scintillation. Specific binding of [^{3}H]DHA (binding in the absence of any added compounds minus that in the presence of 20 μM *l*-alprenolol) was approximately 65 fmol/mg protein. In a typical experiment the total specific binding was 7–8% of the free [^{3}H]DHA and the nonspecific binding was 50% of the total specific binding. The affinity of a compound was estimated by competition binding with [^{3}H]DHA with 5–10 different concentrations of each compound and the IC_{50} (concentration at which specific [^{3}H]DHA binding is inhibited by 50%) was determined visually from a semilog plot. The apparent K_i was calculated according to the formulation of Cheng and Prusoff:[26] $K_i = IC_{50}/(1 + L/KD)$, where K_i is the inhibition constant of the test compound, L is the concentration of [^{3}H]DHA, and the KD is the dissociation constant for [^{3}H]DHA (approximately 1 nM).

Acknowledgment. We are greatly indebted to Drs. S. J. Riggi and F. L. Scott for their support in this work and Drs. W. L. Albrecht, B. E. Watkins, and J. C. Strand for their suggestions during the preparation of this manuscript. Thanks are due to Dr. M. Fedorchuk for his work in chemical synthesis.

Registry No. 1, 84858-29-7; 1·HCl, 84858-18-4; **3**, 84858-17-3; **4**, 106820-96-6; **5**, 106820-97-7; **7** (R = Pr), 87272-33-1; **8**, 87272-33-1; **9**, 84858-38-8; **10**, 84858-53-7; **10**·HCl, 84858-54-8; **11**, 84858-51-5; **12**, 84858-55-9; **13**, 84858-56-0; **13**·maleate, 84858-57-1; **14**, 106820-99-9; **14**·HCl, 106821-05-0; **15**, 106821-00-5; **16**, 106821-01-6; **17**, 106821-02-7; **17**·HCl, 106821-06-1; **18**, 106821-03-8; $PhCH_2CN$, 140-29-4; $PhCO_2COPh$, 93-97-0; *i*-$PrNH_2$, 75-31-0; $PrAlH_2$, 107-10-8; $CH_2{=}CHCH_2NH_2$, 107-11-9; *s*-$BuNH_2$, 13952-84-6; MeNHPr, 627-35-0; *t*-$BuNH_2$, 75-64-9; $BuNH_2$, 109-73-9; $CH_3(CH_2)_4NH_2$, 110-58-7; $CH_3(CH_2)_5NH_2$, 111-26-2; 7-hydroxy-3-phenylflavone, 18651-11-1; resorcinol, 108-46-3; ω-phenylresacetophenone imine hydrochloride, 106821-04-9; ω-phenylresacetophenone, 3669-41-8; epichlorohydrin, 106-89-8; cyclopropanamine, 765-30-0; cyclopentanamine, 1003-03-8; cyclohexanamine, 108-91-8.

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Structure–Activity Relationship in PAF-acether. 3.[1] Hydrophobic Contribution to Agonistic Activity

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The synthesis of some selected PAF-acether homologues with an alkoxy-chain length from C_1 to C_{20} in position 1 is described. All agonist activities are closely correlated among themselves and with the calculated fatty-chain hydrophobicity. After a discussion on recent published results and comparison with our data, we conclude that the ether oxide function is absolutely essential at the glycerol 1-position for potent agonist activity and that potency correlates well with hydrophobicity parameters. We indicate the importance of steric and configurational constraints.

Since its structure was elucidated in 1979 via hemisynthesis from plasmalogens,[2,3] platelet activating factor (PAF-acether) Ib + Ic (natural configuration *R*) (Figure 1) has been increasingly studied for its remarkable biological activities, mainly platelet aggregation,[4] bronchoconstriction,[5] and hypotension.[6] Its biosynthesis was found to occur in a variety of inflammatory cells:[7] basophils,[8] macrophages,[9] neutrophils,[10,11] and platelets themselves.[4] Research interest is now focused on the study of the relationship between specific chemical moieties of this molecule and its biological activities.[12-16]

Here, we report the synthesis of some selected linear-ether-chain homologues of PAF-acether, from C_1 to C_{20}, by a minor modification of the methods we have already described.[17-19] We also studied correlations between lipophilicity of the ether chain and agonistic activities such as platelet aggregation (on washed platelets (WP) and platelet-rich plasma (PRP)), hypotension, bronchoconstriction, and thrombocytepenia.

Chemistry

The PAF-acether homologues described in this paper are listed in Figure 1. Alkylacetylglycerophosphocholines I were prepared by using modified versions of our already published procedures[17-19](Scheme I).

