Synthesis and biochemical studies of analogs of platelet-activating factor bearing a methyl group at C2 of the glycerol backbone

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Abstract Two platelet-activiting factor (PAF) analogs containing a methyl group at C2 of the glycerol moiety were synthesized, and some of their biochemical properties were investigated. 1-*O*-Hexadecyl-2-*C,O*-dimethyl-*rac*-glycero-3-phosphocholine (2-methyl-2-methoxy PAF) was prepared in a synthetic scheme beginning with the etherification of 2-methylpropen-1-ol. A reaction sequence involving hydroxylation, tritylation, alkylation, and detritylation afforded 1-*O*-hexadecyl-2-*C,O*-dimethyl-*rac*-glycerol, which was converted into the phosphocholine. A 2-lyso derivative of this PAF analog (2-methyl-lyso PAF) was synthesized from 1-*O*-hexadecyl-2-*C*-methyl-3-*O*-trityl-*rac*-glycerol. Benzylation followed by detritylation gave 1-*O*-hexadecyl-2-*C*-methyl-2-*O*-benzyl-*rac*-glycerol, which was converted into the phosphocholine compound. Hydrogenolysis afforded 1-*O*-hexadecyl-2-*C*-methyl-*rac*-glycero-3-phospholine (2-methyl-lyso PAF). The 2-methyl-lyso PAF analog served as a substrate for the acetyl-CoA-dependent acetyltransferase that acetylates 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine. However, 2-methyl-lyso PAF did not have a significant effect on the activities of a CoA-independent transacylase or of the acetylhydrolase that inactivates PAF, and thus does not appear to be a substrate or an inhibitor, respectively, for these enzymes. In addition, this analog exhibited only one-half of the antitumor activity of *rac*-1-*O*-alkyl-2-methoxy-*rac*-glycero-3-phosphocholine in human leukemic (HL-60) cells, and elicited no hypotensive response in rats and no platelet-activating activity. In contrast, the 2-methyl-2-methoxy analog of PAF had the same cytotoxic activity against HL-60 cells as *rac*-1-*O*-alkyl-2-methoxyglycero-3-phosphocholine; however, it had no activation effect on rabbit platelets and no hypotensive effect on rats, and it did not inhibit plasma acetylhydrolase activity.—**Bittman, R., N. M. Witzke, T-c. Lee, M. L. Blank, and F. Snyder.** Synthesis and biochemical studies of analogs of platelet-activating factor bearing a methyl group at C2 of the glycerol backbone. *J. Lipid Res.* 1987. **28:** 733–738.

Supplementary key words chemical synthesis of phospholipids • *rac*-1-*O*-hexadecyl-2-*C*-methyl-3-glycerophosphocholine • PAF metabolism

A great deal of information has been compiled about the synthesis and properties of platelet activating factor (PAF) and analogs related to 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (see refs. 1–4 for recent reviews). A wide range of structural variation has been introduced into the groups at the 1, 2, and 3 positions of the glycerol moiety of the lipid in order to explore structure–activity relationships (5–8). However, no analogs have been reported, to our knowledge, in which the steric bulk at the glycerol backbone has been increased by substitution of an alkyl group for hydrogen. Recently, Raman spectral observations indicated that introduction of a methyl group at C1, C2, or C3 of the glycerol moiety of di-*O*-hexadecylglycerophosphocholine perturbs the chain packing arrangements within the hydrophobic portion of the bilayer (9). It is, therefore, pertinent to examine whether increased steric bulk at the glycerol backbone of PAF affects the metabolism of PAF or perturbs the interactions between the lipid and the PAF receptors in biological systems. In this study, we report the syntheses of two PAF analogs bearing a methyl group at C2 of the glycerol moiety. We also report the results of studies of *a)* the cytotoxic activities of these analogs in human leukemic (HL-60) cells and *b)* the enzymatic acetylation of the C2-methyl-lyso PAF analog catalyzed by acetyl-CoA acetyltransferase in microsomes. Comparison of the extent of acetylation of C2-methyl-lyso PAF with that of lyso PAF indicates that the acetyltransferase can react with closely related substrates.

MATERIALS AND METHODS

Chemicals

Methallyl alcohol and benzyl chloride were purchased from Aldrich. The sources of the other chemicals used in the synthetic procedures have been referred to previously (10). 1-*O*-Hexadecyl-2-*O*-methyl-*rac*-glycero-3-phosphochol-

Abbreviation: PAF, platelet-activating factor.

