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NOTE

Synthesis of Hexadecylphospho[methyl-^{14}C]-choline

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SUMMARY

Hexadecylphospho[methyl-^{14}C]-choline was synthesized in two steps from the tetra-n-butyl-ammonium salt of phosphoryl[methyl-^{14}C]-choline and 1-bromohexadecane, and purified by column chromatography on silica gel. The total yield of the radioactive synthesis was 35%, whereas the total yield of the non-radioactive synthesis was 40%. Hexadecylphospho[methyl-^{14}C]-choline may serve as a useful tool for pharmacokinetic and biochemical studies of this new antitumor drug.

Key words: Hexadecylphospho[methyl-^{14}C]-choline, phospholipid analogues, etherlipids

INTRODUCTION

Hexadecylphosphocholine (1) belongs to a new group of etherlipids, the alkylphosphocholines, which exhibit remarkable antitumor activity *in vivo* and *in vitro* [1,2]. The antitumor potential of 1 has been demonstrated in preclinical studies on human leukemia cells and in preliminary clinical studies on the treatment of skin metastases of breast cancer [3,4]. Furthermore, 1 has been recently shown to inhibit different membrane-bound enzymes, e.g.

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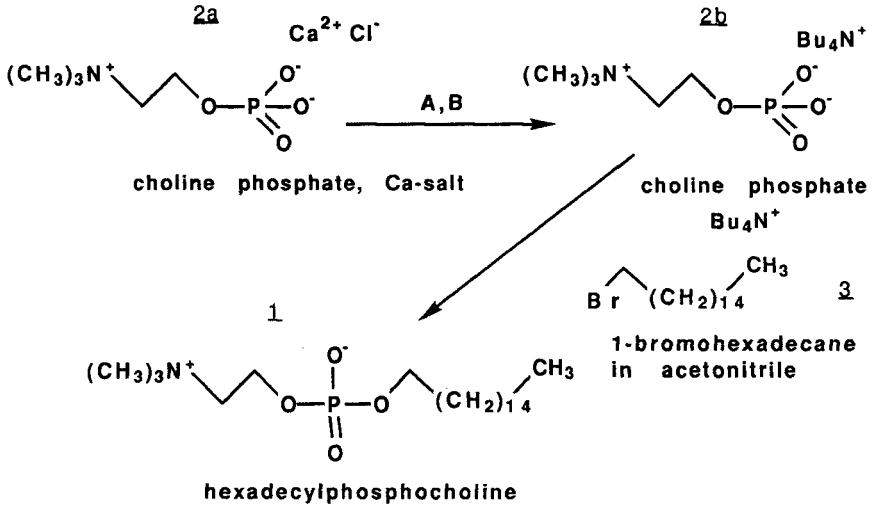
protein kinase C [5-7], K^+/Na^+-ATPase [8] and CTP:phosphocholine cytidylyltransferase [9,10]. Radiolabelled 1 would therefore be useful for pharmacokinetic and metabolic studies, and for investigating the inhibition of the membrane-bound enzymes listed above.

A five-step synthesis of 1 has been described [11,12]. It is possible to use this procedure for the synthesis of hexadecylphospho[methyl-^{3}H]-choline, in which the label is introduced by the methylation of hexadecylphosphoethanolamine with [^{3}H]-methyl iodide [13]. In the present study, we describe an improved synthesis of 1 which is also practicable for the synthesis of hexadecylphospho[methyl-^{14}C]-choline, using commercially available phosphoryl[methyl-^{14}C]-choline.



A) oxalic acid B) aqueous tetrabutylammonium hydroxide (Bu_4NOH)

EXPERIMENTAL

Phosphoryl[methyl-^{14}C]-choline (55 mCi/mmol) was from Amersham (Braunschweig; Germany). Phosphocholine (calcium salt), 1-bromohexadecane, oxalic acid dihydrate and tetrabutyl-ammonium hydroxide (40% in water) were from Fluka Chemie (Buchs; Switzerland). Acetonitrile and toluene were purchased from Rathburn Chemicals Ltd. (Walkerburn Peeblesshire; Scotland). All other chemicals and solvents were from Merck AG (Darmstadt; Germany). For quantification of radioactivity a Berthold LB 2821 HR thin layer chromatography scanner (Berthold; Wildbad; Germany) was used.

Synthesis and characterization of hexadecylphosphocholine.

