Biological Testing Methods. Inhibition of Phenylquinone-Induced Writhing in Mice (PQW). The procedure used in our laboratories is a modification of the method of Siegmund et al.[6] A 0.125% concentration of phenyl-*p*-benzoquinone in a 5% aqueous solution of ethyl alcohol is administered to mice (10 mL/kg, ip). This produces a characteristic "writhe" which is defined as an inward rotation of one or more feet with twisting and turning of the trunk, drawing in of the abdominal wall, lordosis, and arching of the back. A total of 28 male CD-1 Charles River mice (18–30 g) are employed for a time–response. Animals receive food and water ad libitum during their stay in the animal quarters prior to testing. Compounds are tested at 10 mg/kg, sc and are prepared with distilled water, and if insoluble one drop of Tween-80, a surfactant, is added. Compounds are administered in a dosage volume of 10 mL/kg.

Twenty mice (five per group) are administered the test compound at various pretreat times (e.g., 15, 30, 45, and 60 min) prior to phenylquinone injection. Control animals (two per group) receive an equal volume of vehicle. After the administration of phenylquinone, the mice are placed separately into 1-L beakers, and 5 min are allowed to elapse. The mice are then observed for a period of 10 min, and the number of writhes is recorded for each animal. The formula for computing percent inhibition is

$$\frac{(\bar{X}\text{ writhes in control group}) - (\bar{X}\text{ writhes in drug group})}{\bar{X}\text{ writhes in control group}} \times 100\%$$

The time period with the maximum percent of inhibition is considered the peak time. A dose–response is reserved for interesting compounds or those which inhibit writhing by 70% or more. A dose–response is run in the same manner as a time–response except 10 animals per group are tested at the peak time of drug activity. Fifty animals, divided among four drug groups and one vehicle control group, are employed. The mice are normally given four doses of drug, each twice the amount of the preceding dose. An ED_{50} is calculated by a computer linear regression analysis. Compounds were tested in the physical form listed in the tables; i.e., a compound described as a hydrochloride salt was tested as that salt and not as the free base, etc.

The procedures used to determine other biological activities have been described previously in the following references: D'Armour–Smith tail-flick assay,[9] rotorod assay,[11] inhibition of carrageenin-induced rat paw edema,[13] in vitro opiate receptor binding assay.[14]

Acknowledgment. We thank Marc Agnew, Peter Kranak, and John Landis for determining the IR and NMR spectra, and Minh Ma, Janis Little, Karen McCormack, Susan Johnson, and Kenneth Malone for conducting the pharmacological tests, and Wayne Petko for the opiate receptor assay. We also acknowledge Duane Voss for library assistance and Nancy Kurtz for typing the manuscript.

Registry No. **1a**, 92083-16-4; **1b**, 92083-22-2; **1c**, 92083-19-7; **1d**, 92083-25-5; **1e**, 92083-41-5; **1f**, 25699-91-6; **1g**, 92083-31-3; **1h**, 92083-39-1; **2a**, 92083-17-5; **2a**·HCl, 92083-18-6; **2b**, 92083-23-3; **2b**·HCl, 92083-24-4; **2c**, 92083-20-0; **2c**·HCl, 92083-21-1; **2d**, 92083-26-6; **2d**·HCl, 92083-27-7; **2e**, 92083-42-6; **2e**·HCl, 92083-43-7; **2f**, 92083-34-6; **2f**·HCl, 92083-35-7; **2g**, 92083-32-4; **2g**·HCl, 92083-33-5; **2h**, 92083-40-4; **2h**·HCl, 92205-28-2; **3a**, 92083-45-9; **3b**, 92083-44-8; **3c**, 92083-36-8; **3d**, 92083-50-6; **4a**, 92083-48-2; **4a**·HCl, 92083-49-3; **4b**, 92083-46-0; **4b**·HCl, 92083-47-1; **4c**, 92083-37-9; **4c**·HCl, 92083-38-0; **4d**, 92083-51-7; **4d**·HCl, 92083-52-8; **5a**, 92083-68-6; **5a**·HCl, 92083-69-7; **5b**, 92083-70-0; **5b**·HCl, 92083-71-1; **6a**, 92083-61-9; **6b**, 92083-62-0; **6c**, 93040-84-7; **6d**, 92083-64-2; **6e**, 92083-65-3; **6f**, 92083-66-4; **6f**·$Y_2C_4H_4O_4$, 92083-67-5; **7a**, 92083-28-8; **7b**, 92083-53-9; **7c**, 92083-72-2; **8a**, 92083-29-9; **8a**·2HCl, 92083-30-2; **8b**, 92083-54-0; **8b**·$C_4H_4O_4$, 92083-55-1; **8c**, 92083-73-3; **8c**·2HCl, 92083-74-4; 2-FC_6H_4CN, 394-47-8; 3-FC_6H_4CN, 403-54-3; 4-FC_6H_4CN, 1194-02-1; $C_6H_5CH_2COCl$, 103-80-0; EtOCOCl, 541-41-3; CH_3Cl, 74-87-3; C_6H_5Br, 108-86-1; 3-$BrCH_2C_6H_4CN$, 28188-41-2; indole, 120-72-9; indoline, 496-15-1; 5-chloroindole, 17422-32-1; 5-chloroindoline, 25658-80-4; 5-methoxyindole, 1006-94-6; cyclopropanecarbonyl chloride, 4023-34-1.

