A TOTAL SYNTHESIS OF [9',10'-^{3}H]-LABELLED PAF-ACETHER

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SUMMARY

1-O-octadecen-[9',10']-yl 2-O-benzyl sn-glycero-3-phosrylcholine was obtained from 1-O-octadecen-[9',10']-yl sn-glycerol by tritylation, benzylation and phosphorylation with 2-bromoethyl phosphoryl dichloride followed with trimethylamine reaction. Catalytic tritiation of the double bond led to labelled "lyso" PAF-acether from which PAF-acether with a specific radioactivity between 40 and 50 Ci/mmole was obtained by acetylation.

KEY-WORDS

^{3}H-labelled PAF-acether
1-O-octadecen-[9',10']-yl 2-O-benzyl sn-glycero-3-phosphorylcholine
Tritiation.

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1. INTRODUCTION

The value in studying PAF is now beyond question and our previous total syntheses of this compound [1,2] have yielded sufficient quantities to test its effect on platelet aggregation under various conditions [3] as well as other activities [4].

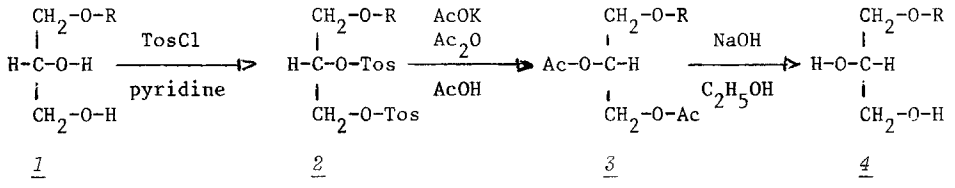
In order to understand its mode of action, it soon became obvious that it was necessary to be able to follow its evolution along enzymatic processes as well as within the cells and metabolically. We have chosen to synthesize a ^{3}H labelled PAF-acether on the ether chain in order to follow the behaviour not only of PAF but also of lyso-PAF.

2. MATERIALS AND METHOD

Solvents were all reagent grade and, unless otherwise specified, were used as received. Column chromatographs were prepared with silica gel 60 from Merck, without special treatment. Analytical thin layer chromatography was performed on TLC-ready plastic sheets F 1500 LS 254 Silica gel 20X20 from Schleicher and Schüll or TLC aluminium sheets Silica gel 60 F 254 precoated 20X20, layer thickness 0.2 mm, from Merck. Spots were revealed with iodine or molybdenum spray according to the method described in ref.[5]. Specific rotations were measured at 20°C on a Perkin Elmer 141 polarimeter. Infrared spectra were recorded on a Pye-Unicam SP3-200 and NMR spectra were obtained using either a Varian EM 360 or a Brücker WP 80 spectrometer, in $CDCl_3$ (reference TMS). Elemental analyses were performed by the Laboratoire de Microanalyses, University Paris VI and the Institut de Chimie des Substances Naturelles (I.C.S.N.), Centre National de la Recherche Scientifique (C.N.R.S.), Gif-sur-Yvette. Specific radioactivity was determined from the amount of tritium used for reduction and the weight of product obtained. The result was confirmed by successive isotopic dilutions in the study of saturation curve.

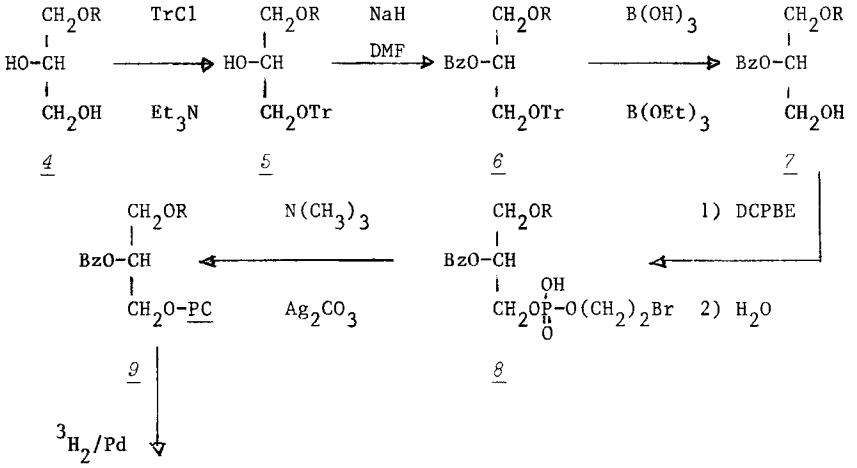
3. RESULTS

Scheme 1



1 2 3 4

Scheme 2



4 5 6 7 8 9 10 11

Tos = p-CH_3-C_6H_4-SO_2 ;

R = $(CH_2)_8$-CH=CH-$(CH_2)_7CH_3$;

R'= $(CH_2)_8$-CH(H)-CH(H)-$(CH_2)_7CH_3$;

Tr = $C(C_6H_5)_3$; Bz = C_6H_5-CH_2 ;

DCPBE = $Cl_2P(O)$-$O(CH_2)_2Br$;

PC = $P(O)(O^-)$-$O(CH_2)_2N^+(CH_3)_3$;

Ac = CH_3-CO .