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ine was obtained from Sigma. The sources of the chemicals used in the biological tests have been cited previously (ref. 11–16).

Enzymatic assays

Rat spleen microsomes were prepared as described previously (12). The activity of microsomal acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase was measured according to Wykle, Malone, and Snyder (11), as modified by Lee (12). The CoA-independent transacylase that acylates 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine was assayed according to the method described by Robinson, Blank, and Snyder (13). Plasma acetylhydrolase was assayed by using 2.5 μl of rat plasma as the enzyme source (14, 15). All incubations were carried out at 37°C for 5 min. CDF (Fisher-344) male rats were the source of the spleens or plasma used in the enzyme studies.

Testing of antitumor, hypotensive, and platelet-activating activities

Antitumor activities in HL-60 cells (16), blood pressure responses in CDF (Fisher-344) male rats (7), and [^{3}H]serotonin release in rabbit platelets (7) were measured as described earlier.

Chromatography and NMR spectroscopy

Thin-layer chromatography was performed on silica gel GF glass plates (Analtech, Newark, DE), and compounds were detected on the plates as described previously (10). NMR spectra at 200 MHz were recorded on an IBM/Bruker WP200 SY FT-NMR spectrometer. Elemental analyses were performed by MicAnal (Tucson, AZ) and Schwarzkopf (Woodside, NY).

RESULTS

2-Methylallyl hexadecyl ether (Scheme 1, *2*)

A mixture of powdered 86% potassium hydroxide (6.8 g, 104 mmol), 1-bromohexadecane (15.3 ml, 50 mmol), and 2-methylpropen-1-ol (*1*, 4.2 ml, 50 mmol) was refluxed in 120 ml of toluene with stirring for 20 hr under a 20-ml Dean-Stark water trap that had been previously filled with toluene. The reaction mixture was cooled below 60°C and hexane (200 ml) was added. After the mixture was cooled to room temperature, the supernatant was decanted from the precipitate of KBr and unreacted KOH. The solution was washed with water (3 × 100 ml), dried with K_2CO_3, and evaporated. The oily residue was chromatographed on silica gel (Baker, 280 g, dried at 120°C for 1 hr) packed in hexane. Elution with hexane and hexane–ether 96:4 (v/v) afforded the product together with a small amount of dihexadecyl ether. The crude product was dissolved in acetone. On cooling at −20°C for 1 hr, dihexadecyl ether precipitated. The precipitate was collected on a chilled Büchner funnel and washed with a small volume of cold acetone. Evaporation of the filtrate gave the product as a colorless oil (10.4 g, 70%), mp 12–13°C. Anal. calc. for $C_{20}H_{40}O$ (296.54) C, 81.01; H, 13.60. Found: C, 80.89; H, 13.52. 200-MHz ^{1}H NMR ($CDCl_3$) δ (ppm): 0.88 (3H, t, J = 6.3 Hz, ω - C$\underline{H}_3$), 1.26 (26H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.58 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 1.73 (3H, s, -C$\underline{H}_3$), 3.38 (2H, t, J = 6.6 Hz, hexadecyl C$\underline{H}_2$ of C_1), 3.86 (2H, s, allylic–C$\underline{H}_2$O–), and 4.87–4.95 (2H, dd, J = 0.7 Hz, =C$\underline{H}_2$).

rac-1-*O*-Hexadecyl-2-methylglycerol (Scheme 1, *3*).

A mixture of 2-methylallyl hexadecyl ether (3.0 g, 10.1 mmol), 90% formic acid (40 ml), and 30% hydrogen peroxide (4 ml) was stirred vigorously at 45°C for 3 hr and then at 35°C for 20 hr. After the reaction mixture was diluted with ice-cold water (60 ml), the precipitate of formate esters was filtered and washed with cold water, then dried briefly, and refluxed for 20 min in a solution of sodium hydroxide (3 g) in water (6 ml) and 95% ethanol (60 ml). The mixture was cooled, and ether (200 ml) and water (200 ml) were added. The ether phase was washed with water (3 × 50 ml), dried with K_2CO_3, and evaporated. Crystallization of the residue (3 g) from 25 ml of hexane gave product *3* (2.3 g, 70%) as white crystals, mp 44.5–45.5°C. Anal. calc. for $C_{20}H_{42}O_3$ (330.56) C, 72.67; H, 12.81. Found: C, 72.55; H, 12.77. 200-MHz ^{1}H NMR ($CDCl_3$) δ (ppm): 0.88 (3H, t, J = 6.3 Hz, ω-C$\underline{H}_3$), 1.13 (3H, s, –C$\underline{H}_3$), 1.26 (26H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.57 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), ~ 2.4 (2H, broad s, -O$\underline{H}$), and 3.34–3.68 (6H, m, –C$\underline{H}_2$O–).