The calcium salt of phosphorylcholine (2a, 50.95 g in 200 ml water) was treated with an aqueous solution of oxalic acid (19.48 g in 230 ml water). The precipitated calcium oxalate was removed by filtering through diatomaceous earth, and the filtrate was titrated with tetra-n-butylammonium hydroxide to pH 9. The resulting solution was evaporated to dryness, and the residue was dissolved in 100 ml toluene. After evaporating to dryness, the residue was dissolved in a further 100 ml toluene, evaporated again and dried over P_2O_5 to yield 65 g 2b as yellow crystals. 2b (52.5 g in 155 ml anhydrous acetonitrile) were reacted with 47.2 ml of 3 at room temperature for 17 h following by refluxing for 3 h. The reaction was monitored by thin layer chromatography (TLC) on silica gel plates using methanol as solvent. Rf values were 0.1 for 1, 0.7 for 2b and 0.8 for 3. The products were evaporated, and the residue was dissolved in methanol. Pure 1 was obtained by column chromatography on silica gel, followed by trituration of the resulting oil with acetone to yield 40% 1, based on the calcium salt of phosphorylcholine.

Synthesis and characterization of hexadecylphospho[methyl-^{14}C]-choline.

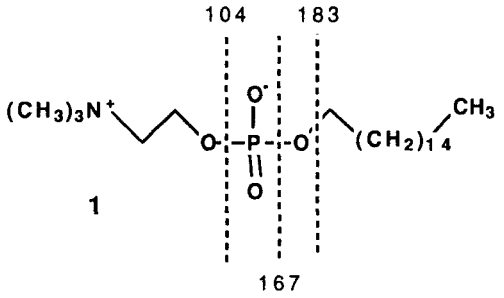
The ammonium salt of phosphoryl[methyl-^{14}C]-choline (0.55 µmol; 30 µCi) was dissolved in 5 ml water and titrated with tetra-n-butylammonium hydroxide to pH 9. The mixture was evaporated to dryness as described above and the yellow residue was dissolved in 5 ml acetonitrile. After addition of 0.55 µmol of 3, the solution was stirred for 17 h followed by refluxing for 3h. The mixture was evaporated to dryness, and the residue dissolved in methanol and purified by column chromatography on silica gel using methanol as eluent. The elution was monitored by counting the radioactivity. The yield of radioactive 1 was 35%, based on the amount of radioactivity incorporated into hexadecylphospho[methyl-^{14}C]-choline.

RESULTS AND DISCUSSION

It has been shown that hexadecylphospho[methyl-^{14}C]-choline (1) and the unlabelled compound are synthesized by a simple two-step procedure from commercially available precursors. The yields after column chromatography were 40% for unlabelled 1 and 35% for [^{14}C]-labelled 1.

The non-radiolabelled product was characterized by fast atomic bombardement mass spectroscopy (FAB-MS), 1H-nuclear magnetic resonance spectroscopy (^{1}H-NMR), elementary analysis and TLC.

FAB-MS: $[M+H]^+$: 408; the following fragments were detectable:



1

^{1}H-NMR (d6-dimethylsulphoxide):

δ = 0.8 (t, 3H, 1-CH_3); 1.3 (m, 26H, [2-14]-CH_2); 1.65 (m, 2H, 15-CH_2); 3.2 (s, 9H, N-Me_3); 3.6 (t, 2H, 18-CH_2); 3.85 (q, 2H,16-CH_2), 4.35 (m, 2H, 17-CH_2).

Elementary analysis (theoretical):

carbon 58.41% (61.9%); hydrogen 11.48% (11.3%); nitrogen 3.40% (3.44%)

TLC: Rf 0.10 (methanol), Rf 0.36 (chloroform/methanol/water, 2:4:1),
Rf 0.01 (chloroform/methanol, 1:1)

The purity and identity of radiolabelled 1 was checked by TLC and radioscanning using two different solvent systems (Fig.1). Radiolabelled 1 comigrated with non-radiolabelled 1.

Furthermore, the biological activity of 1 was determined as described by measuring the inhibition of phosphtidylcholine biosynthesis [9,10].

The previously described synthesis of unlabelled 1 [11,12] was improved by using alkylation to synthesize the phosphodiester bond, a method already described for the synthesis of an affinity ligand for phosphorylcholine-binding proteins [14].