Facile Synthesis of Platelet-Activating Factor and Racemic Analogues Containing Unsaturation in the *sn*-1-Alkyl Chain

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Platelet-activating factor, 1 (PAF, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine), and octadecyl-PAF were synthesized chemically as the racemates. The *sn*-1-*O*-alkyl isomers were isolated after treatment of the racemates with phospholipase A_2 and subsequent reacetylation of the 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholines released. Analogues of PAF containing unsaturated alkyl moieties at the *sn*-1 position (**2**, **4**, **5**) were synthesized by utilizing the methoxyethoxymethyl protecting group as a novel method for preparing unsaturated alkyl lipids. This procedure provides a facile means for preparing unsaturated ether phospholipids of defined structure that may be tritiated to high radiospecific activity for metabolic studies. Unsaturation in the alkyl chain had minimal effect on the bioactivities examined in this study.

The discovery of the biological response initiated by platelet-activating factor (PAF)[1] dates back to 1966 when Barbaro and Zvaifler reported that a mixture of rabbit platelets and stimulated leukocytes released histamine on specific antigen challenge.[2a] Hensen later described this phenomenon as a nonlytic, complement-independent,

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(1) Abbreviations used: PAF = platelet-activating factor, MEM = methoxyethoxymethyl, TLC = thin-layer chromatography, DMAP = (*N*,*N*-dimethylamino)pyridine, PMN = polymorphonuclear neutrophils, NMR = nuclear magnetic resonance, FMLP = *N*-formylmethionylleucylphenylalanine, BSA = bovine serum albumin, GC = gas chromatography, THF = tetrahydrofuran, IR = infrared, EDTA = ethylenediaminetetraacetic acid.

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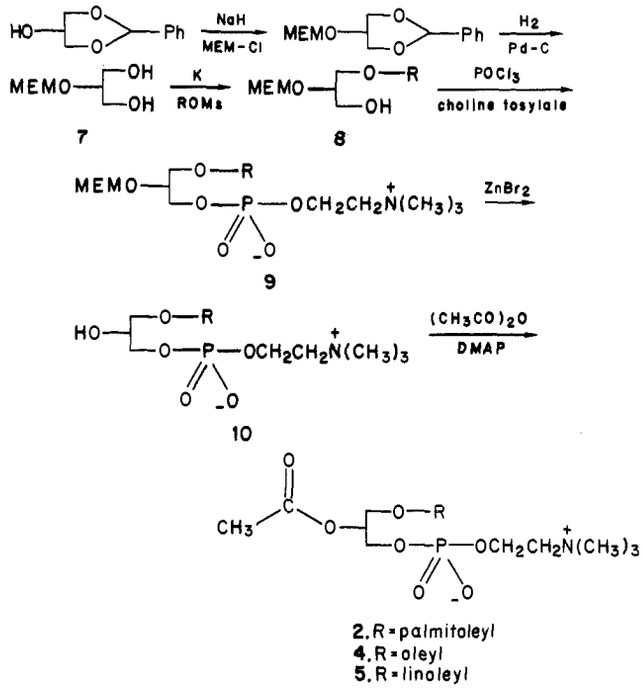
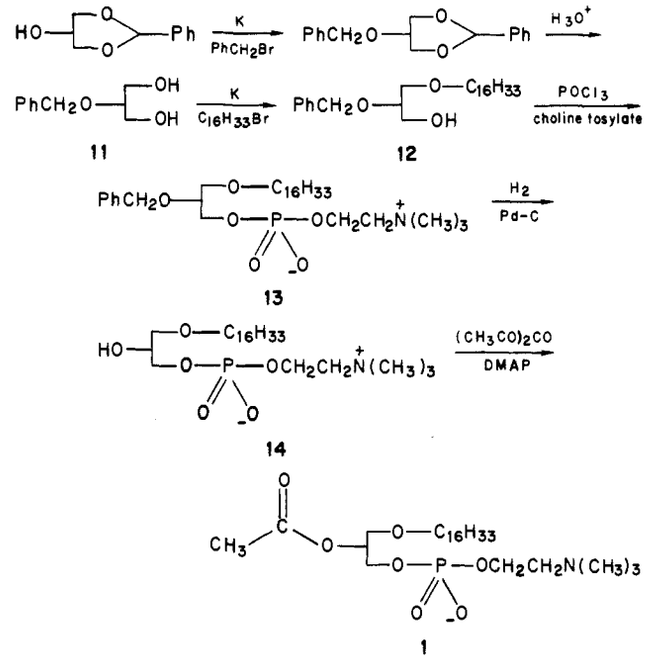
Scheme I



Scheme II



leukocyte-dependent release of a soluble factor from the sensitized leukocyte that reacted with platelets, resulting in the release of vasoactive amines.[2b] Benveniste et al. determined that the antibody involved was of the IgE type, described the methodology for isolation of the soluble factor, and named it platelet-activating factor.[3] The correct chemical structure of PAF as 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine was published independently by two separate groups in 1979.[4,5] The PAF isolated from stimulated leukocytes consisted of a mixture of *sn*-1-alkyl chains (16:0 <10% and 18:0 >90%).[6] A semisynthetic PAF was synthesized from choline plasmalogens isolated from beef heart[4,7] (preparative TLC). These were saturated by hydrogenation over PtO_2 and the 1-*O*-alkyl-*sn*-glycero-3-phosphocholines isolated after base-catalyzed methanolysis. The lyso lipid was acetylated to give a mixture of PAFs whose alkyl chains were 68–78% 16:0 and 22–27% 18:0.[4,5] In addition to its respiratory and circulatory effects,[8] PAF was found to possess potent antihypertensive properties.[7] Several review articles on PAF have been published.[9–12]

