PLATELET ACTIVATING FACTOR (PAF-ACETHER): TOTAL SYNTHESIS OF 1-*O*-OCTADECYL 2-*O*-ACETYL *sn*-GLYCERO-3-PHOSPHORYL CHOLINE

Jean-Jacques GODFROID, Françoise HEYMANS, Elie MICHEL, Catherine REDEUILH, Efroïm STEINER and Jacques BENVENISTE+

Laboratoire de Pharmacochimie Moléculaire, Université de Paris VII, 2, Place Jussieu, 75221 Paris Cedex 05 and *+Laboratoire d'Immunopathologie de l'Allergie et de l'Inflammation, INSERM U 131, 32, rue des Carnets, 92140 Clamart, France*

Received 10 May 1980
Revised version received 3 June 1980

1. Introduction

Platelet-activating factor (PAF), a mediator of anaphylaxis and inflammation, is released from IgE-sensitized antigen-stimulated basophils [1–3]. PAF is a low molecular weight phospholipid which is sensitive to hydrolysis by phospholipases A_2, C, and D, but not by lipase from *Rhizopus arrhizus* or sphingomyelinase C [4]. PAF appeared to be a glycerophospholipid with a choline polar head group devoid of an ester function at position 1 [4,5]. The presence of PAF has been demonstrated in cells from several species including man [3,6,7], rabbit [1–3], rat [8] and pig [4]. PAF has been obtained from rat and mouse peritoneal macrophages upon stimulation by the ionophore A23187 or exposure to phagocytable particles [9]. PAF has been shown to induce aggregation of blood platelets from rabbit [1,3], man [10], rat [11] and guinea pig [12]. The structure of the molecule responsible for PAF activity has been elucidated [13,14]. This was obtained by acetylation of 2-lyso plasmalogens possessing a 3-*O*-phosphoryl choline group [13] or a 3-*O*-phosphoryl ethanolamine group followed by reaction with diazomethane and quaternization with methyl iodide [14]. This result lead us to propose a 1-*O*-alkyl 2-*O*-acetyl *sn*-glycero-3-phosphoryl choline structure and the terminology 'PAF-acether' for this substance. However, total synthesis was necessary to confirm the structure of PAF-acether. Here, we report the total synthesis of 1-*O*-octadecyl 2-*O*-acetyl *sn*-glycero-3-phosphoryl choline, which has the same biological activity as the natural PAF-acether.

2. Materials and methods

2.1. *Chemistry*

Solvents were all reagent grade. Column chromatographs were prepared with Silica gel 40 or 60 from Merck, without any special treatment unless otherwise specified. Melting points were determined on a Büchi capillary melting point apparatus and were uncorrected. Optical rotation values were measured at room temperature in a 5 mm tube using a Rousset-Jouan polarimeter. The purity of each compound was controlled by thin-layer chromatography on silica gel. Infrared spectra were recorded on a PYE UNICAM SP3-200 and NMR spectra were obtained using either a Varian EM 360 or a Varian T 60 spectrometer in $CDCl_3$ with TMS as an internal standard. Elemental analysis were performed by the Laboratoire de Microanalysis, University of Paris VII and the Institut de Chimie des Substances Naturelles (ICSN), Centre National de la Recherche Scientifique (CNRS), Gif s/Yvette.