rac-1-*O*-Hexadecyl-2-methyl-3-*O*-tritylglycerol (Scheme 1, *4*)

To a solution of glycol *3* (0.99 g, 3 mmol) and trityl chloride (0.96 g, 3.4 mmol) in 6 ml of dry toluene was added 0.48 ml (3.4 mmol) of triethylamine. After the mixture was kept at 60°C for 4 days, hexane (50 ml) was added. The mixture was stored at −20°C for 2 hr and then filtered, and the filtrate was evaporated. The residue (2.1 g) was purified by column chromatography on silica gel 60 (Merck, 150 g, dried at 120°C for 2 hr) packed in 1,2-dichloroethane. Elution with this solvent gave product *4*, which crystallized on prolonged drying at 0.5 torr (1.17 g, 68%), mp 45.0–45.5°C. Anal. calc. $C_{39}H_{56}O_3$ (572.88) C, 81.77; H, 9.85. Found: C, 81.69; H, 9.81. 200-MHz ^{1}H NMR (CCl_4–C_6D_6 9:1, v/v): δ 0.89 (3H, t, J = 6.3 Hz, ω-C$\underline{H}_3$), 1.12 (3H, s, –C$\underline{H}_3$), 1.26 (26H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.48 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 2.19 (1H, broad s, –O$\underline{H}$), 2.99 (2H, s, 3-C$\underline{H}_2$O– of glycerol), 3.28–3.44 (4H, m, hexadecyl –C$\underline{H}_2$O– and glycerol 1-C$\underline{H}_2$O–), and 7.09–7.41 (15H, m, trityl).

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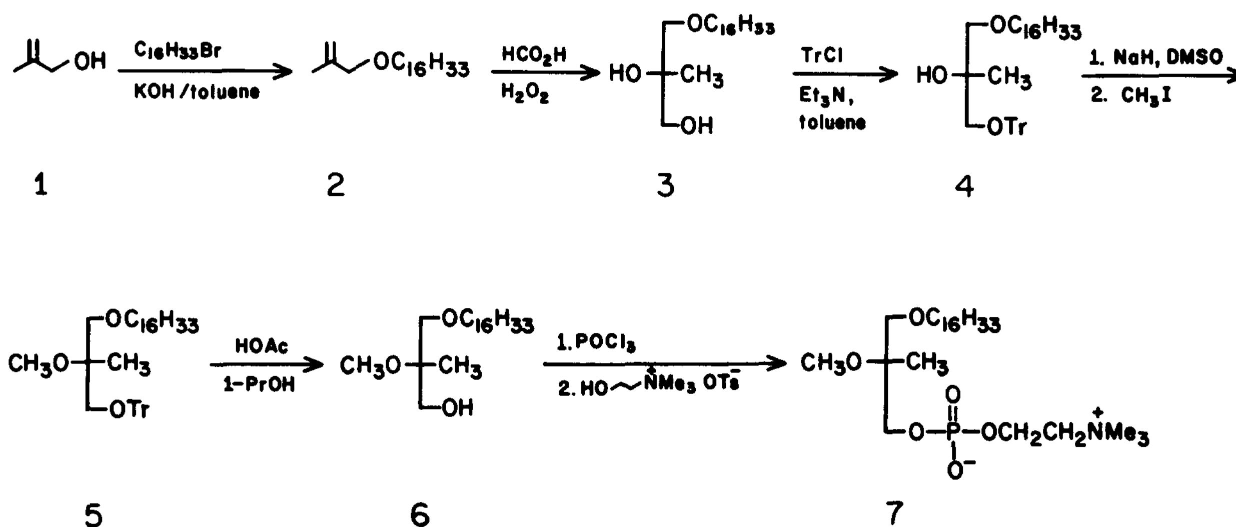

Scheme 1. Reaction sequence for the preparation of 1-*O*-hexadecyl-2-*C,O*-dimethyl-*rac*-glycero-3-phosphocholine (2-methyl-2-methoxy PAF).