Glyceryl ethers 1 were obtained from the potassium salt of 1,2-*O*-isopropylideneglycerol and the corresponding alkyl methanesulfonate (R = C_6–C_{20})[20] or by condensing 1,2-*O*-isopropylideneglycerol with alkyl iodides (R = C_4H_9 and

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Table I. Physicochemical Properties of 1-*O*-Alkyl-2-*O*-acetyl-*rac*-glycero-3-phosphocholine Synthetic Intermediates 1–10

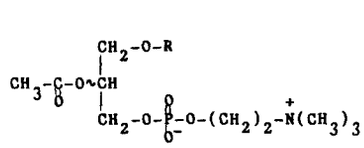
	R_f[c]							
compd	**a**, $C_{20}H_{41}$[a]	**b**, $C_{18}H_{37}$[a,b]	**c**, $C_{16}H_{33}$[b]	**d**, $C_{12}H_{25}$[b]	**e**, $C_{10}H_{21}$[a]	**f**, C_6H_{13}[b]	**g**, C_4H_9[b]	**h**, CH_3[b]
1[d]	0.53 (A)	0.51 (A)	0.48 (A)	0.47 (A)	0.47 (A)	0.40 (A)	0.37 (A)	0.29 (A)
2[e]	0.31 (B)	0.30 (B)	0.29 (B)	0.28 (B)	0.27 (B)	0.25 (B)	0.22 (B)	0.10 (B)
3[e]		0.47 (D)	0.48 (D)	0.52 (D)		0.49 (C)	0.52 (C)	0.65 (C)
4[e]		0.12 (A)	0.12 (A)	0.12 (A)		0.10 (A)	0.07 (A)	0.04 (A)
5[e]		0.39 (F)	0.39 (F)	0.38 (F)		0.35 (F)	0.30 (F)	0.29 (F)
6[e]		0.27 (G)	0.27 (G)	0.26 (G)		0.24 (G)	0.24 (G)	0.24 (G)
7[f]		0.10 (G)	0.10 (G)	0.10 (G)		0.10 (G)	0.09 (G)	0.09 (G)
8[g]	0.28 (D)	0.29 (D)			0.32 (D)			
10[h]	0.35 (F)	0.35 (F)			0.37 (F)			

[a] Method B. [b] Method A. [c] Solvent system in parentheses: A = $CHCl_3$/MeOH, 95:5, v/v (iodine); B = petroleum ether (P)/ether (E), 70:30, v/v (iodine or UV); C = P/E, 80:20, v/v (iodine or UV); D = P/E, 90:10, v/v (iodine or UV); F = $CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v (molybdenum spray or iodine); G = $CHCl_3$/MeOH/NH_4OH, 70:35:7, v/v/v (molybdenum spray or iodine). [d] Melting point according to ref 20 for **1a–1d**; boiling point according to ref 21 for **1e–1g** and to ref 23 for **1h**. [e] Only **2a–2c** were solid and recrystallized in petroleum ether (30–60 °C); mp = 61 °C for **2a**, 55 °C for **2b**, and 36 °C for **2c**; other intermediates from **2** to **6** were viscous oils. [f] Only **7b–7d** were solid and had mp >200 °C with decomposition. [g] Recrystallization solvent = petroleum ether (30–60 °C); mp = 52 °C for **8a** and 49 °C for **8b**; **8e** was a viscous oil. [h] Viscous oil.

Table II. Spectral Data of 1-*O*-Alkyl-2-*O*-acetyl-*rac*-glycero-3-phosphocholine Synthetic Intermediates

compd	IR (KBr):[a] ν (cm^{-1})	^1H NMR:[a] MHz, solvent, δ
1	3380 (OH), 1110 (C—O—C), 1040 (COH)	
2	3450 (OH), 3080, 3050, 3020 (Ar CH), 1590 (Ar C=C), 1080 (C—O—C)	60 MHz, $CDCl_3$, 3.20 (d, 2 H, CH_2OCPh_3), 3.90 (quintet, 1 H, C*H*OH), 7.26 (m, 15 H, Ar H)
3	3080, 3050, 3020 (Ar CH), 1590 (Ar C=C), 1080 (C—O—C)	80 MHz, $CDCl_3$, 3.70 (quintet, 1 H, CHOBz[b]), 4.67 (s, 2 H, OCH_2Ph)
4	3450 (OH), 1125, 1065 (C—O)	60 MHz, $CDCl_3$, 2.00 (s, 1 H, OH, exchanges with D_2O), 4.65 (s, 2 H, OCH_2Ph), 7.36 (large s, 5 H, Ar H)
5	3450 (OH), 1250 (P=O), 1125, 1050, 1030 (C—O—C, P—O)	
6	3400, 1650 (remaining H_2O), 1250 (P=O), 1100, 1080, 1060 (P—OC, C—O—C)	250 MHz, CD_3OD, 3.07 (s, 9 H, $^+N(CH_3)_3$), 3.74 (quintet, 1 H, CHOBz[b]), 3.92 (m,[c] 2 H, CH_2OP), 4.16 (m,[c] 2 H, $POCH_2$)
7	3400 (OH), 1240 (P=O), 1100, 1050 (large, C—O, P—O)	250 MHz, CD_3OD, 3.22 (s, 9 H, $^+N(CH_3)_3$), 3.88 (m,[c] 3 H, C*H*OH and CH_2OP), 4.29 (m,[c] 2 H, $POCH_2$)
8	3100, 3080, 3040 (Ar CH), 1740 (C=O), 1590 (Ar C=C)	60 MHz, $CDCl_3$, 2.00 (s, 3 H, CH_3CO), 5.16 (quintet, 1 H, CHOAc[d])
10	3450 (OH), 1740 (C=O), 1240 (C—O—C ester, P=O), 1080 (large, P—O, C—O—C ether)	

[a] Key peaks. [b] Bz = $CH_2C_6H_5$. [c] ^{31}P spin coupling. [d] Ac = $COCH_3$.



