NOTE

A CONVENIENT SYNTHESIS OF [1-^{14}C]ACETYLCHOLINE IODIDE

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SUMMARY

A convenient synthesis of [1-^{14}C]acetylcholine iodide in two steps is described. β-Dimethylaminoethanol is acetylated with [1-^{14}C]acetic anhydride and the ester β-dimethylaminoethyl [1-^{14}C]acetate quaternised with methyl iodide to afford [1-^{14}C]acetylcholine iodide in an overall material yield of 50-52% and radiochemical yield 18%.

Key words : Synthesis, [1-^{14}C]acetylcholine iodide, β-dimethylaminoethyl [1-^{14}C]acetate, methyliodide, quaternisation

INTRODUCTION

Acetylation of choline chloride with acetyl chloride[1] had been observed to give impure acetylcholine chloride and purification attempts by crystallisation were unsuccessful[2]. However, choline chloride has been acetylated by heating with a mixture of ^{14}C-sodium acetate and acetic anhydride[3] or with ^{14}C-acetic acid and acetyl chloride[4] in the presence of a little acetic acid to yield [1-^{14}C]acetylcholine chloride.

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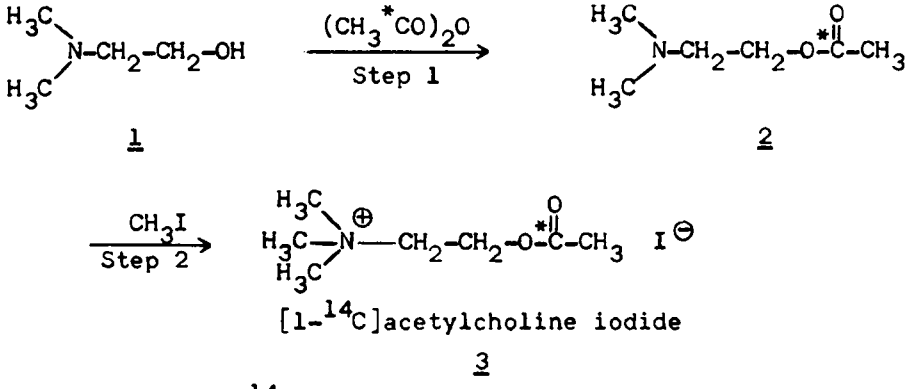
Acetylcholine iodide is less hygroscopic than its chloride salt and pure acetylcholine chloride was thus prepared by reacting the iodide with silver chloride[5].

β-Dimethylaminoethanol reacted smoothly with several acid anhydrides to give the acyl derivatives, in 92-93% yield, which on subsequent quaternisation with methyl iodide afforded the corresponding acylcholine iodides in almost quantitative yield[5,6].

Among the several methods reported for the assay of cholinesterase activity in blood, plasma and tissue homogenates, use of [1-^{14}C]acetylcholine iodide (3) as a substrate in the radiometric assay procedure permits a rapid, specific and accurate determination. This simple method involves a direct quantitation of the [1-^{14}C]acetic acid liberated from enzymic hydrolysis of (3)[7,8,9]. Experimental details for the synthesis of (3), on a 0.5 mmol scale, by the facile method[5] outlined in the scheme below, hitherto unreported in the literature, are described in this paper.

Synthetic scheme:



1 2

[1-^{14}C]acetylcholine iodide

3

* = position of ^{14}C label

β-Dimethylaminoethanol (1) was acetylated with [1-^{14}C]-acetic anhydride in benzene solution under reflux to get β-dimethylaminoethyl[1-^{14}C]acetate (2) which on quaternisation with methyl iodide in ether under gentle reflux afforded [1-^{14}C]acetylcholine iodide (3) (material yield 50-52%; radiochemical yield 18%).

EXPERIMENTAL

Melting and boiling points are uncorrected.

[1-^{14}C]Acetic anhydride (500 μCi; specific activity 10 mCi/mmol) was procured from Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India.

Reagent grade acetic anhydride was distilled over freshly fused sodium acetate, the fraction boiling at 138-140°C was collected and preserved in sealed ampoules (1 ml).

β-Dimethylaminoethanol was purchased from Fluka, A.G., Buchs, Switzerland and distilled (bp 135°C/760 mm) prior to use.

Methyl iodide was distilled and kept over silver powder in an amber coloured bottle.

Reagent grade benzene, was removed of thiophene by shaking twice with conc. H_2SO_4 (50 ml of acid per litre of benzene), distilled and stored over sodium wire.

Ether was dried over anhydrous calcium chloride for two days, filtered and kept dry over sodium wire.

Radioactivity measurements were made with a Nuclear-Chicago, Mark I, Liquid Scintillation counter operating at a carbon-14 counting efficiency of 75% using external standardisation.

The Scintillator (cocktail) contained 4 g PPO and .05 g POPOP per litre of toluene.

Radiometric paper chromatography (RPC) was done on Whatmann No. 1 filter paper in the solvent system n-BuOH (80) : EtOH (20) : AcOH (10) : H_2O (30)[10]. The development distance was 15 cm by the ascending technique. Measurement of radioactivity of (2 x 1 cm) strips of the radiochromatograms was carried out as suspensions in a mixture of methanol (5 ml) and cocktail (10 ml).

Reversed isotope dilution analysis (RIDA) was done by mixing the solutions of the ^{14}C-labelled preparation and the carrier (100 mg of analytically pure unlabelled acetylcholine iodide) in methanol (2 ml). The combined solutions were concentrated to 1 ml and cooled. Ether (2 ml) was added dropwise. The crystalline material was collected by centrifugation, washed with a (2 x 1.5 ml) of ice-cold methanol-ether (1:2 v/v) and dried in vacuo, 100°C for 1 hr. The above procedure was repeated thrice and the specific activities of material from two successive crystallisations were determined. RIDA samples (5 mg) were dissolved in methanol (5 ml) and mixed with cocktail (15 ml) before counting.

β-Dimethylaminoethyl[1-^{14}C]acetate (2):

A solution of [1-^{14}C]acetic anhydride (500 μCi; specific activity 10 mCi/mmol) and inactive acetic anhydride (50 mg)

in dry benzene (5 ml) was treated with a freshly distilled sample of β-dimethylaminoethanol (1) (45 mg) in benzene (0.5 ml) and the mixture refluxed over an oil bath (tempr. 90°C) for 12-15 hrs. The solvent benzene, was removed by distillation. After cooling, the residue was stirred with dry ether (20 ml) and anhydrous potassium carbonate (500 mg) and filtered to get (2) as an ethereal solution.

[1-^{14}C]Acetylcholine iodide (3):

The foregoing ethereal solution of(2), was treated with methyl iodide (0.1 ml) and the mixture was gently refluxed for 6 hrs. and then allowed to stand at 25°C, after closing the reaction flask with a stopper, for 2 days. [1-^{14}C]-Acetylcholine iodide (3) that had separated out as a solid was collected by centrifugation, washed thoroughly with dry ether and dried *in vacuo* at 100°C for 1 hr. Yield 65-70 mg; specific activity 1.33 μCi/mg (material yield 50-52%; radiochemical yield 18%).

The radiochemical purity of (3) when determined by RPC and RIDA as described earlier, was observed to be >99%.

M.P. 157-160°C and mmp (undepressed on admixture with an authentic sample of acetylcholine iodide)[5,6] established the identity of (3) with the unlabelled compound.

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