1-*O*-Hexadecyl-2-*C*,*O*-dimethyl-3-*O*-trityl-*rac*-glycerol (Scheme 1, *5*)

Sodium hydride (48 mg, 2 mmol) was placed in a three-necked flask equipped with a magnetic stirrer, N_2 inlet, addition funnel, and septum injection port. After the flask was flushed with N_2 for 20 min, dimethyl sulfoxide (4 ml) was added and the mixture was stirred for 30 min. A solution of 1-*O*-hexadecyl-2-methyl-3-*O*-trityl-*rac*-glycerol (Scheme 1, *4*) (577 mg, 1.01 mmol) in 1.5 ml of toluene was then injected into the flask. After the mixture was stirred for 15 min, methyl iodide (125 μl, 2 mmol) was added and stirring was continued for 3 hr. Hexane (10 ml) and water (10 ml) were added, and the hexane layer was separated, washed with water (3 × 10 ml), dried with K_2CO_3, and evaporated under vacuum. The 2-*O*-methyl ether *5* was obtained as an oil (575 mg, 97%), which gave only one spot on thin-layer chromatography on silica gel GF plates (solvent, 1,2-dichloroethane, R_f value = 0.51 compared with 0.39 for the corresponding starting 2-methyl-2-lyso compound *4*). This intermediate was, therefore, used in the next step without further purification.

1-*O*-Hexadecyl-2-*C*,*O*-dimethyl-*rac*-glycerol (Scheme 1, *6*)

The detritylation was carried out by refluxing 575 mg (0.98 mmol) of *5* in 5 ml of 1-propanol, 4 ml of glacial acetic acid, and 1 ml of water, with stirring, for 2 hr. The residue obtained after evaporation of the solvents was applied to a column of silica gel (55 g, Merck) packed in chloroform. The column was eluted with chloroform–ethyl acetate 95:5 (v/v), affording the product as an oil (306 mg, 91%) after evaporation of the solvents. Anal. calc. for $C_{21}H_{44}O_3$ (344.58) C, 73.20; H, 12.87. Found: C, 73.42; H, 13.05. 200-MHz ^{1}H NMR ($CDCl_3$) δ (ppm): 0.84 (3H, t, J = 6.4 Hz, ω-C$\underline{H}_3$), 1.08 (3H, s, C$\underline{H}_3$ group at C(2) of glycerol), 1.21 (26H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.53 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 2.50 (1H, t, J = 6.1 Hz, -O$\underline{H}$), 3.26 (3H, s, -OC$\underline{H}_3$), 3.31–3.49 (4H, m, hexadecyl -C$\underline{H}_2$O- and 1-C$\underline{H}_2$O- of glycerol), and 3.55 (2H, d, J = 6.1 Hz, 3-C$\underline{H}_2$O- of glycerol).

1-*O*-Hexadecyl-2-*C*,*O*-dimethyl-*rac*-glycero-3-phosphocholine (Scheme 1, *7*)

The phosphocholine group was incorporated using the procedure described previously (see Scheme 1, *7* of reference 10). Anal. calc. for $C_{26}H_{56}NO_6P \cdot 1.5\ H_2O$ (538.25) C, 58.20; H, 11.08; N, 2.61; P, 5.78. Found: C, 57.81; H, 10.93; N, 2.65; P, 5.49. 200-MHz ^{1}H NMR ($CDCl_3$) δ (ppm): 0.81 (3H, t, J = 6.2 Hz, ω-C$\underline{H}_3$), 1.08 (3H, s, C$\underline{H}_3$ group at C(2) of glycerol), 1.19 (26H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.46 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 3.20 (3H, s, -OC$\underline{H}_3$), 3.31 (11H, m, -C$\underline{H}_2$N and -NC$\underline{H}_3$), 3.70 (6H, m, hexadecyl -C$\underline{H}_2$O- and glycerol 1- and 3-C$\underline{H}_2$O-), and 4.23 (2H, m, POC$\underline{H}_2$CH$_2$-).