Compd	R
Ia	n-$C_{20}H_{41}$
Ib	n-$C_{18}H_{37}$
Ic	n-$C_{16}H_{33}$
Id	n-$C_{12}H_{25}$
Ie	n-$C_{10}H_{21}$
If	n-C_6H_{13}
Ig	n-C_4H_9
Ih	CH_3

Figure 1. Racemic PAF-acether homologues in position 1.

CH_3) in the presence of silver oxide,[21] followed by acid hydrolysis.

Physicochemical properties of the intermediates are summarized in Table I and their spectral data in Table

Table III. Physicochemical Properties and Spectral Data[h] of 1-*O*-Alkyl-2-*O*-acetyl-*rac*-glycero-3-phosphocholines Ia–Ih

compd	R_f[a]	t_R,[b] min (flow rate, mL/min)	mass spectrum,[c,d] m/z [MH]$^+$	[A][e]	[B][f]
Ia	0.20	16–17 (2.5)	580	521	415
Ib	0.19	22 (3)	552	493	387
Ic	0.18	12 (3.3)	524	465	359
Id	0.18	8 (2.5)	468	409	303
Ie	0.18	11 (2.5)	440	381	275
If	0.16	20 (3)	384	325	219
Ig	0.15	14.5 (5)	356	297	191
Ih	0.15	19 (4)	314	255	149[g]

[a] $CHCl_3$/MeOH/NH_4OH, 70:35:7, v/v/v (molybdenum spray or iodine). [b] Retention time in HPLC. [c] General spectrum for Ia–Ih. [d] See ref 24 for details. [e] [A] = [MH]$^+$ – $N(CH_3)_3$. [f] [B] = [MH]$^+$ – [$^-OP(O)O(CH_2)_2N^+(CH_3)_3$] + H, abundant fragment ion corresponding to [B] – H_2O was also present. [g] [B] – CH_3OH at m/z 117 was found in addition. [h] Ia–Ig: ^1H NMR (250 MHz in CD_3OD) δ 0.89 (t, 3 H, CH_3), 1.28 (large s, 2*n* H, $(CH_2)_n$), 1.54 (m, 2 H, $CH_2\alpha$), 2.07 (s, 3 H, $COCH_3$), 3.22 (s, 9 H, $^+N(CH_3)_3$), 3.45 (m, 2 H, $CH_2\beta$,), 3.58 (d, 2 H, $CH_2\gamma$), 3.65 (m, 2 H, $CH_2\zeta$), 4.00 (m, ^{31}P spin coupling, 2 H, $CH_2\delta$), 4.30 (m, ^{31}P spin coupling, 2 H, $CH_2\epsilon$), 5.15 (quintet, 1 H, CHOAc). Compound Ih gave a signal at 3.25 (s, 3 H, CH_3O) for the alkyl chain; others are the same as for Ia–Ig. All products gave satisfactory elemental analyses (C, H, N. P).

II. Chromatographic behavior, ^1H NMR spectral data, and the mean fragmentation peaks in mass spectra are listed in Table III for the terminal compounds I. We

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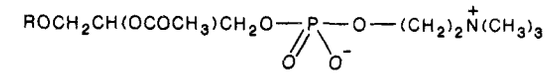
Table IV. Biological Data on 1-*O*-Alkyl-2-*O*-acetyl-*rac*-glycero-3-phosphocholines



compd	R	platelet aggregation:[a] EC_{50}, M WP	platelet aggregation:[a] EC_{50}, M PRP	hypotension:[b] EC_{30}, mol/kg	bronchoconstriction[c] (3 cm H_2O), mol/kg	thrombocytopenia:[c] EC_{50}, mol/kg
	$C_{18}H_{37}$ (*R*)[d]	5.7×10^{-11}	1.9×10^{-8} (1.7–2.2)[f]	4.9×10^{-10} (3.7–6.5)	1.8×10^{-10}	1.8×10^{-10}
Ia	$C_{20}H_{41}$	3.3×10^{-10}	1.9×10^{-7} (1.4–2.5)	2.4×10^{-9} (1.5–6.2)	1.3×10^{-9}	1.8×10^{-10}
Ib	$C_{18}H_{37}$	2.2×10^{-10}	5.7×10^{-8} (4.0–6.6)	1.5×10^{-9} (1.0–1.95)	5.4×10^{-10}	5.4×10^{-10}
Ic	$C_{16}H_{33}$	2.0×10^{-10}	4.5×10^{-8} (3.9–5.1)	1.3×10^{-10} (0.7–1.2)	0.5×10^{-10}	2.0×10^{-10}
Id	$C_{12}H_{25}$	4.5×10^{-10}	2.3×10^{-7} (2.0–2.6)	3.3×10^{-9} (2.3–5.8)	1.2×10^{-10}	0.6×10^{-9}
Ie	$C_{10}H_{21}$	8.5×10^{-9}	1.2×10^{-6} (0.9–1.6)	0.7×10^{-8} (0.6–0.8)	2.5×10^{-10}	0.6×10^{-9}
If	C_6H_{13}	3.1×10^{-7}	1.7×10^{-5} (1.4–1.9)	0.7×10^{-6} (0.3–4.5)	2.6×10^{-8}	1.0×10^{-7}
Ig	C_4H_9	1.8×10^{-6}	1.0×10^{-4}	1.1×10^{-6} (0.7–3.4)	1.0×10^{-6}	1.0×10^{-6}
Ih	CH_3		$>1.0 \times 10^{-4}$ [e]	$>1.6 \times 10^{-6}$ [e]	$>1.6 \times 10^{-6}$ [e]	$>1.6 \times 10^{-6}$ [e]

