TRITIATED HEXADECYL PLATELET ACTIVATING FACTOR (PAF)

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Summary

We report the synthesis of the natural isomer of [^{3}H]-lyso platelet activating factor, [^{3}H]-lyso PAF (1-O-[9′,10′-$^3H_2$]-hexadecyl-2-lyso-sn-glycero-3-phosphocholine) and its conversion by acetylation to [^{3}H]-PAF(1-O-[9′,10′-$^3H_2$]-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine) for use in *in vitro* and *in vivo* metabolic studies. The procedure employed allows the separation of the natural (sn-1-O-alkyl)lyso and unnatural (sn-3-O-alkyl) isomers of the PAF precursors from a racemic mixture. The natural lyso-$\Delta^{9,10}$-1-O-hexadecenyl PAF was reduced with 5.0 Ci of carrier free tritium gas to afford the tritiated lyso PAF at a specific activity of 56 Ci/mmole (115 mCi/mg).

Key Words: PAF, lyso PAF, 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine, deuterium, tritium.

Introduction

Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a metabolically active complex lipid that has been found to be a potent activator of platelets and neutrophils, possesses hypotensive[1] and a number of other activities.[2,3] The anaphylactic properties of PAF are not inhibited by antihistaminic compounds such as pyrilamine[4] and are independent of arachidonic acid cascade metabolites.[5,6] The degranulation action of PAF in neutrophils is

0362-4803/85/111169-06$01.00
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Received March 11, 1985

potentiated,[7] however, by 5-L-HETE, which does not cause degranulation by itself.

The metabolism of PAF is under intensive investigation. PAF appears to be inactivated by a specific cystosolic acetylhydrolase.[8] The synthesis of PAF has been demonstrated in cell free systems from alkylacetylglycerol by a specific choline phosphotransferase[9] or from alkyllysoglycerophosphocholine by a specific acetyltransferase.[10] The importance of the two pathways in whole cells is still under unvestigation, however, there is general agreement that the latter pathway is activated by a number of stimuli in various cells. Indeed, a scheme has been postulated[11] relating membrane alkylacylglycerophosphocholine pools to PAF and eicosatetraenoate metabolite formation. A recent review article on the chemistry and biology of PAF has been published.[12]

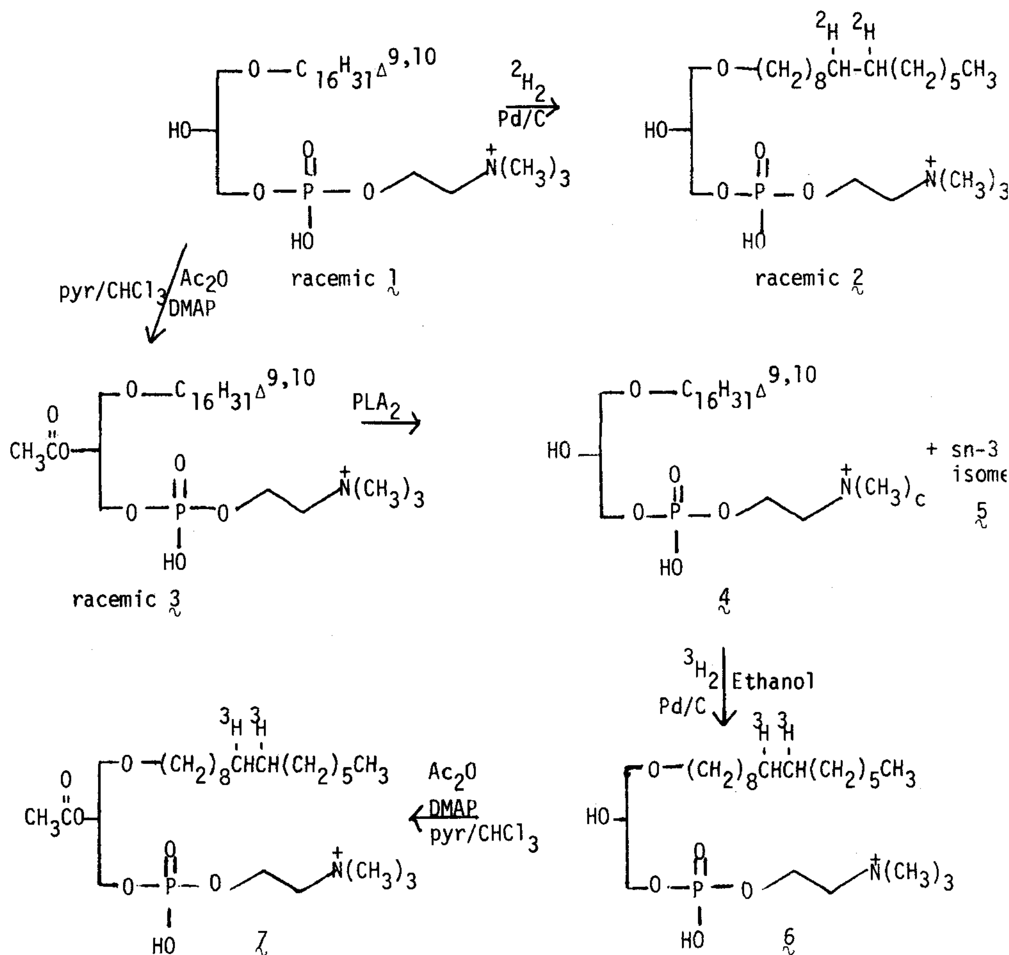
In order to further investigate the metabolism of PAF, we report the synthesis of tritiated hexadecyl PAF (the more active alkyl analogue) at a specific activity of 56 Ci/mmol. The 16:0 species appears to be the major species produced by human neutrophils and is thus of high interest.