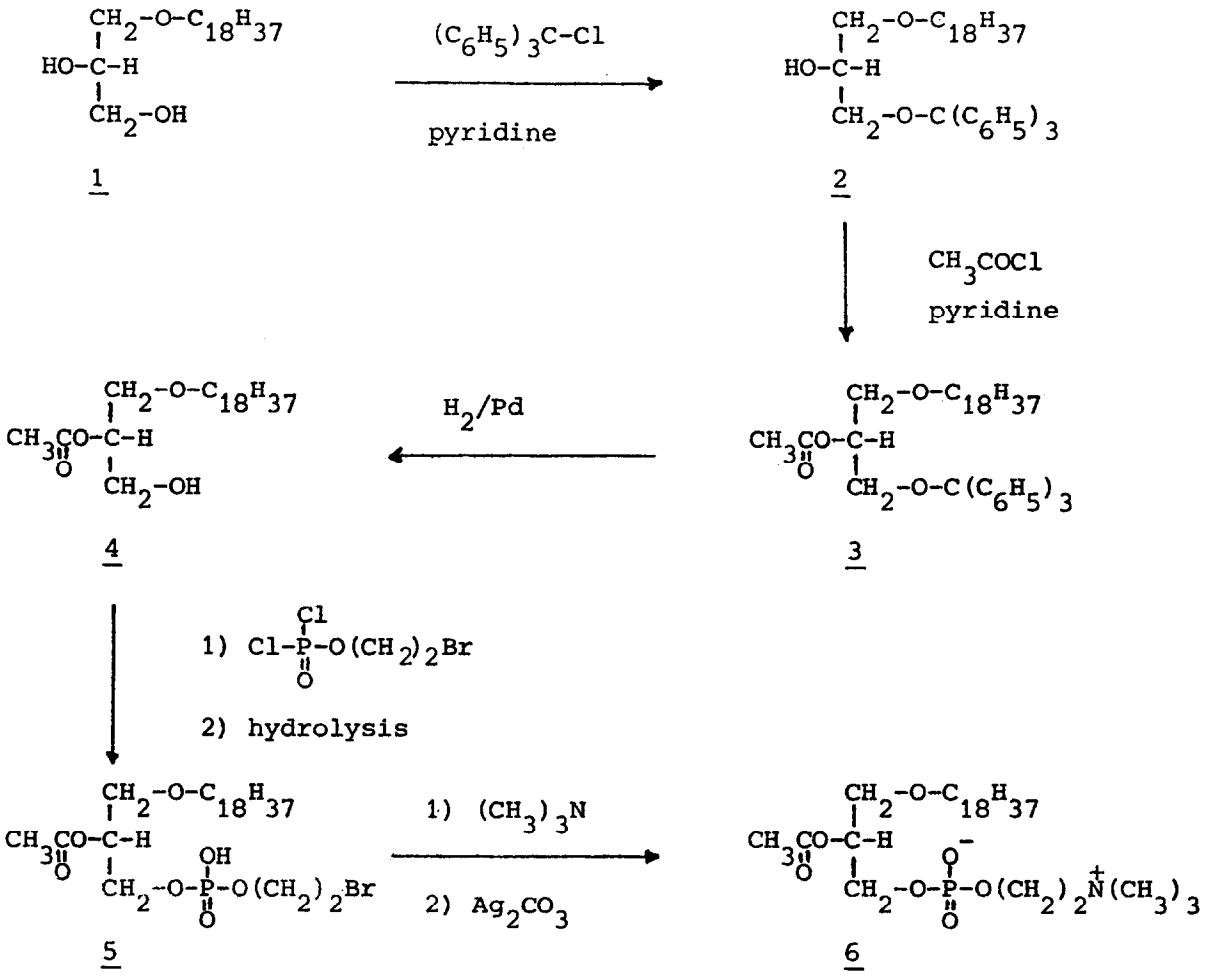
2.2. *Biological assay*

PAF-acether activity was determined in an aggregometer apparatus (Icare, Marseille) by aggregation of 5×10^8 washed rabbit platelets in 300 μl Tyrode's buffer in the presence of 5 μM indomethacin (Sigma Chemicals, St Louis, MO). It was expressed in arbitrary units as measured by the relative chart distance for the aggregation produced by 10 μl PAF-acether containing solution. Preparation of platelets, composition of buffers and assay of PAF-acether have been detailed in [4,5,9].