Naturally occurring octadecyl-PAF has been prepared chemically from D-mannitol in 10 steps,[13] but the procedure had the disadvantage of acetyl migration from the *sn*-2 position. A later paper described the chemical synthesis of racemic octadecyl-PAF in nine steps from isopropylideneglycerol and the *sn*-1-*O*-alkyl isomer in 15 steps from D-mannitol.[14] Semisynthetic PAF has been prepared from beef heart choline plasmalogens[4,7] in five steps but yielded a mixture of hexadecyl- and octadecyl-PAF. A stereoselective synthesis of both enantiomers of PAF from enantiomeric dimethyl tartrates in 11 steps[15] and of the *sn*-3-alkyl isomer[16] of PAF has been reported. Since the semisynthetic and some chemical procedures described above employed hydrogenation, only saturated PAF was obtained. We report the synthesis of racemic hexadecyl- and octadecyl-PAF in six steps from *rac*-1,3-benzylideneglycerol. The *sn*-1-*O*-alkyl isomer can be obtained in two subsequent steps. Unsaturated *rac*-PAF analogues were synthesized to determine what effect unsaturation in the *sn*-1-alkyl chain would have on its biological activity. The unsaturated analogues were synthesized by utilizing the MEM protecting group in place of the benzyl group at what eventually becomes the *sn*-2 carbon. This novel method allows the facile introduction of unsaturated moieties into phospholipid molecules.

Chemistry. The racemic unsaturated analogues of PAF, compounds **2**, **4**, **5** containing palmitoleyl, oleyl, and linoleyl alkyl groups, were prepared as outlined in Scheme I. This scheme offered two advantages over those previously published: (1) the use of the methoxyethoxymethyl (MEM) protecting group allowed the introduction of unprotected unsaturated and polyunsaturated alkyl groups

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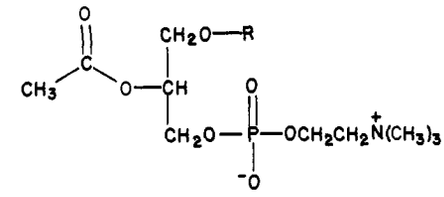
into what eventually became the *sn*-1 position and (2) the *sn*-1-*O*-alkyl isomers isolated from the racemic mixture in two steps by treatment of the racemate with phospholipase A_2, isolation of the *sn*-1-*O*-alkyl lyso lipid by preparative TLC, and reacetylation of the *sn*-2-hydroxyl group. In testing PAF analogues for antihypertensive properties and effects on platelets and neutrophils, one can use the racemic mixture since it has been shown[17] that the *sn*-3-*O*-alkyl isomer was inactive and merely diluted the effect of the *sn*-1-*O*-alkyl isomer. The synthesis of unsaturated PAF analogues began with 1,3-benzylideneglycerol, which was treated with sodium hydride in THF followed by MEM-Cl. The benzylidene group was removed by hydrogenolysis with 5% palladium on charcoal and the resulting 2-MEM-glycerol was then purified by column chromatography. Diol **7** was reacted with potassium metal and the appropriate unsaturated mesylate to give a mixture of products under conditions in which monoalkylation predominated. Diether **8** was isolated by column chromatography and treated with $POCl_3$ and choline tosylate to form the corresponding phosphocholines (**9**). The MEM protecting group was removed by stirring with anhydrous $ZnBr_2$ in CH_2Cl_2. The racemic compounds **2**, **4**, **5** were then prepared by treating the lyso compounds (**10**) with acetic anhydride and (*N*,*N*-dimethylamino)pyridine (DMAP).

A similar procedure was used to prepare saturated PAFs (Scheme II). The potassium salt of 1,3-benzylideneglycerol was reacted with benzyl chloride in benzene and the benzylidene group removed by acid hydrolysis to give 2-*O*-benzylglycerol, compound **11**. This was treated with potassium metal and hexadecyl bromide as previously mentioned to give a mixture in which diether **12** predominated. The phosphocholine moiety was added, the benzyl group removed by hydrogenolysis, and the lyso-PAF acetylated to give *rac*-hexadecyl-PAF (compound **1**). Treatment of the racemate with phospholipase A_2 yielded the *sn*-1-*O*-alkyllyso-PAF, which was subsequently reacetylated to the pure biologically active enantiomer of PAF. Octadecyl-PAF (**3**) was prepared by the same procedure.

The strategies for synthesizing saturated and unsaturated PAF analogues were almost identical. The use of the MEM protecting group instead of the benzyl protecting group is novel in lipid chemistry. The mild conditions employed for introduction and removal of the MEM group at the *sn*-2-hydroxyl allowed the introduction of unprotected alkyl moieties into the lipid molecule, thus simplifying the task of the lipid chemist. Upon acetylation of the lyso lipid, the *sn*-1-*O*-alkyl and *sn*-3-*O*-alkyl isomers can be elaborated from the racemic mixture by the well-known action of phospholipase A_2, which is stereospecific for the *sn*-1-*O*-alkyl isomer.[17]

All final products gave satisfactory elemental analyses (C, H, N, P) as well as ^1H NMR spectra (CD_3OD or $CDCl_3$; see Table III). Mass spectra of the products gave the predicted (M + 1) ions having the proper (M + 2) and (M + 3) ion intensities for the proposed structures: compounds **1**, *m/z* 524 (M + 1); compound **2**, *m/z* 522 (M + 1); compound **3**, *m/z* 552 (M + 1); compound **4**, *m/z* 550 (M + 1); compound **5**, *m/z* 548 (M + 1). A more complete mass spectral analysis of these products will appear elsewhere.

Biological Testing. The procedure for isolation and labeling of rabbit platelets is described elsewhere.[4] Briefly,



1, R = hexadecyl
2, R = *cis*-9-hexadecenyl
3, R = octadecyl
4, R = *cis*-9-octadecenyl
5, R = *cis*,*cis*-9,12-octadecadienyl

Figure 1. Structures of PAF and analogues.

for degranulation, platelet-rich plasma was incubated with 1.0 μCi/mL of [^{14}C]serotonin at 37 °C for 15 min, twice washed as described, suspended at 5×10^7 platelets/0.2 mL in citrate-free Tyrode's buffer (pH 7.3) containing 1 mM Ca^{2+}, incubated at 37 °C for 20 min, and then challenged. After 1 min the suspensions were treated with 20 μL of 6% formalin, placed on ice, and centrifuged (12000*g* × 0.5 min) to obtain supernatant fluid, which was assayed for radiolabel.[18] Results were recorded as the percentage of total cellular label released.