Discussion

The synthesis of racemic [octadecyl-$9',10'$-$^3H_2$]-PAF has been reported previously.[13] The unresolved product was obtained at a specific activity of 40-50 Ci/mmole. The precursor was sn-1-$\Delta^{9,10}$-octadecenyl-2-O-benzylglycerophosphocholine. Unlike our procedure described below which tritiates the lyso compound directly, the previous procedure, due to unsaturation in the alkylchain, does not allow for the prior hydrogenolysis of the benzyl protecting group to produce the lyso precursor. Thus, treatment of the precursor with tritium gas in ethanol/H_2O over palladium oxide both tritiates the alkyl chain as well as debenzylates the product. Not only does this procedure consume tritium which is not included in the structure of the product but it also produces volatile tritiated toluene. In order to produce the more biologically active sn-1-O-hexadecyl-PAF we employed a synthetic strategy which allowed for the direct tritiation of the unsaturated lyso precursor, thereby eliminating the drawbacks mentioned above. This precursor, the natural isomer of lyso $\Delta^{9,10}$-hexadecenyl-PAF was prepared by Surles et al.[14] Treatment of racemic $\Delta^{9,10}$-hexadecenyl-PAF **3** with phospholipase A_2 deacetylates

only the natural isomer 4 which is then separated chromatographically from the unnatural PAF 5. As a model for the tritiation, allowing for more similar isotope effects than between hydrogen and tritium, 1.0 atm of deuterium gas was used to reduce the unsaturated racemic lyso precursor 1 in ethanol over 10% Pd/C at room temperature. High resolution ^{1}H-NMR indicated complete disappearance of the olefinic protons 2 as compared to starting material and authentic product. The identical procedure employing the natural isomer 4 and 5.0 Ci of carrier free tritium gas afforded 1,009 mCi of pure tritiated lyso hexadecyl-PAF 6 at a specific activity of 56 Ci/mmole. A portion of this product was acetylated with acetic anhydride in pyridine-$CHCl_3$ in the presence of 4-dimethylaminopyridine (DMAP) to afford the tritiated hexadecyl-PAF 7 of like specific activity (Figure 1).

Figure 1.



Due to the lack of sufficient volatility and chromophoric absorption, neither the lyso PAF or PAF could be quantitated directly by UV-VIS spectrometry or gas chromatography for specific activity determination. Therefore, in order to convert the tritiated lyso PAF to a more volatile derivative without disturbing the integrity of the label, the lyso PAF was treated with phospholipase C[15] at room temperature for 1 hr in 0.1 M tris buffer and ether (pH=7.4). The resulting hexadecyl-sn-glycerol was then quantitated by gas chromatography using the corresponding tetradecyl-sn-glycerol as an internal standard.

The tritiated lyso PAF 6 and PAF 7 were stored at 4-5° C in absolute ethanol.

Experimental Procedures

All chemicals were used as obtained from the manufacturer. ^{1}H-NMR spectra were obtained on a BRUKER 250 MHz FT spectrometer using $CDCl_3$ (TMS) as solvent. Gas chromatography was performed using a Shimadzu GC-8A chromatograph. Radiopurity was determined using a Packard Radioscanner Model 7201. Tritium was counted using a Packard Liquid Scintillation Counter Model 3255 (internal standard) with Scintiverse R (Fisher) counting solution. Silica gel plates (UV) were used for TLC analyses.

