Tritium Labelled 1-O-Octadecyl-2-O-methyl-rac-glycero-3-phosphocholine (ALP) and 1-S-Hexadecyl-2-O-ethyl-rac-thioglycero-3-phosphocholine (Thio-ALP)

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SUMMARY

The phospholipids, ALP and Thio-ALP, are non-hydrolyzable analogues of platelet activating factor (PAF). Interest in ALP and thio-ALP centers upon their activity as potential antineoplastic agents. A variety of mechanisms of action have been attributed to these compounds including inhibition of a phospholipid cofactor of a phospholipid sensitive Ca^{+2}-dependent protein kinase. Thio-ALP is at least as active as a growth inhibitor of the HL-60 promyelocytic leukemic cell line as is ALP-the most active reference analogue reported in the literature and is approximately twice as active against the BG-1 and BG-3 human ovarian carcinoma cell lines. To aid in further biochemical studies, we report the synthesis of high specific activity tritium labelled ALP and thio-ALP. These products were obtained by palladium catalyzed reduction of 1-O-octadecenyl and 1-S-hexadecenyl precursors with carrier-free tritium gas.

Key Words: ALP, thio-ALP, catalytic reduction, tritium

INTRODUCTION

Alkyl lysophospholipid, 1-O-alkyl-2-O-methyl-rac-glycero-3-phosphocholine,[1] is a non-hydrolyzable analogue of platelet activating factor (PAF).[2,3] Attention has primarily been focused upon the aggregation, degranulation[4,5] and hypotensive[6] properties of PAF while it is the potential antineoplastic properties of ALP[7] and thio-ALP,[8] a sulfur containing analogue, which are of greatest interest. The most active reference ALP analogue reported in the literature is 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine[9] while the

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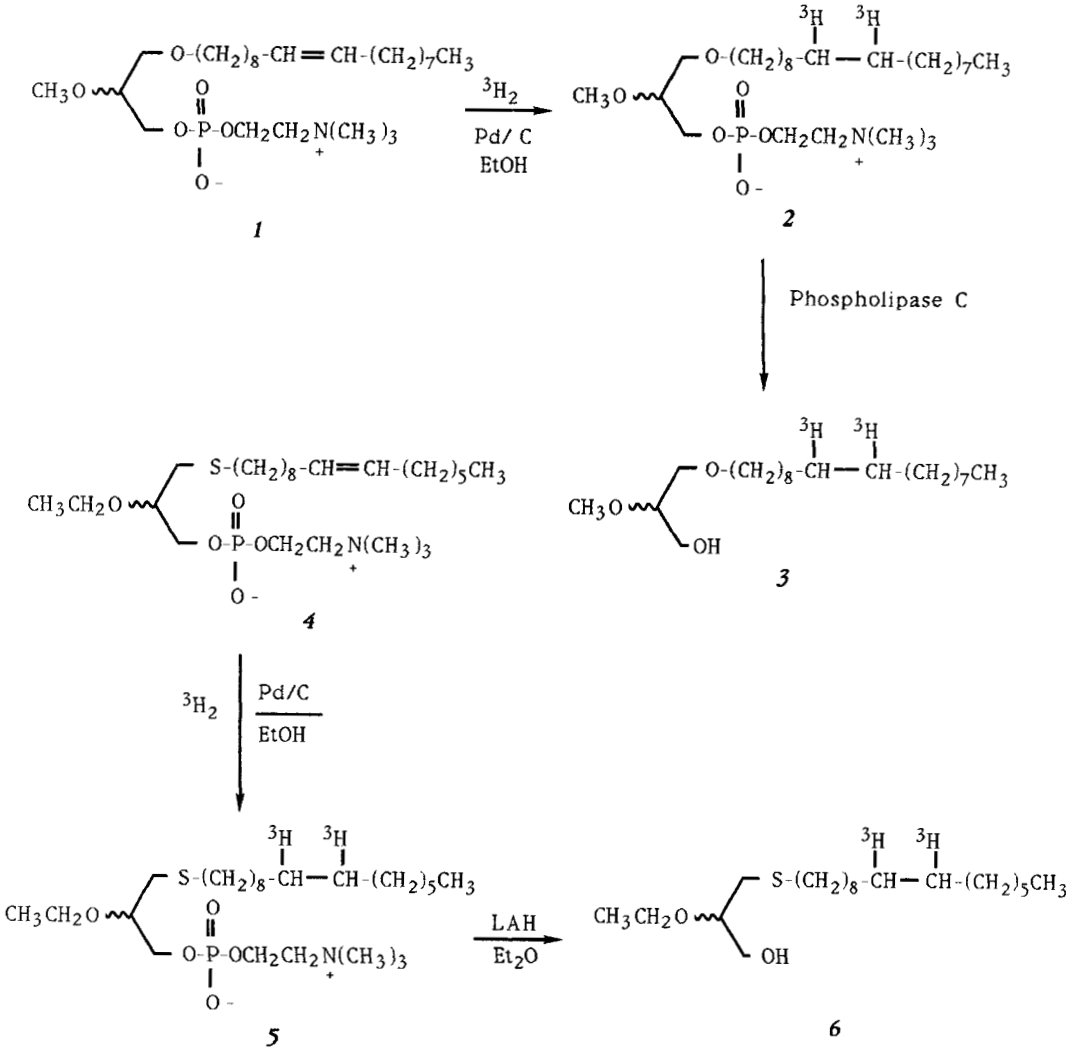
most active thio-ALP analogue reported is 1-S-hexadecyl-2-O-ethyl-rac-thioglycero-3-phosphocholine.[8] Antitumor activity of ALP analogues has been attributed to generation of tumoricidal macrophages,[10,11] reduced alkyl cleavage enzyme activity in tumors,[12] membrane interactions,[13] malignant cell differentiation,[11,14] direct cytotoxicity[7,11] and the inhibition of a phopholipid cofactor of a phospholipid sensitive Ca^{+2}-dependent protein kinase.[15] ALP and its analogues offer an alternative approach to cancer chemotherapy in that these agents appear not to affect DNA synthesis or function directly and are nonmutagenic.[7,14] Since thio-ALP is more lipid soluble than ALP, it may insert more easily into lipid membranes and disrupt membrane function.