1-O-octadecen-[9',10']-yl sn-glycerol 4

The 1-O-octadecen-[9',10']-yl glycerol, 4, was prepared in three steps (scheme 1) from the 3-O-octadecen-[9',10']-yl sn-glycerol, 1, by the method described for the 1-O-octadecyl sn-glycerol [1,2]. The intermediates and their physicochemical properties are given in Table 1.

Table 1

Compound	$[\alpha]_D^{20}$ (concentration[a])
1 : 3-O-octadecen-[9',10']-yl sn-glycerol	+ 1.2° (57mg/ml)
2 : 3-O-octadecen-[9',10']-yl 1,2-ditosyl sn-glycerol[b]	- 0.6° (112mg/ml)
3 : 1-O-octadecen-[9',10']-yl 2,3-diacetyl sn-glycerol	- 5.5° (51mg/ml)
4 : 1-O-octadecen-[9',10']-yl sn-glycerol	- 1.2° (45mg/ml)

[a] in $CHCl_3$. [b] Analyses : found : C : 64.13% ; H : 8.51% ; S : 10.61% $C_{35}H_{54}O_7S_2$ required : C : 64.58% ; H : 8.36% and S : 9.85%.

The 1-O-octadecen-[9',10']-yl sn-glycerol, 4, is the starting product for the synthesis of the 1-O-octadecen-[9',10']-yl 2-O-benzyl sn-glycero-3-phosphorylcholine, 9, following the method already described in ref.[2]. The physicochemical properties of the intermediates 5 to 9 are given below.

1-O-octadecen-[9',10']-yl 3-O-trityl sn-glycerol 5

Oil. Yield : 52.6%. Rf = 0.25 (iodine), light petroleum/diethyl ether (85:15 v/v). Analyses : found : C : 82.37% ; H : 10.01% $C_{40}H_{56}O_3$ required C : 82.14 and H : 9.65. IR ν max : 3460cm^{-1} (OH), 3100, 3070, 3040 and 1600cm^{-1} (triphenylmethyl). NMR ($CDCl_3$) δ ppm : 7.30 (multiplet, 15H, aromatic H), 5.30 (triplet, 2H, -C=C-), 3.88 (multiplet, 1H, -CH-O-), 3.40 (multiplet, 4H, $-CH_2-O-CH_2-$), 3.13 (doublet, 2H, $-CH_2-O-C\Phi_3$), 2.20 (singlet, 1H, OH), 1.93 (multiplet, 4H, $-CH_2-C=C$), 1.23 (24H, $(CH_2)_6-C-C=C$) and 0.81 (triplet, 3H, CH_3). $[\alpha]_D^{20}$ = + 2.1° (c = 35.67mg/ml, $CHCl_3$) (litt. $[\alpha]_D^{22}$ = - 2.8° for the enantiomer [6].

1-O-octadecen-[9',10']-yl 2-O-benzyl 3-O-trityl sn-glycerol 6

Oil. Yield : 77.5%. Rf = 0.47 (iodine), diethyl ether/light petroleum (10:90 v/v). IR ν max : 3100, 3070, 3040 and 1600cm^{-1} (triphenylmethyl and benzyl). NMR ($CDCl_3$) δ ppm : 7.27 (multiplet, 20H, aromatic H), 5.32 (triplet, 2H, -CH=CH-), 4.62 (singlet, 2H, -O-CH_2-Φ), 3.70 (multiplet, 1H, CH-O-benzyl), 3.32 (multiplet, 6H, CH_2-O), 1.95 (multiplet, 4H, CH_2-C=C) and 0.85 (triplet, 3H, CH_3). $[\alpha]_D^{20}$= + 6.3° (c = 36.05mg/ml, $CHCl_3$).

1-O-octadecen-[9',10']-yl 2-O-benzyl sn-glycerol 7

Oil. Yield : 81.5%. Rf = 0.12 (iodine), light petroleum/diethyl ether (70:30 v/v). Analyses : found : C : 77.37% ; H : 11.62% $C_{28}H_{48}O_3$ required C : 77.72 and H : 11.18. IR ν max : 3460cm^{-1} (OH), 3090, 3060, 3020 and 1600cm^{-1} (benzyl and =CH). NMR ($CDCl_3$) δ ppm : 7.30 (multiplet, 5H, C_6H_5), 5.30 (triplet, 2H, -CH=CH-), 4.63 (singlet, 2H, O-CH_2-Φ), 3.50 (multiplet, 7H, CH_2-O and CH-O), 1.96 (multiplet, 5H, CH_2-C=C) and OH), 1.20 (24H, $(CH_2)_6$-C-C=) and 0.81 (triplet, 3H, CH_3). $[\alpha]_D^{20}$= - 10.3° (c = 43.89mg/ml, $CHCl_3$).