1-*O*-Hexadecyl-2-*C*-methyl-2-*O*-benzyl-3-*O*-trityl-*rac*-glycerol (Scheme 2, *8*)

A mixture of the 1-*O*-hexadecyl-2-methyl-3-*O*-trityl glycerol (Scheme 1, *4*) (900 mg, 1.57 mmol), 90% powdered potassium hydroxide (0.60 g, 9.6 mmol), and benzyl chloride (0.60 ml, 5.2 mmol) in 20 ml of toluene was refluxed with stirring under a Dean-Stark water trap for 22 hr. After the mixture had cooled to about 60°C, hexane (40 ml) was added. The mixture was allowed to cool to room temperature, and the hexane phase was extracted with water

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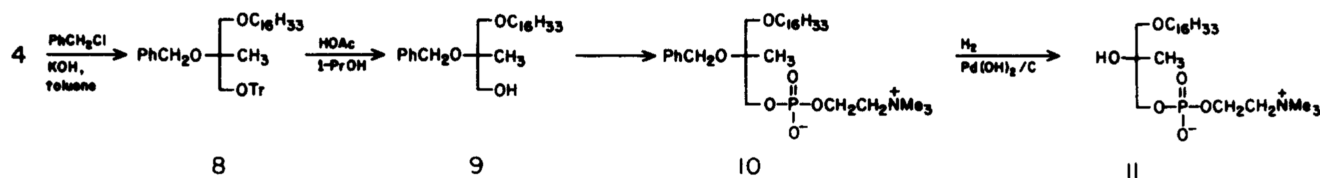

Scheme 2. Reaction sequence for the preparation of 1-*O*-hexadecyl-2-*C*-methyl-*rac*-glycero-3-phosphocholine (2-methyl-lyso PAF).

(3 × 20 ml), dried with K_2CO_3, and evaporated. The residue (1.40 g) was chromatographed on silica gel 60 (Merck, dried at 120°C for 6 hr) using the solvent system hexane–1,2-dichloroethane 55:45 (v/v). After evaporation of the solvents, the product was obtained as an oil (980 mg, 94%). Anal. calc. for $C_{46}H_{62}O_3$ (663.01) C, 83.33; H, 9.43. Found: C, 83.74; H, 9.51. 200-MHz ^1H NMR (CCl_4–acetone-d_6, 4:1) δ (ppm): 0.88 (3H, t, J = 6.3 Hz, ω-C$\underline{H}_3$), 1.21 (3H, s, -C$\underline{H}_3$), 1.26 (26H, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.53 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 3.12 (2H, dd, J = 9.3 Hz, 3-C$\underline{H}_2$O- of glycerol), 3.39–3.64 (4H, m, hexadecyl -C$\underline{H}_2$O- and glycerol 1-C$\underline{H}_2$O-), 4.37 (2H, dd, J = 11.4 Hz, benzyl-C$\underline{H}_2$O), and 7.13–7.46 (20H, m, $C_6\underline{H}_5$-).

1-*O*-Hexadecyl-2-*C*-methyl-2-*O*-benzyl-*rac*-glycerol (Scheme 2, *9*)

A mixture of *8* (0.50 g, 0.75 mmol) in 5 ml of 1-propanol, 4 ml of glacial acetic acid, and 1 ml of water was refluxed, with stirring, for 3 hr. The solvents were evaporated, and the residue was chromatographed on silica gel 60 (Merck, 65 g) packed in chloroform to give the product as an oil which slowly crystallized (0.30 g, 94%), mp 35.0–35.5°C. Anal. calc. for $C_{27}H_{48}O_3$ (420.68) C, 77.09; H, 11.50. Found: C, 77.30; H, 11.61. 200-MHz ^1H NMR ($CDCl_3$) δ(ppm): 0.88 (3H, t, J = 5.9 Hz, ω-C$\underline{H}_3$), 1.25 (29H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}, and C$\underline{H}_3$ group at C(2) of glycerol), 1.56 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 2.47 (1H, broad s, -O$\underline{H}$), 3.41–3.65 (6H, m, hexadecyl-C$\underline{H}_2$O- and glycerol 1- and 3-C$\underline{H}_2$O-), 4.56 (2H, s, benzyl -C$\underline{H}_2$O), and 7.25–7.36 (5H, m, $C_6\underline{H}_5$-).

1-*O*-Hexadecyl-2-*C*-methyl-2-*O*-benzyl-*rac*-glycero-3-phosphocholine (Scheme 2, *10*)