Both ALP and thio-ALP have been shown active against growth of the HL-60 promyelocytic leukemic cell line and the BG-1 and BG-3 human ovarian carcinoma cell lines.[16] The compounds showed equal potency against the former cell line while thio-ALP was twice as potent against the latter. These ether lipids have been shown to inhibit the effects of phorbol diester tumor promotors.[17] In order to extend the biochemical studies of these interesting agents, we prepared ALP and thio-ALP at high specific activity labelled with tritium in the 1-O-octadecyl and 1-S-hexadecyl side chains, respectively.

DISCUSSION

We previously reported the synthesis of high specific activity tritium labelled PAF (56 Ci/mmol).[18] A similar route to obtain the labelled ALP and thio-ALP appeared attractive. Thus, both lipids were first produced by palladium catalyzed reduction of the corresponding Δ^9-1-O-octadecenyl **1** and hexadecenyl **4** precursors, respectively, with hydrogen gas at 1.0 atm and room temperature as a model for tritiation. Complete reduction was evidenced by disappearance of the olefinic proton resonances at δ5.35 ppm in both products. Upon treatment with 5.0 Ci of carrier-free tritium gas under similar conditions, 1422 mCi of ALP **(2)** at 56 Ci/mmole and 112 mCi of thio-ALP **(5)** were obtained. Since neither product contains a suitable UV chromaphore for specific activity determination, both were converted to the dephosphorylated derivatives **3** and **6** respectively, so that mass quantitation could be carried out by gas chromatography using an internal

SCHEME I.



standard. For ^{3}H-ALP, dephosphorylation was accomplished enzymatically using phospholipase C while thio-ALP was dephosphorylated using LAH by the procedure of Wood and Snyder.[19] The latter procedure proved superior for these derivatives since the enzymatic treatment proceded sluggishly. However, while the dephosphorylated ALP was successfully quantitated by gas chromatography, the labelled thio-ALP decomposed completely under gas chromatography conditions at 235°C.

EXPERIMENTAL PROCEDURES

All chemicals were used as obtained from the manufacturer. Melting points were obtained on a MEL-TEMP melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a JEOL FX-60 60 MHz FT spectrometer using $CDCl_3$ (TMS) as solvent. Radiopurity was determined using a Bioscan BID 100 Image Analyzer with silica gel 60 thin layer plates. Tritium was counted using a Packard Tricarb Minaxi Liquid Scintillation Counter Model 4000 (external standard) with Scintiverse^{R} (Fisher) counting solution. Gas chromatographic analysis was performed using a Shimadzu GC-8A gas chromatograph. The unsaturated precursors to the labelled ALP and thio-ALP were prepared by a similar procedure.[20]

Reduction of Δ^{9}-1-O-Octadecenyl-2-O-methyl-rac-glycero-3-phosphocholine and Δ^{9}-1-S-Hexadecenyl-2-O-ethyl-rac-thioglycero-3-phosphocholine with Hydrogen Gas as a Model for Tritiation. In both cases, approximately 0.1 mmol of unsaturated precursor (**1** and **4**) in 3.0 mL of absolute ethanol was stirred for 4.0 h at room temperature with 30 mg of 10% Pd/C under 1.0 atm of hydrogen. The catalyst was filtered off through a Celite/Na_2SO_4 pipet column and the filtrate evaporated in vacuo to afford essentially pure saturated ALP and thio-ALP as evidenced by complete disappearance of the olefinic protons at δ 5.35 ppm. ^{1}H-NMR data for these compounds has been reported.[8]