3. Results

3.1. *Synthesis*

The 1-*O*-octadecyl *sn*-glycerol 1 (scheme 1), prepared in eight steps from D-mannitol by the methods in [15–17], and adapted to our use, was converted into the 1-*O*-octadecyl 3-*O*-trityl *sn*-glycerol 2 as proved by IR spectra: 3080, 3040, 1600 cm^{-1} for the trityl group and NMR spectra: δ ppm 7.26 (multiplet, 15H, aromatic H) (see table 1). The 2 derivative was acetylated to yield the 1-*O*-octadecyl 2-*O*-acetyl 3-*O*-trityl *sn*-glycerol 3: IR, 1735 cm^{-1} for ν C=O; NMR, δ ppm 2.00 (singlet, 3H, CH_3CO), 5.16 (quintuplet-like, J = 6 Hz, 1H, CH. After reduction by H_2 on charcoal/Pd, 3 gave the 1-*O*-octadecyl 2-*O*-acetyl *sn*-glycerol 4: IR, 3450 cm^{-1} ν OH, 1735 cm^{-1} ν C=O; NMR, δ ppm 4.93 (quintuplet-like, J = 6 Hz, 1H, CH), proof that the acetyl group was retained in the 2-position. 4 was then treated by 2-bromoethylphosphoryl dichloride in dry ether by the method in [18] simplified. After hydrolysis and chromatography, the 1-*O*-octadecyl 2-*O*-acetyl *sn*-glycero-3-phosphoryl 2′-bromoethanol 5 was obtained as shown by NMR spectra and elemental analysis. 5 was treated by an excess of trimethylamine according to [19] and by silver carbonate to give the 1-*O*-octadecyl 2-*O*-acetyl *sn*-glycero-3-phosphoryl choline 6. The NMR signal for $(CH_3)_3N^+$ appeared at 3.36 ppm as a broad singlet (9H) and the elemental analysis was in significant agreement with



Scheme of synthesis

Table 1
Physicochemical results

Compound no.	Purification	Yield (%)	m.p. (°C)	$[\alpha]_D^{22.5}$[a]	Silica gel TLC R_F (development)	Analyses	
						Calc.	Found
1	recryst. (hexane)		71[b]	−0.86°	0.10[c] (iodine)	C: 73.25	72.97
						H: 12.79	12.85
2	Silica gel 40 column[d]	99.5	55	+1.08°	0.60[c]	C: 81.86	82.01
						H: 9.89	10.08
3	Silica gel 60 column[d]	81	44	+8.90°	0.80[c]	C: 80.25	80.33
						H: 9.55	9.59
4	Silica gel 60 column deactivated[e]		Surfused	Not measured	0.24[c] (iodine)	C: 71.51	71.46
						H: 11.91	11.91
5	Silica gel 60 column[f]	61	46	−3.05°	0.84[g] (molybdenum spray[h])	C: 52.35	51.81
						H: 8.72	8.80
						Br: 13.96	13.10
6	Silica gel 60 column[i]	59	Decomposition from 200°C	−3.38°	0.00[g] (molybdenum spray[h])	C: 60.98	61.39
						H: 10.53	10.64
						N: 2.54	2.60[i]
						P: 5.63	6.01[i]

[a] Conc., 1 g in 100 ml $CHCl_3$; [b] Lit., 71–72°C [20]; [c] Ether–light petroleum (b.p. 30–60°C) 30:70 (v/v); [d] Ether–light petroleum (b.p. 30–60°C) 15:85 (v/v); [e] Ether–light petroleum (b.p. 30–60°C) 30:70 (v/v); [f] Solvent system in increasing order of polarity: chloroform, chloroform–methanol (85:15, v/v), chloroform–methanol (70:30, v/v) and chloroform–methanol (50:50, v/v); [g] Chloroform–methanol (70:30, v/v); [h] See [21]; [i] Solvent system in gradually increasing order of polarity: chloroform–methanol (70:30, v/v) to chloroform–methanol (30:70, v/v) and methanol alone; [i] $N/P = 0.96$

calculated values. The chemical ionization mass spectrum using isobutane as reactant gas showed the MH^+ peak *m/e* 552 [5,20]. Although the treatment with silver carbonate in order to obtain the internal salt exhibited a slight deacetylation into a lyso-derivative as observed by high pressure liquid chromatography. Other methods of synthesis will be described elsewhere.

3.2. *Characterization of natural and synthetic PAF-acether*

Several criteria were used to verify that the synthetic compound 6 exhibited the same biological characteristics as that of natural PAF-acether: aggregation of platelets by natural or synthetic PAF-acether 6 was not influenced by indomethacin. PAF-acether 6 containing solution was completely inactivated after incubation for 10 min at 37°C with phospholipase A_2 (Boehringer, Mannheim). Natural and synthetic PAF-acether 6 migrated on silicic acid thin-layer chromatography between sphingomyelin and lysophosphatidylcholine, with an R_F 0.35 and exhibited identical retention time on silicic acid high pressure liquid chromatography [5]. Synthetic PAF-acether 6 exhibited spec. act. 13 units/ng (arbitrary units as defined in section 2.2.), as compared to 6 units/ng for the semi-synthetic PAF-acether.