A solution of 185 μl (1.32 mmol) of dry triethylamine in 2 ml of alcohol-free, dry chloroform was placed in a flask equipped with a magnetic stirring bar and a dropping funnel closed with a drying tube. After the solution was stirred at 0°C for 30 min, 67 μl (0.72 mmol) of freshly distilled phosphorus oxychloride was added, and then a solution of *9* (0.29 g, 0.69 mmol) in 1.3 ml of chloroform was added dropwise. The mixture was stirred for 5 min at 0°C and was then kept for 30 min at room temperature. A solution of choline *p*-toluenesulfonate (275 mg, 1.0 mmol) in 5 ml of dry pyridine was added rapidly, and stirring was continued at room temperature for 20 hr. Then water (0.52 ml) and sodium bicarbonate (0.26 g) were added, and the mixture was stirred vigorously for 30 min. The volatile solvents were removed on a rotary evaporator, and the residue was dissolved in 100 ml of chloroform. The chloroform solution was washed with water (30 ml), 5% HCl (2 × 30 ml), water (30 ml), 5% sodium carbonate solution (2 × 30 ml), and water (30 ml), then dried with K_2CO_3 and evaporated. The residue was chromatographed on silica gel 60 (Merck, 60 g) packed in chloroform–methanol 65:25 (v/v). Elution with this solvent system and then with chloroform–methanol–water 65:25:4 (v/v/v) gave product *10* as crystals (0.26 g, 59%), mp 219–221°C (dec.), on repeated evaporations after additions of 2-propanol. Anal. calc. for $C_{32}H_{60}NO_6P \cdot 3H_2O$ (639.86) C, 60.07; H, 10.40; N, 2.19; P, 4.84. Found: C, 59.24; H, 10.31; N, 2.17; P, 4.95. 200-MHz ^1H NMR ($CDCl_3$) δ (ppm): 0.88 (3H, t, J = 6.2 Hz, ω-C$\underline{H}_3$), 1.16 (3H, d, J = 6.1 Hz, C$\underline{H}_3$ group at C(2) of glycerol), 1.25 (26H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.54 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 3.09 (9H, s, -NC$\underline{H}_3$), 3.38–3.49 (6H, m, hexadecyl -C$\underline{H}_2$O-, glycerol 1-C$\underline{H}_2$O-, and -C$\underline{H}_2$N-), 3.85 (2H, m, glycerol 3-C$\underline{H}_2$O-), 4.13 (2H, m, -POC$\underline{H}_2$CH$_2$-), 4.56 (2H, s, benzyl -C$\underline{H}_2$O), and 7.20–7.35 (5H, m, $C_6\underline{H}_5$-).

1-*O*-Hexadecyl-2*C*-methyl-*rac*-glycero-3-phosphocholine (2-methyl-lyso PAF) (Scheme 2, *11*)

A mixture of the benzyl-PC *10* (235 mg, 0.37 mmol) and 20% $Pd(OH)_2$ on carbon (100 mg, Pearlman's catalyst) in methanol (9 ml) and water (1 ml) was stirred under a hydrogen atmosphere for 20 hr, then filtered on a bed of Celite 545. The catalyst was washed with methanol. The filtrate was evaporated, and the residue was dried by re-evaporation after several additions of 2-propanol, yielding the product *11* (202 mg, 100%), mp 228–230°C (dec.). Anal. calc. for $C_{25}H_{54}NO_6P \cdot 3H_2O$ (549.73) C, 54.62; H, 11.00; N, 2.55; P, 5.63. Found: C, 56.17; H, 10.99; N, 2.58; P, 5.61. 200-MHz ^1H NMR ($CDCl_3$) δ (ppm): 0.88 (3H, t, J = 6.3 Hz, ω-C$\underline{H}_3$), 1.20 (3H, d, J = 6.1 Hz, C$\underline{H}_3$ group at C(2) of glycerol), 1.26 (26H, m, hexadecyl C$\underline{H}_2$ groups, C_3–C_{15}), 1.51 (2H, m, hexadecyl C$\underline{H}_2$ of C_2), 3.39 (9H, s, -NC$\underline{H}_3$), 3.19–3.99 (9H, m, hexadecyl 1,3-C$\underline{H}_2$O-, glycerol 1,3-C$\underline{H}_2$O-,

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$-CH_2N-$, and $-OH$), 4.37 (2H, m, $-POCH_2CH_2-$), and ~4.7 (shoulder, H_2O); no aromatic or benzylic protons were present.

Effect of 2-methyl-lyso PAF (*11*) on the CoA-dependent acetyltransferase and CoA-independent transacylase

Several biological properties of the 2-methyl-lyso PAF analog (*11*) were measured. **Table 1** indicates that the free hydroxyl group of this analog can be acetylated by an enzyme system similar to the acetyl-CoA acetyltransferase that is involved in the formation of PAF through the acetylation of lyso PAF; these data show that the acetylation of the 2-methyl-lyso PAF analog is concentration dependent. Utilization of the 2-methyl-lyso PAF analog as a substrate by the same acetyltransferase is substantiated by the competition data shown in **Table 2**. When 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine is used as the substrate (25 μM final concentration), the specific activity of the acetyltransferase decreased more than 2.5-fold when 2-methyl-lyso PAF (10 μM final concentration) was added to the reaction mixture. However, the 2-methyl-lyso PAF analog did not influence the activity of the CoA-independent transacylase (13) that acylates [^{3}H]-1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine. In these experiments with rat spleen microsomes as the enzyme source, the 2-methyl-lyso PAF analog (7.5 μM or 15 μM) did not affect the rate of CoA-independent transacylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (5, 10, 20, or 25 μM).

