Notes

Structure-Activity Relationship in PAF-acether. 2. *rac*-1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)propyl]glycerol

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Two products without phosphoryl groups, 1-*O*-octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)propyl]glycerol and its quaternary salt, were synthesized from 1-*O*-octadecyl-2-*O*-benzylglycerol. In comparison with PAF-acether, they lost aggregating and bronchoconstrictive activities and did not show any antagonistic effects.

The PAF-acether (platelet activating factor), or 1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine, is a mediator of anaphylaxis and inflammation. It aggregates rabbit, guinea pig, and human platelets; for washed rabbit platelets, the EC_{50} values are 5.7×10^{-11} M for the *R* form and 2.2×10^{-10} M for the racemate form.[1]

The influence of the nature of various substituents at positions 1 and 2 on the biological activity was examined to determine the necessity for the presence of the glycerol skeleton. In a previous work[2] we reported the synthesis of 1-[2-(acetyloxy)docosyl]phosphocholine and we found that the absence of an ether group at the 1-position led to a dramatic decrease in aggregating activity (3.9×10^{-5} M).

Likewise, Wykle et al.[3] described the synthesis of PAF analogues lacking oxygen at the 2-position and found that a nonester group (*n*-propyl) can replace the acetyl group with retention of activity (4×10^{-9} M).

Here, we report the total synthesis of the 1-*O*-octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)propyl]glycerol and its quaternary salt, a compound that possesses the glycerol skeleton but in which the phosphoryl group is replaced by a γ-amino ether.

Chemistry. 1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)propyl]glycerol hydrochloride (**4**) was synthesized as outlined in Scheme I. The starting material was 1-*O*-octadecyl-2-*O*-benzylglycerol (**1**), obtained in three steps (tritylation, benzylation, detritylation) from 1-*O*-octadecylglycerol as described previously.[1,4] Compound **1** reacted with γ-(dimethylamino)propyl methanesulfonate prepared according to Toldy[5] to give 1-*O*-octadecyl-2-*O*-benzyl-3-*O*-[γ-(dimethylamino)propyl]glycerol (**2**). The removal of the benzyl group by catalytic reduction (H_2/Pd) gave **3**. Acetylation of **3** by acetyl chloride yielded 1-*O*-octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)propyl]-glycerol hydrochloride (**4**). The quaternary ammonium salt prepared by the classical treatment of **2** with methyl iodide yielded **5**. For biological test purposes, the chloride ammonium salt is preferred to iodide. The conversion to chloride was accomplished by evaporation, on a steam bath through a distillation condenser, of a solution of **5** in excess methanolic hydrogen chloride.[6] This conversion was accompanied by complete debenzylation and gave [3-(1-*O*-octadecylglycero-3)propyl]trimethylammonium chloride (**6**). Acetylation by acetyl chloride afforded [3-(1-*O*-octa-