The procedures for human neutrophil isolation and bioassay are described elsewhere.[19] Briefly, for PMN degranulation, cells (>96% PMN, fewer than 5 platelets/100 PMNs, and no erythrocytes) were suspended in Hanks' buffer (37 °C) at 2.6×10^6 cells/mL for 20 min, treated with cytochalasin B (5 μg/mL) for 2–4 min, and challenged for 5 min. The suspension was then placed on ice and centrifuged (200*g* × 4 min; 4 °C) to obtain supernatant fluid, which was assayed for granule-bound (lysozyme and β-glucuronidase) and cytosolic (lactic acid dehydrogenase) enzymes. To study desensitization of this response, cells were preincubated with an indicated stimulus for 8 min before exposure to cytochalasin B. Cytochalasin B is necessary for the degranulating actions of all the stimuli studied here and when added 8 min after cell stimulation supports minimal enzyme release. However, these pretreated cells are fully capable of responding to a fresh stimulus provided the second stimulus does not utilize the exact same mechanism as the first.[17,18] All results are reported as net enzyme release, which is the percentage of total cellular enzyme released by stimulated cells minus the release found for unstimulated but otherwise identically handled cells. None of the stimuli or conditions used here cause net release of lactic acid dehydrogenase. This indicates that the reported release of granule-bound enzymes is not due to cell lysis.

Results and Discussion

The compounds **1**–**5** (Figure 1) were analyzed by 250-MHz ^1H NMR spectroscopy (Table III). Compounds **1** and **3** gave spectra identical in all respects with that published previously[14] for octadecyl-PAF. The spectra of the unsaturated analogues (**2**, **4**, **5**) differed from that of the saturated PAF compounds only in their cis double bond protons and those adjacent to the double bonds. The multiplicity of the absorption signal for the double bonded protons (a doublet of triplets for the monounsaturated analogues and an octet for the diunsaturated analogue) indicated a stronger coupling with the adjacent methylene protons than with each other. Compound **2** is currently

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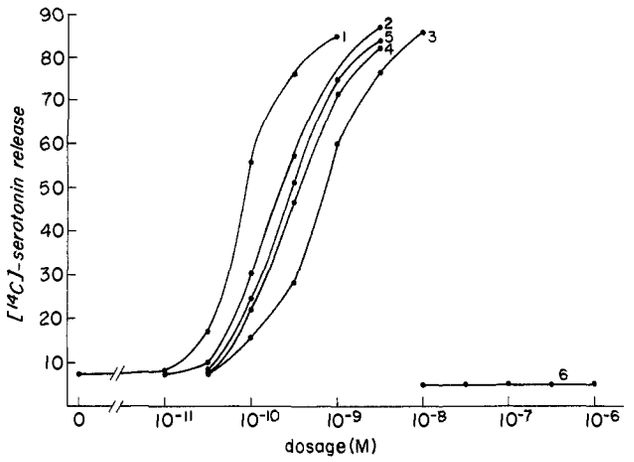

Figure 2. Platelet release of [^{14}C]serotonin induced by various analogues of PAF.

Table I. Relative Potencies of *rac*-PAF Analogues in Stimulating Rabbit Platelet Release of [^{14}C]Serotonin and Human PMN Release of Lysozyme[a]

	response[b]	
analogue	ED_{50}: platelet serotonin release, Molar	ED_{10}: PMN lysozyme release, Molar
1 [16:0]	2×10^{-10}	8×10^{-9}
2 [16:1]	5×10^{-10}	1×10^{-8}
3 [18:0]	8×10^{-10}	6×10^{-8}
4 [18:1]	4×10^{-10}	1.3×10^{-8}
5 [18:2]	3×10^{-10}	9×10^{-9}
6 [18:1]-lyso	$>10^{-6}$	$>10^{-6}$

[a] Dose–response curves for each analogue were generated as in Figure 2. Values causing 50% of total cellular [^{14}C]serotonin or 10% net release of lysozyme were extrapolated on curves averaged from at least five dose–response experiments. [b] The correlation coefficient for the two responses was 0.94 ($p < 0.001$).

being tritiated to high radiospecific activity for our metabolic studies.

The five compounds were tested for their ability to affect platelets and neutrophils by measuring [^{14}C]serotonin release from prelabeled rabbit platelets and lysozomal enzyme release from human PMN (Table I). Figure 2 gives dose–response curves for the rabbit platelet-activating actions (i.e., [^{14}C]serotonin release) of the saturated hexadecyl- and octadecyl-PAFs (**1** and **3**) and their unsaturated analogues (**2**, **4**, **5**). Five of the analogues tested were effective at nanomolar and lower dosages, but compound **6**, the 2-lyso analogue of **4**, was inactive at all dosages. This is in agreement with previous reports which demonstrated that substitution at the *sn*-2 position was essential for bioactivity.[4,17,20] The five compounds, which varied the moiety at the *sn*-1 position, were approximately equipotent. It was clear, however, that *sn*-1-*O*-hexadecyl-PAF (**1**) was slightly but significantly more active than the *sn*-1-*O*-octadecyl-PAF and that increasing unsaturation tended to increase the potency in the octadecyl series (**3–5**) but not the hexadecyl series (**1**, **2**). A similar potency profile was found for these compounds in stimulating human PMN release of lysozyme (Table I) and β-glucuronidase (data not shown) as well as hypotensive responses in SHR rats (data not shown). The potencies of these compounds in each in vitro assay (Table I) correlated highly (correlation coefficients = 0.94).

