evaporated under reduced pressure, the remaining HBr was eliminated by addition and evaporation of several small portions of water. The solid residue was taken with benzene and filtered, and the solution was evaporated to dryness affording 2,171 g 1-bromo-3-phthalimidopropanone-2-^{14}C (77% based on K^{14}CN), which after recrystallization from ethanol melted at 147-148° C, form benzene [15].

δ-Aminolevulinic acid-4-^{14}C hydrochloride. A suspension in dimethylformamide (recently distilled) of 5,712 g di-t-butyl malonate sodium derivative, prepared by treating the ester with sodium metal at room temperature in anhydrous ether, was added to a solution of 2,711 g 1-bromo-3-phthalimidopropanone-2-^{14}C in dimethylformamide and the mixture was stirred for 24 hr at room temperature, after which the reddish solution was evaporated to dryness under reduced pressure. The crystalline residue was taken up with 20 ml dioxane and saturated with gaseous HCl at 0° C, and kept at room temperature overnight. The dioxane was then evaporated and the residue suspended in HCl-acetic acid (1 : 1) and refluxed for 24 hr. The solution was evaporated to dryness, taken up with distilled water and evaporated (once more). This operation was repeated three times, and finally the residue was suspended in

water, filtered and washed until the solution was colourless. The yellow filtrate contained δ-aminolevulinic acid-4-^{14}C and other ninhydrin-positive impurities, as well as a radioactive substance whose presence could not be detected with ninhydrin [2]. The crude acid hydrochloride solution was poured onto a Dowex 50 W × 12 (H^+) column (100 × 1 cm) and eluted (6 ml/hr) at room temperature with 0.05 N HCl, collecting 100 fractions of 3 ml each [8]. The elution was completed with 600 ml 1 N HCl. No destruction of the product was observed. The fractions containing the pure product were frozen-dried, yielding 0,826 g δ-aminolevulinic acid-4-^{14}C (50 % based on $K^{14}CN$). The fraction containing δ aminolevulinic acid and glicine -l-^{14}C, were frozen-dried together with solution (1), and the mixture was separated into its components by chromatography on cellulose column (4 × 40 cm), using as solvent the upper phase (at 24° C) of butanol-acetic acid-water (31.5 : 5 : 13.5) at a ratio of 6 ml/hr. 200 fractions of 3 ml each were collected, and those containing pure J-aminolevulinic acid-4-^{14}C hydrochloride were frozen-dried to afford 0,102 g of the product (6 % based on $K^{14}CN$), with added to the previous quantity gives a overall yield of 56 %.

Radioactivity measurements were done with a "liquid scintillator", a value of 0.47 mCi/mM was obtained.

By using the enzyme, δ-aminolevulinic dehidratase, the biological activity of the product was demonstrate.

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