[a] In vitro on rabbit platelets; WP = washed platelets, PRP = platelet-rich plasma. [b] On anesthetized rat. [c] On anesthetized guinea pig. [d] Synthetic PAF-acether in its natural configuration (*R*) used as a reference. [e] Considered as inactive. [f] Values in parentheses are 95% confidence intervals.

Table V. Squared Correlation Coefficient Matrix of Lipophilic Character Σf and Biological Data

	Σf[a]	pE WP[b,c]	pE PRP[b,c]	pE HYPO[b]	pE BRON[b]	pE THR[b]
Σf	1.000					
pE WP	0.850	1.000				
pE PRP	0.824	0.980	1.000			
pE HYPO	0.726	0.908	0.935	1.000		
pE BRON	0.470	0.787	0.812	0.830	1.000	
pE THR	0.762	0.910	0.892	0.884	0.871	1.000

[a] See Experimental Section. [b] –log (effective concentration). [c] Aggregation.

determined that when R decreased from C_{20} to C_1, the lability of the acetyl group and also the phosphoryl group increased so much for R = C_4H_9 or CH_3 that we obtained a mixture of the two positional isomers which were separated by HPLC chromatography, at the last step. This fact was observed by Pluncktun and Dennis.[22] Since the last two compounds were highly water soluble, the yields were poor.

Biology and Discussion

All the homologues were tested (i) in vitro for their ability to aggregate washed rabbit platelets (WP) and platelet-rich plamsa (PRP), (ii) in vivo for bronchoconstriction and thrombocytopenic effects in guinea pigs, and (iii) for hypotensive activity in rats. The results are listed in Table IV as effective concentrations.

The qualitative structure–activity relationships[12-16] were thoroughly investigated (i) in order to show which moieties of the molecule are important to retain agonist activities and (ii) to separate platelet stimulation and bronchoconstriction from hypotensive activity.

Our aim here was not to prepare a new generation of antihypertensive drugs, but to study the influence of alkoxy-chain hydrophobicity (lipophilicity) in position 1 from C_1 to C_{20} as a function of platelet stimulation in vitro (on washed rabbit platelets and on platelet-rich plasma) and in some in vivo activities: bronchoconstriction, hypotension, and thrombocytopenia. The lipophilicity (hydrophobicity) was evaluated according to the hydrophobic fragmental constant (f) system, as described by Rekker and De Kort[25] (see Experimental Section).

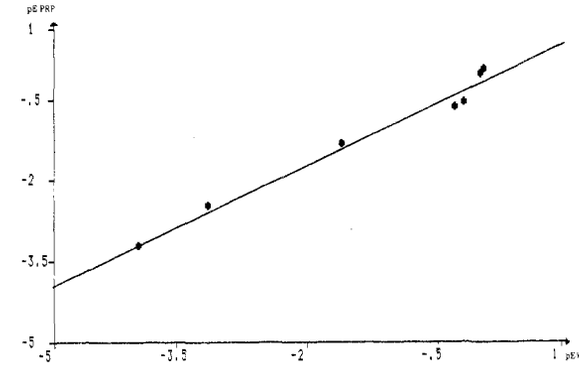


Figure 2. Relationship between pE PRP = –log (EC_{50} PRP) and pE WP = –log (EC_{50} WP) for compounds Ia–Ig. The regression equation is pE PRP = –0.156 (±0.195) + 0.773 (±0.098) pE WP. $n = 7$, $s = 0.199$, $r = 0.989$, $F(1,5) = 238.98$.

The first interesting result is that measurements performed in these series by two independent groups (Institut Pasteur and Roussel-Uclaf) on PRP and WP are closely correlated (Figure 2). Furthermore, all the agonist activities are also closely correlated among themselves and with the calculated ether oxide chain lipophilicity, as shown by the orthogonality matrix (Table V).

The data point corresponding to the Ih analogue was excluded because it was inactive at 10^{-4} M doses before lysis (cf. Table IV). These data indicate that there is no possibility of separating platelet stimulation and bronchoconstriction from hypotension by means of chain-length variation.