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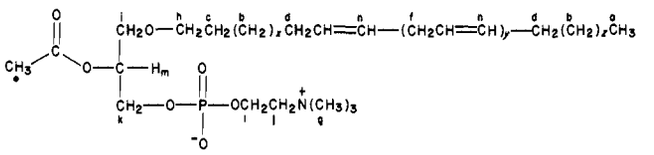
Table II. Selective Desensitizing Actions of *rac*-PAF Analogues on the Human PMN Degranulation Response[a]

desensitizing agent (dosage, nM)	degranulating agent[d]	
	PAF[b] (200 nM)	FMLP (17 nM)
1 [16:0] (8)	−0.2 ± 0.6*[c]	23.6 ± 2.0**
2 [16:1] (10)	1.4 ± 0.9*	29.5 ± 3.1**
3 [18:0] (60)	−1.2 ± 0.7*	22.0 ± 2.5
4 [18:1] (10)	0.3 ± 0.6*	28.2 ± 3.9**
5 [18:2] (9)	0.1 ± 0.5*	26.3 ± 2.5**
6 [18:1]-lyso (1000)	9.6 ± 1.8	15.4 ± 2.3
FMLP (17)	11.9 ± 1.4	6.2 ± 1.4*
BSA	12.0 ± 1.4	17.0 ± 2.1

[a] PMNs were incubated with the indicated dosage of desensitizing agent (in BSA) or BSA alone for 8 min, treated with 5 μg/mL of cytochalasin B for an additional 2–4 min, and challenged with 200 mM PAF or 17 mM FMLP for 5 min. [b] PAF used as the degranulating agent was 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, whereas compounds **1–5** are racemic mixtures. [c] Net β-glucuronidase release, ±SEM, for 14 experiments. Assays of lysozyme release gave similar results. [d](*) Indicates values significantly lower ($p < 0.05$, Student's unpaired *t*-test) than those found for cells pretreated with BSA alone. (**) Indicates values significantly greater ($p < 0.05$, Student's unpaired *t*-test) than those found for cells pretreated with BSA alone.

Table III. 250-MHz NMR Spectral Data for Compounds **2**, **4**, **5**



2, $x = 5$, $y = 0$, $z = 4$ (CD_3OD)
4, $x = 5$, $y = 0$ $z = 6$ (CD_3OD)
5, $x = 5$, $y = 1$, $z = 3$ ($CDCl_3$)

assignment	mult[a]	**2**, δ (ppm) [no. H]	**4**, δ (ppm) [no. H]	**5**, δ (ppm) [no. H]
a	t	0.90 [3]	0.90 [3]	0.89 [3]
b	m	1.29 [18]	1.33 [22]	1.29 [16]
c	m	1.53 [2]	1.56 [2]	1.53 [2]
d	m	2.03 [4]	2.04 [4]	2.06 [4]
e	s	2.10 [3]	2.06 [3]	2.11 [3]
f	t			2.79 [2]
g	s	3.36 [9]	3.16 [9]	3.32 [9]
h	m	3.42 [2]	3.37 [2]	3.42 [2]
i	d	3.57 [2]	3.50 [2]	3.59 [2]
j	m	3.79 [2]	3.54 [2]	3.74 [2]
k	m	3.95 [2]	3.88 [2]	3.95 [2]
l	m	4.29 [2]	4.14 [2]	4.29 [2]
m	p	5.17 [1]	4.97 [1]	5.17 [1]
n	m	5.39 [2]	5.17 [2]	5.40 [4]

[a] Singlet, s; doublet, d; multiplet, m; pentet, p.

The data in Table II demonstrated that each compound desensitized human PMNs to PAF but not an oligopeptide stimulus, *N*-formylmethionylleucylphenylalanine (FMLP). These data indicated that each analogue activated PMNs by a similar mechanism which was quite distinct from that used by FMLP and therefore were most probably biological as well as structural analogues.

Experimental Section

All chemicals were used as provided by the supplier without further purification unless otherwise indicated. All melting points were obtained on a Hoover Meltemp apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer PE 297 instrument, proton nuclear magnetic resonance spectra were obtained on either a JEOLCO 60-MHz or a Bruker 250-MHz spectrometer, and mass spectra were obtained on a Ribermag R10-10 quadrupole mass analyzer interfaced to a PDP-8A minicomputer. Samples were introduced by the desorption chemical ionization technique using ammonia as the chemical ionization gas. Elemental analyses of final products were performed by either Atlantic Microlabs, Inc. or Galbraith Laboratories, Inc. TLC was

performed with Baker-flex IB-F flexible silica gel plates, developed in lined tanks and stained with iodine vapors. Gas chromatograms were run on a Shimadzu GC-8A instrument equipped with a 6-ft glass column (2 mm i.d.) packed with 2% OV-17 on Supelcoport with helium as the carrier gas (30 mL/min).