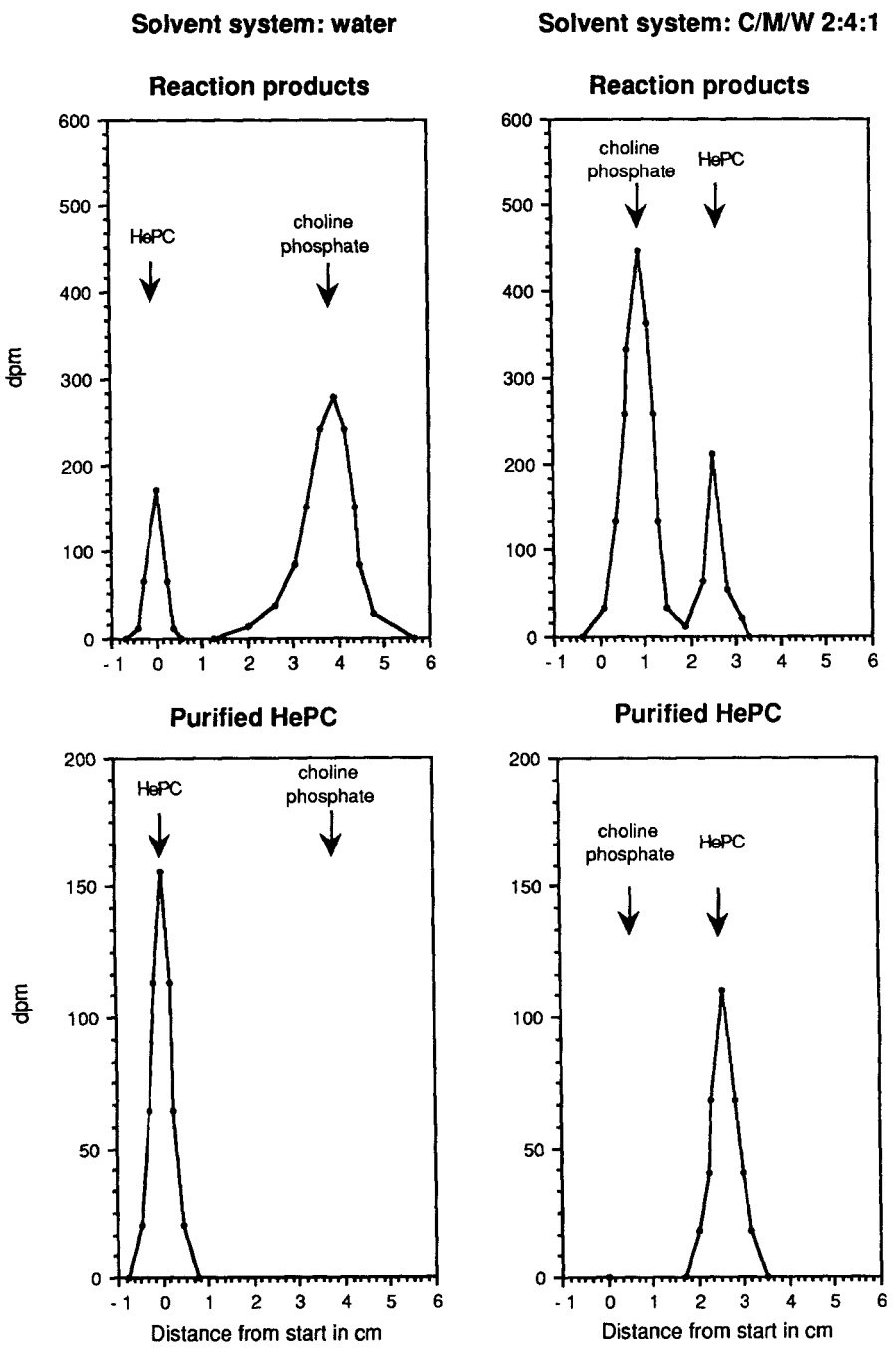


Figure1: **TLC-Separation of radiolabelled compounds.** 5 µl of the reaction mixture and 5 µl of the purified radiolabelled hexadecylphosphocholine (HePC) were applied to silica gel TLC plates and separated using water or chloroform/methanol/water (2:4:1, by volume) as solvent systems. HePC and choline phosphate were located by comparing the Rf values of unlabelled standards.

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