Racemic 1-O-[Hexadecyl-9´,10´-$^2H_2$]-sn-glycero-3-phosphocholine (2). Racemic $\Delta^{9,10}$-hexadecenyl-lyso PAF (1) (43.7 mg, 0.088 mmol) and 20 mg of 10% Pd/C in 3.0 ml of absolute ethanol were stirred for 4 h under 1.0 atm of deuterium gas. The catalyst was removed by filtration through a Celite pipet column and the filtrate evaporated to afford 28.9 mg (66%) of solid. ^{1}H-NMR indicated loss of olefinic protons ^{1}H-NMR ($CDCl_3$, TMS) δ 4.33[m,2H, $OC\underline{H_2}CH_2N$], 3.93[m,2H,$C\underline{H_2}OP$], 3.71[t,2H,$OCH_2C\underline{H_2}N$], 3.42[m,4H,$C\underline{H_2}OC\underline{H_2}$], 3.25 [s,9H,$N(C\underline{H_3})_3$], 1.58[m,2H,$OCH_2C\underline{H_2}$], 1.41[m,2H,C$\underline{H}DC\underline{H}$D], 1.28[m,22H,$(C\underline{H_2})n$] and 0.92 [t,3H, $CH_2C\underline{H_3}$].

1-O-[Hexadecyl-9´,10´-$^3H_2$]-sn-glycero-3-phosphocholine (6). A mixture of 9.0 mg (0.018 mmol) of the natural isomer of the $\Delta^{9,10}$-hexadecenyl precursor 4 and 10.0 mg of 10% Pd/C in 1.0 ml of absolute ethanol was stirred for 4 h at room

temperature under 5.0 Ci of carrier free tritium gas. The catalyst was removed by filtration through a Celite pipet column. The filtrate was evaporated in vacuo and the residue taken up in 100 ml of methanol and evaporated. The residue was then taken up in 500 ml of absolute ethanol as a stock solution. TLC radioscan ($CHCl_3$-MeOH-NH_4OH 70:35:7) indicated a pure product 6. Liquid scintillation counting indicated a yield of 1,009 mCi (96%) of the lyso product with specific activity of 56 Ci/mmole (115 mCi/mg).

1-0-[Hexadecyl-9′,10′-$^3H_2$]-2-acetyl-sn-glycero-3-phosphocholine (7). A 300 mCi portion of the lyso product 6 was stirred at room temperature for 16 h with 2.0 mg of DMAP and 50 µl of acetic anhydride in 1.5 ml of $CHCl_3$-pyridine 4:1. The mixture was evaporated under N_2 and the residue chromatographed on a 20 x 20 cm x 0.25 mm silica gel plate ($CHCl_3$-MeOH-NH_4OH 70:35:7) using authentic PAF as an R_f reference. The appropriate band was removed and eluted with $CHCl_3$-MeOH 1:1. The silica gel was filtered off, the filtrate evaporated in vacuo and the residue taken up in 50 ml of absolute ethanol as a stock solution to afford 168 mCi (56%) of pure PAF with specific activity of 56 Ci/mmole (103 mCi/mg).

Specific Activity Determination. A 161 mCi portion of the lyso product 6 was stirred vigorously for 1 h in pH=7.4 0.1 M Tris buffer (1.0 ml) and 3.0 ml of ether with 50 units of Bacillus cereus phospholipase C. The ether phase was drawn off and the aqueous phase extracted twice with $CHCl_3$-MeOH (2:1). The combined organic phases showed ~ 25% conversion to the 1-0-alkyl-sn-glycerol by TLC-radioscan. The 1-0-alkyl-sn-glycerol was purified by column chromatography (CH_2Cl_2-MeOH 8:2), counted and quantitated by gas chromatography using the corresponding tetradecyl-sn-glycerol as an internal standard with a previously calibrated FID detector.

Acknowledgements

The authors wish to thank Mr. J. R. Surles and Dr. C. Piantadosi of the Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, N.C. for their aid in the synthetic

strategies employed and Mr. Chris Wyrick and Mr. George Taylor of the Research Triangle Institute, Research Triangle Park, N.C. for technical assistance with the tritium gas reduction. This work was supported by National Institutes of Health grants HL28491, HL26257, HL27799, HL26818, AI17287, and 5 T32 ES-07126 (JSM).

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