In addition, the close correlation among the platelet-stimulation measurements observed above made it possible to compare literature results on other PAF-acether ho-

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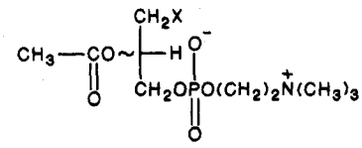
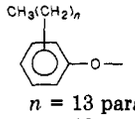
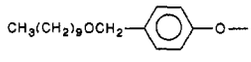
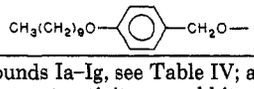
Table VI. Analogues of PAF-acether Used in Discussion, Calculated Hydrophobicity of the Chain in Position 1, and Relative Aggregant Activity



X	compd[a]	Σf[b]	RA[c]
$CH_3(CH_2)_nO$—			
n = 19	Ia	8.97	0.300
n = 17	Ib	7.73	1.000
n = 15	Ic	6.89	1.268
n = 11	Id	4.81	0.248
n = 9	Ie	3.78	0.048
n = 5	If	1.70	0.003
n = 3	Ig	0.66	0.001
			
n = 13 para	**2a**[d]	8.69	0.302
n = 13 meta	**2b**[d]	8.69	0.364
n = 15 ortho	**2c**[d]	9.73	0.002
$CH_3(CH_2)_nCH{=}CH(CH_2)_8O$—			
n = 5	**3a**[e]	6.44	1.600
n = 7	**3b**[e]	7.58	2.000
$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_8O$—	**3c**[e]	7.22	2.667
			
R = $CH_3(CH_2)_{14}$—	**4a**[f]	6.72	0.009
R = $CH_3(CH_2)_{16}$—	**4b**[f]	7.75	0.004
$CH_3(CH_2)_{17}S$—	**5a**[g]	9.01	0.000
$CH_3(CH_2)_n$—			
n = 15	**6a**[h]	8.49	0.005
n = 16	**6b**[i]	9.00	0.022
n = 18	**6c**[j]	10.04	0.000
$CH_3(CH_2)_{15}OCH_2$—	**7a**[k]	7.41	0.015
$CH_3O(CH_2)_2O(CH_2)_2O(CH_2)_8O$—	**8a**[l]	1.70	0.007
$CH_3(CH_2)_9O(CH_2)_2O(CH_2)_2O$—	**8b**[l]	3.81	0.280
	**9a**[m]	5.51	0.01–0.02[n]
	**9b**[m]	5.51	

[a] For compounds Ia–Ig, see Table IV; all are racemic mixtures except compounds **4a** and **4b** (*R* configuration). [b] See Experimental Section. [c] Relative aggregant activity on rabbit platelets except compounds **9a** and **9b**; see footnote *n* and the text. [d] From ref 14. [e] From ref 16. [f] From ref 12. [g] From ref 26. [h] From ref 14. [i] From ref 27. [j] From ref 13. [k] From ref 28. [l] From ref 29. [m] From ref 30. [n] Values estimated by the authors on human platelets.

mologues with ours (Ia to Ig). We expressed relative platelet-stimulating activities (RA) as follows:

$$RA = \frac{EC_{50}\ C_{18}\ PAF\ (R \text{ or } R,S)}{EC_{50}\ \text{analogue}\ (R \text{ or } R,S)}$$

both values taken from the same publication (cf. references in Table VI). Table IV shows that all the agonist activities increase with the lipophilicity. More specifically, the platelet aggregation in vitro in the rabbit is maximum for a C_{16}–C_{18} chain and decreases with the homologue in C_{20} Ia. Table VI presents all the homologues substituted in position 1 published to date[13,14,16,27-30] and for which the relative activity has been calculated as stated above, except for homologues **9a** and **9b**, for which the authors[30] have not mentioned the EC_{50}; in contrast they have provided estimated relative values on human platelets shown in Table VI.

By comparison of the relative activities, it can be observed that (i) no matter what their nature is, ether oxide chain homologues, i.e., alkylphenoxy (**2a** and **2b**), alkenoxy (**3a**, **3b**, and **3c**), or polyalkoxy (**8a** and **8b**) have an activity approximately equal to or greater than the corresponding *n*-alkoxy homologue (Ia–Ig) at the same hydrophobicity value of the chain, (ii) the elimination of oxygen (ether oxide function) and its replacement by a sulfur (thioether, **5a**), by a CH_2 (**6a**, **6b**, and **6c**), or by an ester function (**4a** and **4b**) dramatically decreases the platelet-aggregating activity, (iii) passage from a glycerol skeleton to a butane-1,2,4-triol skelton (**7a**) significantly decreases the activity, (iv) the steric-hindrance effect around the ether function can also decrease or cancel the biological activity (**2c**). Wissner et al.[28] have demonstrated that the steric hindrance on the glycerol skeleton itself canceled all activity.