Unsaturated Mesylates. The procedure of Baumann and Mangold[21] was followed. To a three-neck round-bottom flask equipped with a nitrogen inlet, mechanical stirrer, liquid addition funnel, and a drying tube (Drierite) were added 4.9 g (18.3 mmol) of oleyl alcohol (Sigma) and 25 mL of dry, distilled pyridine (stored over KOH). The vessel was cooled to 0 °C in an ice/H_2O bath with stirring under a steady stream of nitrogen. Methanesulfonyl chloride (2.2 mL, 28.2 mmol; Aldrich) was added to the stirred reaction mixture over 5 min through the dropping funnel. The ice bath was removed and the reaction mixture allowed to come to room temperature and stirred vigorously under nitrogen for 5 h. The reaction mixture was washed into a separatory funnel with 50 mL of Et_2O and 35 mL of oxygen-free H_2O. The aqueous layer was drawn off and stored under nitrogen in a stoppered flask in an ice bath while the nonaqueous layer was extracted with 15 mL of H_2O, 4 × 15 mL of 2 N H_2SO_4 (degassed), and 15 mL of H_2O. These aqueous layers were combined, labeled as acid layer I, and stored under nitrogen in a stoppered flask over ice. The original nonaqueous layer was then extracted with 3 × 15 mL of 1% K_2CO_3 (degassed) and 15 mL of H_2O. These aqueous layers were combined, labeled as basic layer I, and stored under nitrogen in a stoppered flask over ice; the original nonaqueous layer was dried over anhydrous Na_2SO_4 and stored under nitrogen in a stoppered flask over ice. Basic layer I and the original aqueous layer were combined and extracted with 50 mL of Et_2O; this ether layer was then extracted with acid layer I, 15 mL of 2 N H_2SO_4 (degassed), 15 mL of H_2O, 2 × 15 mL of 1% K_2CO_3, and (degassed) 15 mL of H_2O, then combined with the original nonaqueous layer, and dried over Na_2SO_4 under nitrogen in a stoppered flask on ice. Gas chromatography (2% OV-17 on Supelcoport, T_{col} = 200 °C) showed no oleyl alcohol. The drying agent was filtered, and the solvents were removed on a rotary evaporator to give a yellow, oily residue that was dried under high vacuum for 3 h. This residue was taken up in 25 mL of absolute EtOH and placed at −30 °C overnight. The white crystals that formed were filtered at −15 °C on a chilled Buchner funnel and dried under high vacuum to give 4.2 g (66% based on alcohol) of product which had a melting point of 10–11 °C (lit. mp 9 °C, corr). Infrared spectroscopy of the product showed the absence of any OH bands and the presence of the S–O stretch (1080–1030 cm^{-1}).

Linoleyl mesylate (5.9 g) was prepared in 93% yield from linoleyl alcohol (18.4 mmol, Sigma) with >99% purity as measured by gas chromatography (column T = 200 °C). Palmitoleyl methanesulfonate was obtained from Nu Check Prep, Inc.

2-*O*-[(Methoxyethoxy)methyl]glycerol (7) from 1,3-Benzylideneglycerol. 1,3-Benzylideneglycerol was prepared by the method of Verkade and van Roon[22] [mp 82–83 °C; R_f [95/5 (v/v) $CHCl_3/Et_2O$] = 0.25]. The method of Corey et al.[23] was used to prepare 2-*O*-[(methoxyethoxy)methyl]-1,3-benzylidene glycerol. To a three-neck round-bottom flask equipped with a magnetic stir bar, reflux condenser, nitrogen bubbler, and liquid addition funnel was added 150 mL of THF and the flask cooled to 2 °C with stirring in an ice bath. Sodium hydride [5.32 g of a 50% oil dispersion (112.2 mmol)] was added to the THF to form a slurry after which a solution of 20 g (111.2 mmol) of 1,3-benzylideneglycerol in 150 mL of THF was dripped into the reaction vessel over 20 min. After hydrogen evolution ceased (about 10 min), a solution of 16.6 g (133.2 mmol) of methoxyethoxymethyl chloride (MEM-Cl) in 40 mL of THF was dripped in slowly and the reaction stirred for 1 h at 2 °C at which time TLC showed no starting material remained. Water (40 mL) was added cautiously to quench any excess hydride reagent and the mixture stirred at 2 °C for 2 h. The reaction mixture was washed into a separatory funnel with 200 mL of Et_2O and the nonaqueous layer separated and extracted with 3 × 150 mL of H_2O; the layers were combined and extracted with 200 mL of Et_2O. The nonaqueous layers were combined and dried over anhydrous sodium sulfate overnight.

The drying agent was removed by filtration, and the solvents were stripped on a rotary evaporator to give 34.2 g of a yellow oil that solidified below room temperature. TLC [95/5 (v/v) $CHCl_3/Et_2O$] revealed two spots, neither of which was starting material. The entire crude product was subjected to hydrogenolysis (2.0 g of 5% Pd/C in 300 mL of absolute EtOH) in a Parr shaking hydrogenator (room temperature, 30 psig), the catalyst filtered, and solvent removed on a rotary evaporator to give 18 g of a yellow oil. The product was chromatographed on 200 g of silica gel 60 (E. M. Merck) in $CHCl_3$ and eluted with 500 mL of 95/5 (v/v) $CHCl_3$/MeOH and then 1000 mL of 9/1 (v/v) $CHCl_3$/MeOH, collecting 20-mL fractions. Fractions 32–50 were combined and stripped of solvents to give 7.5 g material (38% based on 1,3-benzylideneglycerol) as a slightly yellow oil that was pure by TLC [R_f [9/1 (v/v) $CHCl_3$/MeOH] = 0.41]. Elemental analysis (C, H) and IR and NMR spectra were consistent with the structure proposed. On flash distillation of an aliquot of the product, a boiling point of 175–180 °C (8 torr) was obtained.

***rac*-1-*O*-Alkyl-2-*O*-[(methoxyethoxy)methyl]glycerols (8).** The procedure of Baumann and Mangold[21] was used to introduce the 1-*O*-alkyl groups. To a three-neck round-bottom flask equipped with a magnetic stir bar, reflux condenser, drying tube (Drierite), liquid addition funnel, and nitrogen inlet was added 75 mL of benzene which was heated to reflux with stirring and 0.48 g (12.15 mmol) of freshly cut potassium metal was added. When the potassium had formed a fine shot, 4.3 g (24.3 mmol) of 2-*O*-[(methoxyethoxy)methyl]glycerol in 20 mL of dry benzene was added rapidly and the reaction vigorously stirred at reflux for 4 h until all the potassium metal had reacted. Oleyl mesylate (4.2 g, 12.15 mmol) dissolved in 20 mL of benzene was slowly added to the reaction mixture over 15 min and the reaction allowed to proceed at reflux overnight.