[9,10-$^{3}H_2$(N)]-1-O-Octadecyl-2-O-methyl-rac-glycero-3-phosphocholine (^{3}H-ALP) (2). A solution of 13.8 mg (0.026 mmol) of 1 in 1.0 mL of absolute ethanol was stirred for 4.0 h at room temperature with 10 mg of 10% Pd/C under 5.0 Ci (0.086 mmol) of carrier-free tritium gas. The catalyst was filtered off through a Celite/Na_2SO_4 pipet column and the column washed with 4.0 mL of methanol. The combined filtrates were evaporated in vacuo and the residue immediately dissolved in 50 mL of absolute ethanol and again evaporated to remove exchangable tritium. The residue was then dissolved in 500 mL of absolute ethanol as a stock solution and was counted. A yield of 1,422 mCi (94% radiochemical yield) of > 98% pure product was obtained with a specific activity of 56 Ci/mmol (105 mCi/ mg). See specific activity determination below under preparation of 3.

[9,10-$^3H_2$(N)]-1-O-Octadecyl-2-O-methyl-rac-glycerol (3). In order to determine the specific activity of 2 by gas chromatography, **3** was prepared. A 500 mCi aliquot of 2 was evaporated *in vacuo* and the residue dissolved in 3.0 mL of ether and this solution was stirred for 4.0 h with 1.0 mL of freshly prepared Tris buffer (pH 7.4) containing 150 units of phospholipase C. The ether layer was removed and the aqueous layer was extracted with 3.0 mL of CH_2Cl_2-MeOH (2:1). The combined organic layers were evaporated *in vacuo* and the residue purified on a pipet column of silica gel (CH_2Cl_2-MeOH 95:5) to afford 8.2 mCi of 3 which was quantitated by gas chromatography using 1-O-hexadecyl-2-O-benzyl-rac-glycerol as an internal standard. A specific activity of 56 Ci/mmol was obtained for both 2 and **3**.

[9,10-$^3H_2$(N)]-1-S-Hexadecyl-2-O-ethyl-rac-thioglycero-3-phosphocholine (^{3}H-thio-ALP) (5). A solution of 10.0 mg (0.019 mmol) of 4 in 1.0 mL of absolute ethanol was stirred for 4.0 h at room temperature with 30.0 mg of 10% Pd/C under 10.0 Ci (0.172 mmol) of carrier-free tritium gas. The catalyst was filtered off through a Celite/Na_2SO_4 pipet column and the column washed with 4.0 mL of methanol. The combined filtrates were evaporated *in vacuo* and the residue dissolved in 50 mL of absolute ethanol and evaporated *in vacuo* to remove exchangeable tritium. The residue was dissolved in 20 mL of absolute ethanol and counted to afford 1,370 mCi (124% radiochemical yield) of 5 which was approximately 85% radiochemically pure.

1-S-Hexadecyl-2-O-ethyl-rac-thioglycerol. In a flame dried reaction flask under N_2 was added 2.0 mL (2.0 mmol) of a 1.0 M solution of lithium aluminum hydride to a solution of 25.0 mg (0.048 mmol) of unlabelled thio-ALP in 20 mL of anhydrous ether. After stirring at reflux for 30 min, the reaction was quenched by careful addition of ice water followed by 4% HOAc. The mixture was extracted with ether and the extracts were dried (Na_2SO_4) and evaporated *in vacuo* to afford crude product which had an identical R_f to authentic material prepared as a synthetic precursor to thio-ALP. TLC indicated complete disappearance of starting material. The crude product was column chromatographed on silica gel ($CHCl_3$) to afford pure product. ^{1}H-NMR was identical to that of the authentic synthetic precursor.[8]

[9,10-$^{3}H_{2}$(N)]-1-S-Hexadecyl-2-O-ethyl-rac-glycerol (6). An aliquot containing 25.0 mCi of 5 was evaporated in vacuo and the residue immediately dissolved in 20 mL of ether. Lithium aluminum hydride (4.0 mmol, 4.0 mL of 1.0 M solution in ether) was added to the above solution and the reaction was stirred at reflux for 40 min. TLC-radioscan indicated that all starting material had been consumed. The reaction was quenched by careful addition of water followed by 4% HOAc. The ether phase was removed, dried (Na_2SO_4) and evaporated in vacuo. The residue was immediately column chromatographed on silica gel (CH_2Cl_2-ether 95:5) to afford 4.7 mCi of product. A known quantity of 1-O-octadecyl-2-O-ethyl-rac-glycerol as an internal standard was added to the above product and samples of the mixture were subjected to gas chromatography at 235°C as described above for **3**. No product peak could be detected in the chromatograph compared to comparable analysis using unlabelled product; however, earlier eluting peaks were present indicating that the labelled product would not withstand the temperature conditions required for gas chromatographic analysis. For use in biochemical assays, 5 will be diluted with unlabelled material and the specific activity determined gravimetrically.

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