

**AN ELEGANT SYNTHESIS OF [1-¹⁴C] HEXADECYL-
TRIMETHYLAMMONIUM BROMIDE [CTAB]**

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SUMMARY

Hexadecyltrimethylammonium bromide, a cationic detergent having a wide range of applications, was synthesised from [1-¹⁴C] hexadecanoic acid via its methyl ester, alcohol and bromide, in an overall yield of 84%. It had a specific activity of 6.30 uCi/mmol and a radiochemical purity of 97.6%.

Key Words: [1-¹⁴C] Hexadecanoic acid, [1-¹⁴C] Methyl hexadecanoate, [1-¹⁴C] Hexadecanol, [1-¹⁴C] Hexadecyl bromide, [1-¹⁴C] Hexadecyltrimethylammonium bromide, [1-¹⁴C] Cetyltrimethylammonium bromide.

INTRODUCTION

Hexadecyltrimethylammonium bromide or Cetyltrimethylammonium bromide (CTAB) is a cationic detergent and it has numerous applications as a laboratory reagent, apart from being an antiseptic^{1a,b}. It finds use as a phase transfer catalyst in the reduction of arenes and heterocyclic compounds², as a precipitant for nucleic acids^{3a,b} and mucopolysaccharides⁴ and in the solubilization of biomembranes⁵. Recently, it has been employed for the permeabilization of yeast cells^{6,7} whereby the transport of small molecules such as

mono- and disaccharides across the cell membrane is greatly facilitated. For understanding the mechanism of permeabilization, radioactive CTAB was required in this laboratory.

Generally, CTAB is prepared by bubbling trimethylamine through an alcoholic solution of n-hexadecyl bromide⁸. The latter is derived from hexadecanol which itself is obtained commercially by reduction of hexadecyl chloride¹. This method however could not be directly adopted for a semi-micro scale preparation, especially with the radioactive starting material. Incidentally, an interesting application of [1-¹⁴C] CTAB viz., the determination of surface area of powders and flat surfaces, has been reported⁹, but no mention is made about its origin. Hence, we designed a new preparative procedure starting from [1-¹⁴C] hexadecanoic acid (I) (fig 1), highlights of which are as follows: (i) amenability for carrying out on a millimolar scale, (ii) simple reaction conditions and easy work-up, (iii) convenient handling of the radioactive starting material as well as the intermediates, (iv) near quantitative yields in each step and (v) minimal loss of radioactivity.

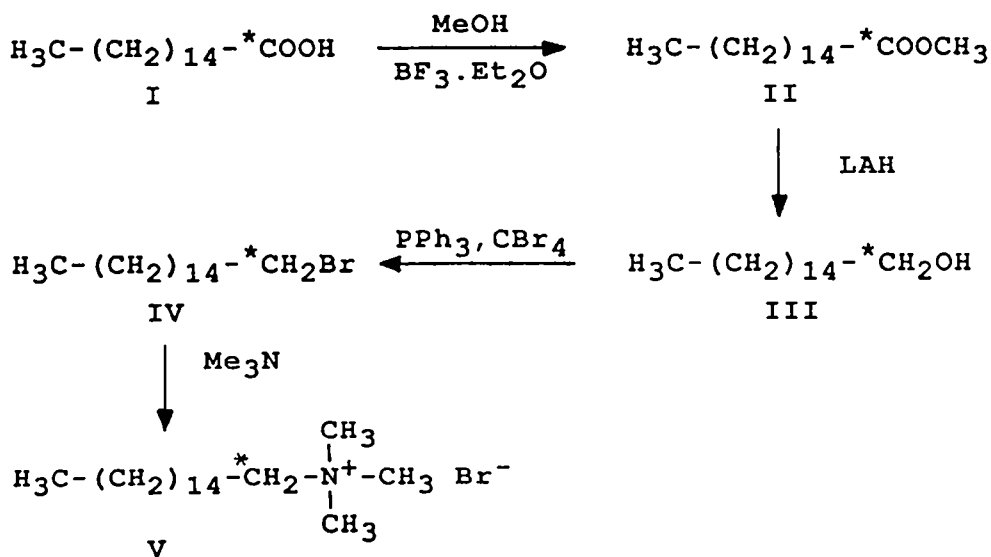


Fig 1. Synthetic scheme for [1-¹⁴C] hexadecyltrimethylammonium bromide [CTAB]

EXPERIMENTAL

ANALYTICAL PROCEDURES

Radioactivity Measurements: Liquid scintillation counting (Beckman LS-100) was used to determine the specific activities of all the materials. The activity was measured after transferring the sample into vials containing 10 ml Bray solution [methanol 10%; ethylene glycol 2%; naphthalene 6%; 2,5- diphenyloxazole (PPO) 0.4%; 1,4-bis (5-phenyloxazolyl)benzene (POPOP) 0.002% in p-dioxane].

Melting Point: Determined in glass capillary and uncorrected.