It was difficult to assess specific activity of natural PAF, given the lack of ponderous material. However, it was approximately between the semi-synthetic and the synthetic substances.

4. Discussion

These data demonstrate clearly that we succeeded in synthetizing a compound the structure of which corresponded to that of PAF-acether. The synthetic compound 6 met with the biological and physicochemical criteria used for characterization of natural PAF-acether. Moreover, none of the intermediate compounds exhibited any platelet-aggregating activity observed only when full synthesis was completed, indicating specificity of the structure exhibited by this compound. Preliminary studies have shown that the 2-*O*-propionyl and the 2-*O*-butyryl derivatives were much less active than the 2-*O*-acetyl compound in inducing platelet aggregation (in preparation). However, if this study shows clearly that the specific activity of the 1-*O*-octadecyl compound is very high,

as compared to the semi-synthetic substance obtained from natural plasmalogens, there still remains uncertainty on possible variations in length of the 1-*O*-alkyl chain.

Acknowledgements

The authors wish to thank Dr Judith Polonsky (ICSN, CNRS, Gif s/Yvette) and Professor Andrée Marquet (University of Paris VI and CNRS) for fruitful discussions and technical assistance. We are grateful to Dr P. Warren for the chemical ionization mass spectrum. This work was supported, in part, by grant ANVAR 78 338201 from SPECIA and DGRST ACC 79-7-0982.

References

[1] Benveniste, J., Henson, P. M. and Cochrane, C. G. (1972) J. Exp. Med. 136, 1356–1377.
[2] Siraganian, R. P. and Osler, A. G. (1971) J. Immunol. 106, 1244–1251.
[3] Benveniste, J. (1974) Nature 249, 581–582.
[4] Benveniste, J., Le Couedic, J. P., Polonsky, J. and Tencé, M. (1977) Nature 269, 170–171.
[5] Tencé, M., Polonsky, J., Le Couedic, J. P. and Benveniste, J, (1980) Biochimie 3, 62.
[6] Camussi, G., Mencia-Huerta, J. M. and Benveniste, J. (1977) Immunology 33, 523–534.
[7] Lewis, R. A., Goetzl, E. J., Wassermann, S. I., Valone, F. H., Rubin, R. H. and Austen, K. F. (1975) J. Immunol. 114, 87–92.
[8] Kater, L. A., Austen, K. F. and Goetzl, E. J. (1965) Fed. Proc. FASEB 34, 967.
[9] Mencia-Huerta, J. M. and Benveniste, J. (1979) J. Immunol. 9, 409–415.
[10] Benveniste, J., Le Couedic, J. P. and Kamoun, P. (1975) Lancet i, 344.
[11] Fesüs, L., Csaba, B, and Muszbek, L. (1977) Clin. Exp. Immunol. 27, 512–515.
[12] Vargaftig, B. B., Lefort, J., Prancan, A. V., Chignard, M. and Benveniste, J. (1979) Haemostasis 8, 171–182.
[13] Demopoulos, C. A., Pinckard, R. N. and Hanahan, D. J. (1979) J. Biol. Chem. 254, 9355–9358.
[14] Benveniste, J., Tencé, M., Varenne, P., Bidault, J., Boullet, C. and Polonsky, J. (1979) CR Acad. Sci. Paris 289, sér. D, 1037–1040.
[15] Lands, W. E. M. and Zschocke, A. (1965) J. Lipids Res. 6, 324–325.
[16] Palameta, B. and Kates, M. (1966) Biochemistry 5, 618.
[17] Baumann, W. J. and Mangold, H. K. (1964) J. Org. Chem. 29, 3055–3057.
[18] Von Hirt, R. and Berchtold, R. (1958) Pharm. Acta Helv. 33, 349–356.
[19] Diembeck, W. and Eibl H. (1979) Chem. Phys. Lipids 24 237–244.
[20] Warren, P., Bardey, B., Lonvevialle, P. P. and Das, B. C. (1977) Bull. Soc. Chim. Fr. 886.
[21] Chocko, G. K. and Hanahan, D. J. (1968) Biochim. Biophys. Acta 164, 252–271.
[22] Dittmer, J. C. and Lester, R. L. (1964) J. Lipids Res. 5, 126–127.