Effect of *7* and *11* on plasma acetylhydrolase

We also tested the ability of the 2-methyl-lyso and 2-methyl-2-methoxy analogs of PAF to influence the activity of the acetylhydrolase responsible for the removal of the acetate group from PAF (14, 15). Only a slight inhibition (18%) of acetylhydrolase activity was found when 9 μM compound *11* was co-incubated with 1 μM PAF and 2.5 μl of rat plasma, and no inhibition was seen with compound *7*.

TABLE 1. Concentration-dependent acetylation of 2-methyl-lyso PAF (*11*) by rat spleen microsomes

Concentration of 2-Methyl-lyso PAF	Specific Activity
μM	*nmol/min per mg protein*
0	0.45 ± 0.05
10	0.50 ± 0.10
15	0.65 ± 0.05
20	1.30 ± 0.10
25	2.40 ± 0.10

Five separate experiments were performed. The results shown represent the average of duplicate assays conducted in one of the five experiments. Incubation conditions were identical to those described previously (12).

TABLE 2. Effect of 2-methyl-lyso PAF (*11*) on the activity of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase in rat spleen microsomes

Concentration of 1-Alkyl-2-lyso-GPC	Concentration of 2-Methyl-lyso PAF	Specific Activity
μM	*μM*	*nmol/min per mg protein*
0	0	not detectable
25	0	3.2 ± 0.7
25	10	1.2

Results are averages of two separate experiments with duplicate determinations. Incubation conditions were identical to those described previously (12).

Hypotensive and antitumor properties of *7* and *11*

Tests were done to compare the hypotensive response, serotonin release, and antitumor activities of the 2-methyl-lyso (*11*) and 2-methyl-2-methoxy (*7*) PAF analogs with those previously reported for PAF and *rac*-1-alkyl-2-methoxy-glycerophosphocholine (7, 16). The 2-methyl-lyso and 2-methyl-2-methoxy PAF analogs elicited no hypotensive responses when rats were given up to 2 nanomoles intravenously; in contrast, 25 picomoles of PAF administered to the same rats caused a reduction in blood pressure of about 50%, with a recovery period of about 5 min. Also, the 2-methyl-lyso PAF analog is only approximately one-half as effective in killing HL-60 cells as the *rac*-1-*O*-alkyl-2-methoxyglycero-3-phosphocholine under identical conditions (see ref. 16 for experimental conditions). On the other hand, the cytotoxic activity of the 2-methyl-2-methoxy analog of PAF *7* toward HL-60 cells was identical to that of *rac*-1-*O*-alkyl-2-methoxyglycero-3-phosphocholine. Finally, the 2-methyl-2-lyso PAF analog (0.2, 2.0, or 20 μM) could not induce platelet serotonin release, whereas the 2-methyl-2-methoxy analog at 20 μM exhibited a serotonin release activity similar to that observed with 0.17 nM PAF.

DISCUSSION

In the present study a methyl group has been substituted for hydrogen at C2 of the glycerol moiety of lyso PAF and its 2-*O*-methyl derivative in order to obtain information about the molecular requirements involved in some of the biological activities of PAF. Recent studies have also shown that the acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase can utilize the 1-acyl analog (12). The present study represents the first investigation of whether the acetyltransferase has a specific requirement for the H-C-OH group at the *sn*-2 position. Our results indicate that when the hydrogen is replaced with a methyl group, the acetyltransferase still recognizes this analog as a substrate. It is not known whether the acetylated product

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(C2-methyl-2-acetyl-PAF) possesses any biological activity. The methyl-lyso analog *11* possesses no hypotensive or platelet-activating properties, and its cytotoxic action toward HL-60 cells is only half as effective as *rac*-1-alkyl-2-methoxy-glycero-phosphocholine, a known antineoplastic lipid (16) that we have used as a reference compound. In contrast, the 2-methyl-2-methoxy analog of PAF (*7*) had a cytotoxic potency toward the HL-60 cells equal to that of the reference compound. Our results indicate that further exploration of lyso-PAF analogs with various substituents at the *sn*-2 position could produce useful competitive inhibitors for the acetyltransferase responsible for PAF biosynthesis in cells where PAF production is stimulated by inflammatory agents. Such inhibitors could control PAF levels formed via the acetyltransferase reaction, since non-inflammatory products could be formed instead of PAF.