Determination of Radioactive purity by TLC: 2 mg of [1-¹⁴C] CTAB was chromatographed on thin layer of silica gel [Kieselgel, E. Merck G (type 60) 20x20 cm, 0.25 mm thick] using isopropanol:water:acetic acid (70:28:2 by volume) as developing solvent. An authentic sample of CTAB (Sisco-Chem, India) was also run on the same plate and exclusively exposed to iodine. The silica gel layer corresponding to standard CTAB was scraped off into a vial containing 10 ml of Bray solution and the radioactivity measured.

SYNTHESIS

1. Methyl hexadecanoate (II): Esterification of hexadecanoic acid(I)

[1-¹⁴C] Hexadecanoic acid (1.056 mg; 6.3 uCi, 4.12 umol) [Isotope division, Bhabha Atomic Research Centre, Bombay, India] in 70% ethanol (1 ml) was diluted with 256 mg (1 mmol) of ordinary hexadecanoic acid (palmitic acid, BDH, England) in 20 ml of benzene. Then the solvents were slowly distilled off when all the water got removed azeotropically. The residue was dissolved in 10 ml methanol (Karl Fischer grade), 0.6 ml of boron trifluoride etherate added and the mixture refluxed on a water bath for a period of 2h. It was further stirred with 250 mg of solid sodium hydrogen carbonate for 15 min and filtered through a G-2 sintered funnel. The solid was

washed with 5 ml portions (3 times) of methanol and the combined filtrates evaporated to dryness to get a waxy residue (II).

2. [1-¹⁴C] Hexadecanol (III): Lithium aluminium hydride (LAH) reduction of (II)

II (step 1) was taken in anhydrous diethyl ether (10 ml) and added dropwise to a slurry of 100 mg of LAH in diethyl ether (10 ml) under stirring. The mixture was set to reflux on a sand bath. After 3 h of refluxing, the heating bath was removed and 1 ml of 5% aqueous oxalic acid was added dropwise under stirring. This resulted in a gray mass coagulating from the ethereal medium. The coagulated mass was filtered through a G-2 sintered funnel and washed with diethyl ether (10 ml x2). Combined filtrates were dried over anhydrous sodium sulphate (2 g) and concentrated to obtain a solid (III).

3. [1-¹⁴C] Hexadecyl bromide (IV): Reaction of III with triphenylphosphine-carbon tetrabromide

To III (step 2) in diethyl ether (10 ml), an ethereal solution of carbon tetrabromide (0.664 g in 10 ml, 2 mmol) was added followed by triphenylphosphine (0.524 g, 2 mmol) in small portions under stirring and cooling in a cold water bath. The mixture was later stirred for 2h at room temperature and filtered through a glass wool plug. The plug was washed with diethyl ether (10 ml x2) and the combined filtrate and washings were concentrated to 10 ml. To the concentrate, silica gel (100-200 mesh, 1 g) was added and the remaining solvent removed carefully on a hot water bath. The adsorbed material was loaded onto a column of silica gel (10 g) built in hexane and the column eluted with the same solvent to give a pure fraction of IV (TLC, hexane) which on evaporation gave a residue (267 mg, 87.5%).

4. [1-¹⁴C] CTAB (V): Condensation of (IV) with trimethylamine

A gas generation assembly consisting of a 250 ml round bottomed flask, fitted with a 100 ml pressure equalising funnel and a side arm connected to a gas bubbler with a teflon tube, was set up.

80 ml of 30% w/v aqueous trimethylamine solution was added dropwise to a warm solution (45°C) of 10% aqueous potassium hydroxide taken in the round bottomed flask. The evolved gas was let to bubble through an acetone solution of IV (10 ml) (Step 3) when a white solid started to separate. When no more precipitation occurred, the mixture was filtered at the pump and the solid collected on filter paper disc. The mother liquor (filtrate) was concentrated to get an additional amount of solid. The combined solids were washed successively with dry acetone and diethyl ether (5 ml each). The product (V) recrystallized from hexane:chloroform (5:1 v/v, 3 ml) in colourless plates [(306 mg), overall yield 84%; m.p. 234-237°C(d), lit.¹ >230°C(d); radiochemical purity 97.6%. (by co-TLC with an authentic sample, R_f value 0.7)¹⁰].

The radiochemical yield of [1-¹⁴C] hexadecyltrimethylammonium bromide or [1-¹⁴C] CTAB (V) was 5.05 uCi (80 %) at a specific activity of 0.017 uCi/mg., 6.3 uCi/mmol.

ACKNOWLEDGEMENTS

We are grateful to Dr. D. Rajagopal Rao, Chairman, Food Sciences Division, who initiated us into this work and offered advice throughout to its accomplishment. Our thanks are also due to Dr. S.G. Bhat for the helpful discussions.

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10. [$1-^{14}\text{C}$] CTAB was comparable with the authentic sample in its efficacy to permeabilize yeast cells (*K. fragilis*).
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