Compounds **9a** and **9b** have a hydrophobicity that should bring their activity to the level of homologues with an *n*-alkoxy chain in C_{14}–C_{12} such as compound Id, for example. Despite comparison difficulties with all the other results (for discussion see above), it is possible to suppose

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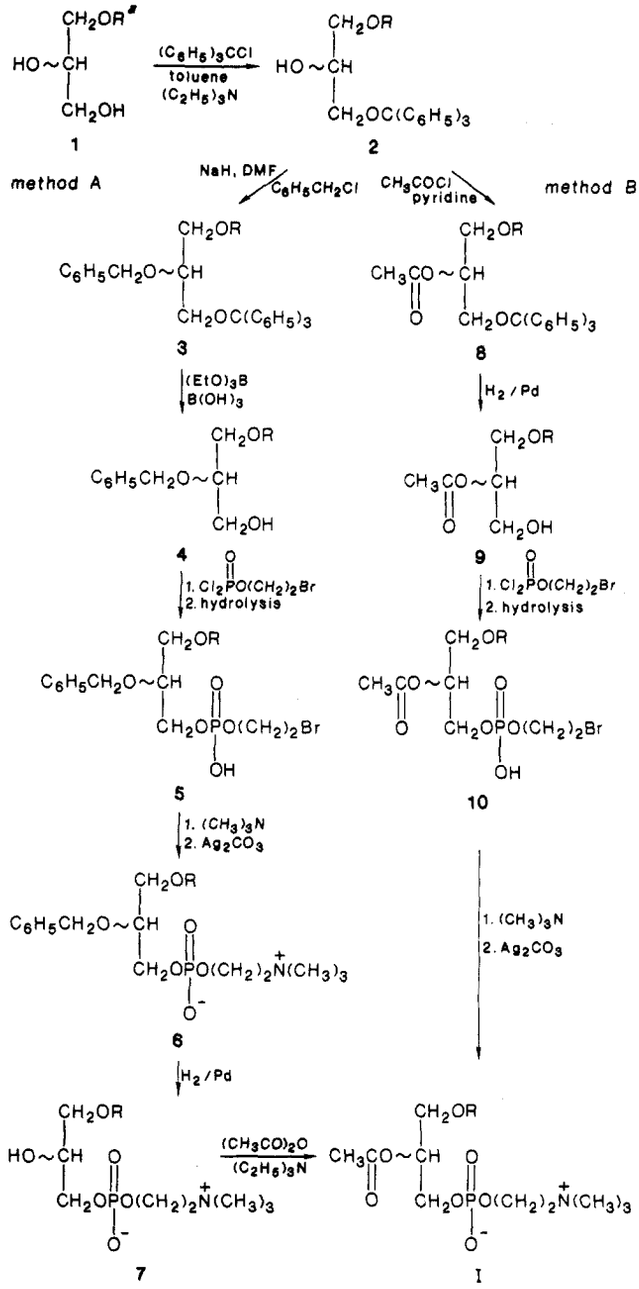
Scheme I



[a] For R, see Figure 1.

that modifications of an electronic nature have occurred and that there are configurational constraints that are perhaps more effective on human platelets.

A QSAR study is being performed and should allow analyzing these various factors better.

In conclusion, discussion remains open on the mode of PAF-acether action.[31] Its action may be not only direct on one (or more) proteinic receptor(s) but also indirect: for instance, it may modify local membrane fluidity. This is reminiscent of diacylglycerols which also stimulate protein kinase C activity.[32] The data presented here indicate a strong contribution of 1-ether oxide chain hydrophobicity to the activities of PAF-acether as well as the need for the 1-*O*-alkyl ether function for maximal agonist activities. Such a hypothesis must take account of possible steric and configurational constraints around this function.

Experimental Section

Materials and Methods. A. Chemistry. Solvents were all reagent grade. The purity of each product was checked by TLC on silica gel plates (Kieselgel 60 F_{254}, thickness 0.2 mm). Column chromatography was performed on silica gel (Merck, particle size 0.063–0.200 mm for normal chromatography, middle particle size 15 μm for flash chromatography), without any special treatment. HPLC was conducted on a Waters 201 U/6000 high-pressure liquid chromatograph equipped with a differential refractometer. Melting points were determined on a hot-stage microscope (Reichert Thermovar). The structure of all compounds was confirmed by IR (Pye-Unicam SP3-200 instrument) and ^1H NMR (Varian EM 360 or Brucker 250-MHz instrument) in $CDCl_3$ or CD_3OD with Me_4Si as an internal standard.[33] Chemical-ionization mass spectra were obtained at 220 °C on a modified AEI spectrometer.[34] Elemental analyses were consistent with the proposed structures (C, H, N, P).

In the synthesis, only modifications of the original methods[17-19] are described here. Physicochemical properties and spectral data are reviewed in Tables I–III.

1-*O*-Alkyl-2-*O*-tritylglycerol 2. Tritylation of 1-*O*-alkylglycerol 1 (25 mmol) was performed by Ph_3CCl (30 mmol) and Et_3N (60 mmol) in boiling toluene (75 mL) for 3 h. After the usual treatment,[18] the remaining oil was dissolved in petroleum ether, allowing insoluble triphenylmethanol to crystallize. After filtration, flash chromatography using 5–15% ether in petroleum ether yielded pure **2** (80%).

Method A. 1-*O*-Alkyl-2-benzylglycerol 4. Compound **2** was reacted with NaH and benzyl chloride in DMF to give **3**.[18] Compound **4** was obtained by detritylation of **3** (8 mmol) with boiling mixture of $B(OH)_3$ (80 mmol) and $(EtO)_3B$ (10 mL) and purified by flash chromatography using petroleum ether/ether (80:20, v/v) as eluent (yield 75%).