The flask was cooled to 4 °C in an ice/H_2O bath and moist Et_2O (20 mL) followed by oxygen-free H_2O (200 mL) was added. The aqueous layer was separated and extracted with 2 × 150 mL of Et_2O; the nonaqueous layers were combined and dried over anhydrous sodium sulfate. The drying agent was filtered, and the solvents were removed on a rotary evaporator to give 6.0 g of crude yellow oil. The crude material was chromatographed on 100 g of silica gel 60 with 95/5 (v/v) $CHCl_3$/MeOH as eluent, followed by chromatography on 75 g of silica gel 60 with diethyl ether as the eluent to give 2.9 g of pure, colorless oil (56% based on starting mesylate).

In a similar manner *rac*-1-*O*-linoleyl-2-*O*-[(methoxyethoxy)-methyl]glycerol and *rac*-1-*O*-palmitoleyl-2-*O*-[(methoxyethoxy)-methyl]glycerol were prepared in 55% and 80% yields, respectively. The compounds prepared had an R_f value of 0.30 (diethyl ether) and 0.59 [95/5 (v/v) $CHCl_3$/MeOH]. IR and NMR spectra were consistent with the proposed structures.

***rac*-1-*O*-Alkyl-2-*O*-[(methoxyethoxy)methyl]glycero-3-phosphocholines (9).** The procedure of Brockerhoff and Ayengar[24] was used to prepare the phosphocholines. Phosphorus oxychloride (7.56 mmol) was added to a reaction vessel equipped with a magnetic stir bar, condenser, liquid addition funnel, and nitrogen bubbler. A solution of 1-*O*-oleyl-2-*O*-[(methoxyethoxy)methyl]glycerol (6.0 mmol) and triethylamine (7.56 mmol) in 38 mL of EtOH-free $CHCl_3$ was added to the stirring $POCl_3$ over 5 min and the solution heated to 60–65 °C for 30 min. The reaction mixture was cooled to room temperature, 3.8 mL of dry pyridine followed by 13.2 mmol of choline tosylate was added, and the reaction was stirred for 5 h at room temperature. The reaction was quenched by the addition of 2.0 mL of H_2O and the $CHCl_3$ layer extracted successively with 15 mL of H_2O, 2 × 15 mL of 5% HCl, and 15 mL of H_2O, using MeOH to break the emulsions that formed during the extraction procedure. The $CHCl_3$ layer was dried over anhydrous sodium sulfate. After filtration and solvent removal on a rotary evaporator, 2.7 g of a viscous yellow oil was obtained. The crude material was chromatographed on silica gel 60 with a discontinuous gradient of 100-mL aliquots of 3/1, 1/1, 1/3 (v/v) chloroform/methanol

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followed by 200 mL of methanol to give 1.4 g of pure product as a viscous clear oil (52% yield). The material had an R_f value of 0.48 [70/35/7 (v/v/v) $CHCl_3$/MeOH/NH_4OH]. The linolenyl and palmitoleyl analogues were prepared in the same manner in 48% and 30% yields, respectively.

***rac*-1-*O*-Alkyl-2-acetylglycero-3-phosphocholines.** The procedure of Corey et al.[23] was used to remove the MEM protecting group. The MEM ether **9** (1.63 mmol) and anhydrous zinc bromide (8.14 mmol) were stirred at room temperature in 10 mL of CH_2Cl_2 for 24 h at which time the reaction was poured into a separatory funnel and the CH_2Cl_2 layer was extracted with 3 × 15 mL of H_2O. The nonaqueous layer was dried over anhydrous sodium sulfate, the drying agent filtered, and the solvent removed on a rotary evaporator to give 0.8 g of an opalescent solid. The product was chromatographed on 30 g of silica gel 60 as before with a discontinuous gradient of $CHCl_3$/MeOH to give 0.52 g (62% yield) of pure lyso lipid as a clear semisolid. The material had an R_f value of 0.32 [70/30/7 (v/v/v) $CHCl_3$/MeOH/NH_4OH] and IR and NMR spectroscopy was consistent with the proposed structure. The lysolinoleyl- and lysopalmitoleylphosphocholines were prepared in the same manner in 80% and 93% yields, respectively.

The lyso lipids were acetylated by using the procedure of Gupta et al.[25] The lyso lipid (0.27 mmol) and (*N*,*N*-dimethylamino)-pyridine (0.27 mmol) were dissolved in a mixture of 1.2 mL of EtOH-free $CHCl_3$ and 0.3 mL of pyridine. To this solution 3.0 mL of freshly distilled acetic anhydride was added, the vessel stoppered under nitrogen, and the mixture stirred with a magnetic stir bar for 24 h. The reaction mixture was blown down under a stream of nitrogen, and the lipids were extracted by the procedure of Bligh and Dyer[26] to give 191 mg of crude brown product. This was purified on silica gel preparative TLC plates [eluent: 70/35/7 (v/v/v) $CHCl_3$/MeOH/NH_4OH] to give 157 mg (99%) of product as an off-white solid having an R_f value of 0.47. The linoleyl- and palmitoleyl-PAF analogues (**4** and **2**, respectively) were prepared in a similar manner in 16% and 36% yields, respectively. All three unsaturated PAF analogues gave satisfactory elemental analyses (C, H, N, P), and NMR spectra (Table III) were consistent with the proposed structures.

2-*O*-Benzylglycerol (11). To a three-neck round-bottom flask equipped with a magnetic stir bar, reflux condenser, drying tube, and liquid additional funnel was added 800 mL of benzene. The benzene was heated to reflux and then 14.0 g (356 mmol) of freshly cut potassium metal was added and the mixture stirred to form potassium shot. A solution of 64.0 g (356 mmol) 1,3-benzylideneglycerol in 600 mL of benzene was added over 1 h to the stirring refluxing suspension and the reaction refluxed for 7 h until no potassium metal remained. At this time 45.0 g (336 mmol) of benzyl chloride was added to the reaction mixture over 30 min and the reaction allowed to proceed at benzene reflux overnight.