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REFERENCES

1. O'Flaherty, J. T., and R. L. Wykle. 1983. Mediators of anaphylaxis. *Clin. Lab. Med.* **3:** 619–643.
2. Lee, T-c., and F. Snyder. 1985. Function, metabolism, and regulation of platelet activating factor and related ether lipids. *In* Phospholipids and Cellular Regulation. J. F. Kuo, editor. CRC Press, Boca Raton, FL. 1–39.
3. Snyder, F. 1985. Chemical and biochemical aspects of platelet activating factor: a novel class of acetylated ether-linked choline-phospholipids. *Med. Res. Rev.* **5:** 107–140.
4. Hanahan, D. J. 1986. Platelet activating factor: a biologically active phosphoglyceride. *Annu. Rev. Biochem.* **53:** 483–509.
5. Satouchi, K., R. N. Pinckard, L. M. McManus, and D. J. Hanahan. 1981. Modification of the polar head group of acetyl glyceryl ether phosphorylcholine and subsequent effects upon platelet activation. *J. Biol. Chem.* **256:** 4425–4432.
6. Tence, M., E. Coeffier, F. Heymans, J. Polonsky, J. J. Godfroid, and J. Benveniste. 1981. Structural analogs of platelet-activating factor (PAF-acether). *Biochimie.* **63:** 723–727.
7. Blank, M. L., E. A. Cress, T-c. Lee, B. Malone, J. R. Surles, C. Piantadosi, J. Hajdu, and F. Snyder. 1982. Structural features of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) required for hypotensive and platelet serotonin responses. *Res. Commun. Chem. Pathol. Pharmacol.* **38:** 3–20.
8. O'Flaherty, J. T., W. L. Salzer, S. Cousart, C. E. McCall, C. Piantodosi, J. R. Surles, M. J. Hammett, and R. L. Wykle. 1983. Platelet-activating factor and analogues: comparative studies with human neutrophils and rabbit platelets. *Res. Commun. Chem. Pathol. Pharmacol.* **39:** 291–309.
9. Lewis, E. N., R. Bittman, and I. W. Levin. 1986. Methyl-group substitution at C(1), C(2), or C(3) of the glycerol backbone of a diether phosphocholine: a comparative study of bilayer chain disorder in the gel and liquid crystalline phases. *Biochim. Biophys. Acta.* **861:** 44–52.
10. Witzke, N. M., and R. Bittman. 1986. Convenient synthesis of racemic mixed-chain ether glycerophosphocholines from fatty alkyl allyl ethers: useful analogs for biophysical studies. *J. Lipid Res.* **27:** 344–351.
11. Wykle, R. L., B. Malone, and F. Snyder. 1980. Enzymatic synthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a hypotensive and platelet-aggregating lipid. *J. Biol. Chem.* **255:** 10256–10260.
12. Lee, T-c. 1985. Biosynthesis of platelet activating factor: substrate specificity of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine: acetyl-CoA acetyltransferase in rat spleen microsomes. *J. Biol. Chem.* **260:** 10952–10955.
13. Robinson, M., M. L. Blank, and F. Snyder. 1985. Acylation of lysophospholipids by rabbit alveolar macrophages: specificities of CoA-dependent and CoA-independent reactions. *J. Biol. Chem.* **260:** 7889–7895.
14. Blank, M. L., T-c. Lee, V. Fitzgerald, and F. Snyder. 1981. A specific acetylhydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid). *J. Biol. Chem.* **256:** 175–178.
15. Blank, M. L., M. N. Hall, E. A. Cress, and F. Snyder. 1983. Inactivation of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by a plasma acetylhydrolase: higher activities in hypertensive rats. *Biochem. Biophys. Res. Commun.* **113:** 666–671.
16. Hoffman, D. R., J. Hajdu, and F. Snyder. 1984. Cytotoxicity of platelet activating factor and related alkyl-phospholipid analogs in human leukemia cells, polymorphonuclear neutrophils, and skin fibroblasts. *Blood.* **63:** 545–552.

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