1-O-octadecen-[9',10']-yl 2-O-benzyl sn-glycero-3-phosphoryl-2"-bromoethanol 8

Oil. Yield : 65.7%. Rf = 0.19 (molybdenum spray), MeOH/$CHCl_3$ (15:85 v/v). Analyses : found : C : 58.86% ; H : 8.35% $C_{30}H_{52}BrO_6P$ required C : 58.15 and H : 8.46. IR ν max : 3440cm^{-1} (OH), 3080, 3040 and 3020cm^{-1} (benzyl and =C-H), 1250cm^{-1} (P=O), 1130cm^{-1} (P-OH), 1080 and 1040cm^{-1} (P-O-alkyl). NMR ($CDCl_3$) δ ppm : 7.25 (multiplet, 5H, C_6H_5), 5.32 (triplet, 2H, -CH=CH-), 4.62 (singlet, 2H, O-CH_2-Φ), 3.67 (multiplet, 12H, CH_2O, CHO, OH and CH_2Br), 1.95 (multiplet, 4H, CH_2-C=C), 1.20 (24H, $(CH_2)_6$-C-C=) and 0.80 (triplet, 3H, CH_3). $[\alpha]_D^{20}$= + 2.2° (c = 30.25mg/ml, $CHCl_3$).

1-O-octadecen-[9',10']-yl 2-O-benzyl sn-glycero-3-phosphorylcholine 9

Oil. Yield : 39%. Rf = 0.39 (molybdenum spray), $CHCl_3$/MeOH/NH_4OH (34%, d=0.89) (70:35:7 v/v/v). Analyses : found : C : 62.60% ; H : 9.82% ; N : 2.67%: P : 4.37% $C_{33}H_{60}NO_6P$, $2H_2O$ required C : 62.52 ; H : 10.18 ; N : 2.21 and P : 4.37. IR ν max : 3070, 3040 and 3020cm^{-1} (P=O), 1090 and 1060cm^{-1} (P-O-alkyl). NMR ($CDCl_3$) δ ppm : 7.26 (multiplet, 5H, C_6H_5), 5.32 (triplet, 2H, -CH=CH-), 4.56 (singlet, 2H, O-CH_2-Φ), 3.13 (singlet, 9H,

$+N(CH_3)_3$, 1.93 (multiplet, 4H, CH_2-C=C), 1.23 (24H, $(CH_2)_6$-C-C=) and 0.86 (triplet, 3H, CH_3). $[\alpha]_D^{20}$ = + 2.0° (c = 31.27mg/ml, $CHCl_3$).

1-O-[9',10'-di^{3}H]-octadecyl sn-glycero-3-phosphorylcholine 10

3.16mg (5µmoles) of 9 were dissolved in 1ml of pure methanol, transferred to a tritiation vial and then frozen. Palladium oxyde catalyst (27mg in EtOH/H_2O 20:80 v/v) was then added and the reacting vial connected to an automatic gas transfer unit [9]. After a vacuum of 10^{-4} Torr was reached, pure tritium gas (80Ci) was introduced, compressed to 1.1 bar and the catalyst was flushed (15 min.) onto the still frozen solution. After thawing, the reaction mixture was kept at 20°C and magnetically stirred for 4 hours. The absorption of tritium gas produced a reduction of pressure (0.52 bar). The solution was frozen again and the unreacted tritium gas removed. The catalyst was separated from the reacting solution by filtration over Millipore (Millex F.G., 0.2µ) and labile tritium atoms eliminated by successive washes and evaporations with a large volume of methanol. The first attempts to purify the 10 reaction mixture were recovered by thin layer chromatography on silica gel with $CHCl_3$/MeOH/H_2O (70:35:7 v/v/v) as eluent. The autoradiogram and ^3H scanning revealed a peak co-migrating with 2-lyso PAF-acether and corresponding to the spot detectable with Dittmer reagent (molybdenum spray). The product 10 is obtained by organic extraction ($CHCl_3$/MeOH, 1:1 v/v), transferred into an Eppendorf microtest tube and evaporated under nitrogen.

1-O-[9',10'-di^{3}H]-octadecyl 2-O-acetyl sn-glycero-3-phosphorylcholine 11

An aliquot of the dry residue precedently obtained (6nmoles, 0.3mCi) was suspended in 200µl pyridine and 3µl acetic anhydride and incubated at 95°C. Quantitative evolution of acetyl incorporation into 10 was checked by thin layer chromatography (every five minutes) and the reaction product was finally stopped after 1 hour and purified through preparative thin layer chromatography using the same solvent system as above. The specific radioactivity of 11 obtained by this process was found to be close to 40-50Ci/mmole.

4. DISCUSSION

The synthesis of the labelled product is nearly the same as for the unlabelled which we have already described [2]. This method has the advantage that the label is added at the next to last step (9→10) which facilitates the manipulations and yields a highly radioactive product.

Lyso-PAF labelled on the ether chain has already been obtained by Paltauf [10] starting from an oleyl glycerol reduced with tritium.

The specific yield of this product was only 1.27mCi/mmole whereas our method gave a radiolabelled lyso-PAF with a specific radioactivity of 5.10^4mCi/mmole and a PAF-acether between 4.10^4 and 5.10^4mCi/mmole.

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