The reaction was cooled to room temperature and solvent removed on a rotary evaporator to give an orange solid which was dissolved in a mixture of 600 mL of Et_2O and 400 mL of H_2O. The aqueous layer was separated and extracted with 3 × 150 mL of Et_2O, and the ether layers were combined, washed with 200 mL of saturated NaCl solution, and dried over anhydrous sodium sulfate. After filtering, the solvent was removed on a rotary evaporator to give a crude off-white solid that was recrystallized from 600 mL ether at 5 °C. The product was filtered and dried under vacuum to give 73.7 g (77%) of 2-*O*-benzyl-1,3-benzylideneglycerol, mp 77–78 °C.[27] This material was refluxed for 5 h in a mixture of 200 mL of H_2O, 130 mL of EtOH, and 26 mL of concentrated H_2SO_4. The benzaldehyde was steam distilled and the product extracted into 3 × 300 mL of Et_2O. The ether layer was dried over anhydrous magnesium sulfate. After filtering, the solvent was removed on a rotary evaporator to give 60.5 g of crude material. This was chromatographed on 300 g of silica gel 60 with 1000 mL of 9/1 (v/v) $CHCl_3$/MeOH as eluent to give 49.8 g (87%) of 2-*O*-benzylglycerol (**11**) that solidifies at −15 °C and has a melting point of 36–38 °C. IR and NMR spectra were consistent with the proposed structure.

***rac*-1-*O*-Hexadecyl-2-*O*-benzylglycerol (12).** The product was synthesized in the manner of compound **8** with 15.0 g (82.4 mmol) of 2-*O*-benzylglycerol and equimolar (61.8 mmol) amounts of potassium metal and hexadecyl bromide as starting materials, yielding 29.6 g of crude product as a pale viscous oil that solidifies below room temperature. The material was chromatographed on 300 g of silica gel 60 eluting with 2400 mL of $CHCl_3$ followed by 800 mL of 95/5 (v/v) $CHCl_3$/Et_2O to give 9.6 g (38%) of compound **12**.

***rac*-Hexadecyl-PAF (1).** Compound **13** (10.5 g) was prepared from compound **12** (22.2 mmol) in 63% yield in a manner similar to that for compound **9**. The material had an R_f value of 0.47 [70/38/7 (v/v/v) $CHCl_3$/MeOH/NH_4OH].

The racemic lyso compound was prepared by hydrogenolysis of the benzyl group. To 80 mL of absolute MeOH was added 1.1 g (1.86 mmol) of compound **13** and 0.11 g of 10% Pd/C. The mixture was hydrogenated on a Parr hydrogenator at room temperature for 3 h (30 psig) at which time the catalyst was filtered and the solvent removed on a rotary evaporator to give 0.9 g of lyso lipid (99%). The material had an R_f value of 0.30 [70/35/7 (v/v/v) $CHCl_3$/MeOH/NH_4OH].

The lyso lipid was acetylated in the manner of compound **3** to give *rac*-hexadecyl-PAF, compound **1**, in 89% yield.

Conversion of *rac*-PAF to L-PAF. The phospholipase A_2 treatment as described by Wells and Hanahan[28] was used to prepare the natural 1-*O*-alkyllyso-PAF. The lipid (200 mg) was suspended in 200 mL of 95/5 (v/v) Et_2O/MeOH and 8 mg of phospholipase A_2 (*O. hannah*) was added in 1.25 mL of buffer containing 50 mM Tris-HCl (pH 7.5), 25 mM $CaCl_2$ and 1 mM EDTA. The reaction mixture was stirred at room temperature overnight, and the lipids were then recovered by a Bligh and Dyer extraction.[26] The natural 1-*O*-alkyllyso-PAF was purified by preparative TLC [50/25/8/4 (v/v/v/v) $CHCl_3$/MeOH/HOAc/H_2O] and reacetylated as before[25] to give 1-*O*-alkyl-PAF in 40% overall yield from the racemate.

Note Added in Proof: After this manuscript was submitted for publication a symposium proceedings[29] appeared in which compound **4** was radiolabeled. The synthetic procedure[30] differed significanly from our synthetic strategy, taking two additional steps, consuming tritium gas in the hydrogenolysis of the 2-benzyl ether, and producing tritiated toluene which is volatile and presents an additional environmental hazard.

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Registry No. 1, 77286-68-1; **2**, 93135-76-3; **3**, 85026-88-6; **4**, 93220-78-1; **5**, 93135-77-4; **6**, 93220-79-2; **7**, 93135-78-5; 8 (R = linoleyl), 93135-79-6; 8 (R = palmitoleyl), 93135-80-9; 8 (R = oleyl), 93135-81-0; **9** (R = oleyl), 93135-82-1; **9** (R = palmitoleyl), 93135-83-2; **9** (R = linoleyl), 93135-84-3; **10** (R = linoleyl), 93135-87-6; **10** (R = palmitoleyl), 93135-88-7; 11, 14690-00-7; **12**, 18678-94-9; **13**, 18678-98-3; 14, 17364-21-5; oleyl alcohol, 143-28-2; oleoyl mesylate, 35709-09-2; linoleyl mesylate, 51154-39-3; palmitoleyl methanesulfonate, 93135-85-4; linoleyl alcohol, 506-43-4; 1,3-benzylideneglycerol, 1708-40-3; 2-memo-1,3-benzylideneglycerol, 93135-86-5; choline tosylate, 55357-38-5; 2-*O*-benzyl-1,3-benzylideneglycerol, 68728-34-7; hexadecyl bromide, 112-82-3.

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