1-*O*-Alkyl-2-*O*-benzylglycero-3-phospho-2′-bromoethanol 5. To a solution of **4** (7.6 mmol) and Et_3N (13 mmol) in 15 mL of dry diethyl ether was added dropwise 13 mmol of (2-bromoethyl)phosphoryl dichloride in 10 mL of dry ether. The mixture was stirred at room temperature for 5 h. Et_3N (2.5 mL) and 1 mL of H_2O were added, and the mixture was refluxed for 2 h with stirring and then evaporated. The residue was extracted with ether and washed with water. After elimination of the solvent, the crude product was purified by normal column chromatography using $CHCl_3$/MeOH (95:5, 90:10, then 85:15, v/v) as eluent (yield 70%).

1-*O*-Alkyl-2-*O*-benzylglycero-3-phosphocholine 6. An excess of Me_3N gas was dissolved in an ice-bath-cooled solution of **5** (0.44 mmol) in a mixture of $CHCl_3$/2-propanol/DMF (40mL) (3:5:5, v/v/v). The solution was stirred and heated (50 °C) by using an oil bath until **5** disappeared, as indicated by TLC (12 h). After cooling, Ag_2CO_3 (0.4 mmol) was added and the mixture refluxed for 1 h. The cooled grey solution was filtered through paper and the paper washed with $CHCl_3$ and MeOH. After evaporation, the crude product was purified by normal column chromatography using $CHCl_3$/MeOH (70:30 to 30:70, v/v) as eluent.

1-*O*-Alkylglycero-3-phosphocholine 7. Compound **7** was prepared by hydrogenolysis of the benzyl group of **6**. A mixture of **6** (4.6 mmol) and Pd/C (10%) in absolute EtOH (40 mL) was hydrogenated with stirring at 40 °C for 12 h (20 psi). After filtration on paper, the catalyst was washed several times with $CHCl_3$ and MeOH. Evaporation of the solvents gave the "lyso" lipid **7** (99%).

1-*O*-Alkyl-2-*O*-acetylglycero-3-phosphocholine I. Compound **7** (0.36 mmol) was acetylated by using freshly distilled $(CH_3CO)_2O$ (0.12 mmol) and Et_3N (0.16 mmol) in $CHCl_3$ (10 mL). The mixture was refluxed for 24 h with stirring. After cooling, the solvent and excess Et_3N and $(CH_3CO)_2O$ were eliminated. The

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(33) Laboratoire de Chimie Organique Structurale, Université P. et M. Curie, 75230 Paris Cedex 05, France.
(34) Institut de Chimie des Substances Naturelles, CNRS, 91190 Gif-sur-Yvette, France.

residue was dissolved in $CHCl_3$, filtered on Millipore apparatus, and purified by HPLC, using a μ-Porasil column (diameter 7.8 mm, length 30 cm) and CH_2Cl_2/MeOH/H_2O (60:50:5, v/v/v) as eluent (for retention times, see Table III).

Method B. 1-*O*-Alkyl-2-*O*-acetylglycerol 9. A mixture of **8** (15.2 mmol), obtained by acetylation of **2**, $CaCO_3$ (2 g), and Pd/C (10%) in 60 mL of $CHCl_3$ was hydrogenated with stirring at 40 °C for 18 h (20 psi). After filtration and evaporation, the residue including **9** and triphenylmethane was used without purification in the next step to avoid acetyl migration.

1-*O*-Alkyl-2-*O*-acetylglycero-3-phosphocholine I. Compound **9** was converted into the corresponding I via reaction of Me_3N with the alkylacetylglycerophosphobromoethanol **10** in the same way as described for **6** in method A.

B. Biological Methods. For all experiments, PAF-acether or analogues were dissolved in ethanol at 10 mg/mL and diluted in saline. Activities (EC_{50}, EC_{30}) were calculated from the dose–response curve by a polynomial adjustment method followed by a linear least squares regression analysis (GLM procedure from SAS (Statistical Analysis System)).

Platelet Aggregation in Washed Rabbit Platelets (WP). The platelet-aggregating activity of PAF-acether and its analogues was performed on washed rabbit platelets as described previously.[35] Aggregation was measured by using a concentration of 1.5×10^8 platelets treated by 0.1 mM lysine (acetyl salicylate) from Egic-Joullie (Aspegic) and stirred in 300 μL of Tyrode's gelatin[36] in the presence of the complex CP/CPK (1 mM, 10 units/mL). Activity was expressed as the final concentration of each compound required for 50% of the maximum aggregation induced by a standard solution of synthetic PAF-acether (concentration: 2×10^{-10} M). Results shown in Table IV are the mean of three independent experiments.

Platelet Aggregation in Platelet-Rich Plasma (PRP). Platelet-aggregation studies were performed by the method of Born and Croos.[37] Blood was collected in 3.2% sodium citrate (1 mL for 9 mL of blood) by cardiac puncture from conscious male New Zealand rabbits (≃3 kg). Platelet-rich plasma was obtained by centrifuging blood at 300*g* for 10 min at room temperature. The platelet concentration was adjusted to 300 000 μL^{-1} with platelet-poor plasma. Platelet aggregation was monitored by continuous recording of light transmission in a dual-channel recorder. Various concentrations of PAF-acether or its analogues (40μL) were added to 0.4 mL of incubated and stirred PRP.

Aggregation induced by analogues was compared to that obtained with PAF-acether with produced 100% aggregation at 5 to 8×10^{-8} M. The concentrations of drugs required to produce 50% aggregation (EC_{50}) were calculated.

Rat Hypotension. Male albino rats (Sprague–Dawley) weighing 280–300 g were anesthetized with sodium pentobarbital (50 mg/kg) by intraperitoneal injection. PAF-acether and its analogues were injected via a cannulated jugular vein, and arterial pressure was registered from a cannulated artery by means of a Statham P 23 ID pressive transducer. Drugs were injected at three cumulative doses at 20-min intervals, to obtain a lowering or arterial diastolic blood pressure (BP) to 40% of initial value. Hypotensive activity was maximal 1 min after administration of compounds, and the doses required to lower BP by 30% (EC_{30}) were calculated.

Bronchoconstriction in the Guinea Pig. Male Hartley guinea pigs (450–500 g) were anesthetized with ethyl carbamate (1.25 g/kg ip). The jugular vein was then cannulated for administration of agonists, the carotid artery for blood sampling, and the trachea for artificial ventilation. Respiration was arrested with pancuronium bromide (2 mg/kg), and the animal was respirated by means of a Harvard pump (50 stokes/min, 10 mL/kg). Resistance to lung inflation was measured by a modification of the Konzett–Rossler overflow technique.[38] The resistance to lung inflation (in cm H_2O) was measured 1 min after drug administration. The dose that induced a bronchoconstriction of 3 cm H_2O was calculated (five animals/dose). This effect was obtained with 0.18 nM PAF-acether. Each animal received only one dose of test compound since guinea pigs show tachyphylaxis to PAF-acether. One minute before and after injection, a blood sample was taken for platelet counting. The doses inducing 50% thrombocytopenia were calculated (EC_{50}).

C. Lipophilicity. Lipophilicity values of the glycerol position 1 moieties were calculated according to the hydrophobic fragmental constant system. For compounds Ia–Ih: $\Sigma f = f$(aliphatic O) + $nf(CH_2)$ + $f(CH_3)$, where n is the number of methylene groups in the chain. For **2a–2c**, **9a**, and **9b**: $\Sigma f = f(C_6H_4)$ + f(aromatic O) + f(alkyl chain). For **3a–3c**: $\Sigma f = f$(aliphatic O) + $nf(CH_2)$ + $f(CH_3)$ + $n'f$(CH=CH), where n and n' are the frequency of each kind of fragment. For **4a** and **4b**: $\Sigma f = f$(aliphatic O-CO) + f(alkyl chain). For **5a**: $\Sigma f = f$(aliphatic S) + f(alkyl chain). For **6a–6c** and **8a** the calculations were the same as described above. For compounds **9a** and **9b** a correction of proximity was performed. Computer-assisted multiple regression analyses were performed, which yielded the regression equations together with statistical parameters, adjusted for the degrees of freedom. Regression coefficients are given in equations with their standard errors.

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Registry No. **1a**, 106759-46-0; **1b**, 10438-95-6; **1c**, 6145-69-3; **1d**, 100165-14-8; **1e**, 92219-12-0; **1f**, 106972-45-6; **1g**, 98451-40-2; **1h**, 36887-04-4; **2a**, 106689-11-6; **2b**, 86334-56-7; **2c**, 82002-20-8; **2d**, 86483-56-9; **2e**, 106881-89-4; **2f**, 106881-90-7; **2g**, 106881-91-8; **2h**, 106972-46-7; **3b**, 106972-47-8; **3c**, 82002-21-9; **3d**, 106881-92-9; **3f**, 106881-93-0; **3g**, 106881-94-1; **3h**, 106881-95-2; **4b**, 89104-47-2; **4c**, 18678-94-9; **4d**, 106972-48-9; **4f**, 92948-22-6; **4g**, 106881-96-3; **4h**, 106972-49-0; **5b**, 106972-50-3; **5c**, 106972-51-4; **5d**, 106881-97-4; **5f**, 106881-98-5; **5g**, 106881-99-6; **5h**, 106905-65-1; **6b**, 43043-61-4; **6c**, 18678-98-3; **6d**, 70641-54-2; **6f**, 106882-00-2; **6g**, 106882-01-3; **6h**, 106882-02-4; **7b**, 72490-82-5; **7c**, 17364-21-5; **7d**, 70641-52-0; **7f**, 106882-03-5; **7g**, 106882-04-6; **7h**, 106882-05-7; **8a**, 106882-06-8; **8b**, 89496-74-2; **8c**, 106882-07-9; **10a**, 106882-08-0; **10b**, 106972-52-5; **10c**, 106882-09-1; Ia, 106972-53-6; Ib, 85026-88-6; Ic, 77286-68-1; Id, 91423-78-8; Ie, 106882-10-4; If, 106882-11-5; Ig, 106882-12-6; Ih, 106882-13-7; $(C_6H_5)_3CCl$, 76-83-5; $Cl_2P(=O)O(CH_2)_2Br$, 4167-02-6.

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