Scheme I

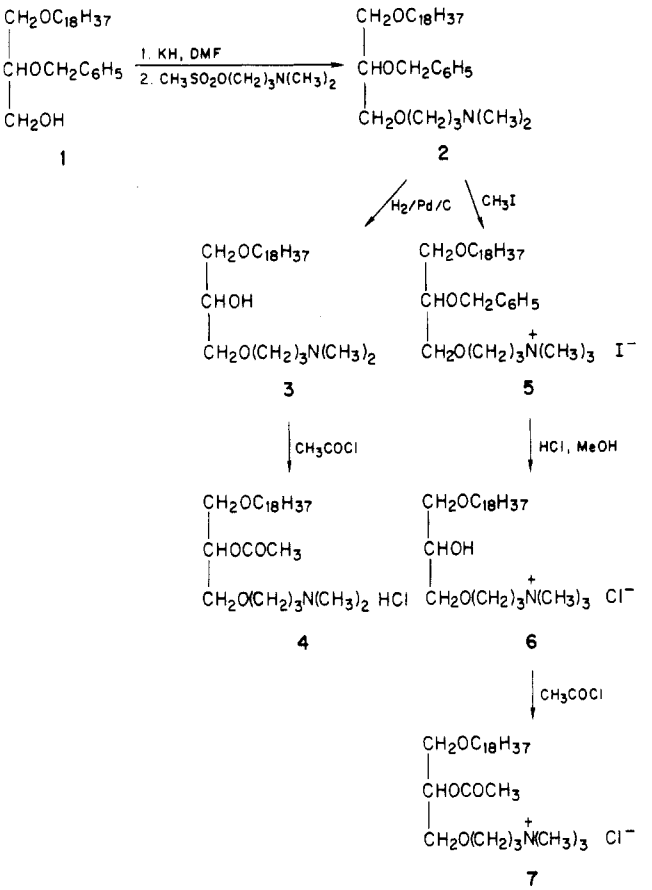

Table I. Platelet Aggregating Activity in Vitro

compd	act.: EC_{50},[a] M
(*R*)-PAF-acether	$(5.7 \pm 0.5) \times 10^{-11}$
(*RS*)-PAF-acether	$(2.2 \pm 0.7) \times 10^{-10}$
4	$(1.7 \pm 0.4) \times 10^{-7}$ [b]
7	$>4 \times 10^{-6}$ [c]

[a] Final molar concentration for 50% of maximal aggregation of washed rabbit platelets induced by a standard solution of synthetic PAF-acether, mean ± 1 SD of three experiments. [b] No lysis at these doses. [c] Lysis at 10^{-5} M.

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Table II. Platelet Aggregating and Bronchoconstrictive Activities in Vivo

compd	bronchoconstriction concn	cm of H_2O	%[a]	aggregation,[b] %
(*R*)-PAF-acether	33 ng/kg	17.2		48
4	33 ng/kg	0	100	0
	66 ng/kg	1.2		0
7	520 μg/kg	0	100	0

[a] Percent of decrease of bronchoconstrictive activity with regard to PAF-acether. [b] Percent of drop in platelet counts between 2 min before and 1 min after injection.

decyl-2-*O*-acetylglycero-3)propyl]trimethylammonium chloride (7).

Biological Results and Discussion

1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)-propyl]glycerol hydrochloride (4) and its ammonium salt 7 were tested for their aggregating activity in vitro and in vivo and for their bronchoconstrictive activity.

In Vitro Biological Assay. Table I shows the platelet aggregating activity of the two compounds. Compound 4 is 10^4 times less active than (*R*)-PAF-acether; 7 was not found to aggregate until 10^{-6} M concentration.

In Vivo Testing: Bronchoconstrictive and Platelet Aggregating Activities. The results are summarized in Table II. No significant platelet aggregation or bronchoconstriction activities were obtained. The two compounds seem inactive, especially the quaternary ammonium salt. They neither inhibit PAF-acether nor affect PAF-acether-induced bronchoconstriction.

The different tests show that a phosphoryl group at the 3-position plays a role in PAF-acether activities. 1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)propyl]-glycerol hydrochloride (4) and its quaternary ammonium salt 7 lost any PAF-acether characteristic activity and are not antagonist to PAF. These results are not due to the fact that the two compounds are racemic: (*R*)-PAF-acether and the racemate (*RS*) form have very close activities (Table I).

Experimental Section

Materials and Methods. A. Biological Methods. In Vitro Biological Assay. The platelet aggregating activity of PAF-acether and compounds 4 and 7 was performed on washed rabbit platelets as described previously.[7] Aggregations were assessed on 1.5×10^8 platelets treated with 0.1 mM Aspegic and stirred in 300 μL of Tyrode's gelatin in the presence of the complex CP/CPK (1 mM/10 U per 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. Results shown in Table I are the mean of ±1 standard deviation (SD) of three independent experiments. Platelet lysis was investigated by measuring the lactate dehydrogenase activity in platelet supernatants according to the method of Wroblewski and La Due.[8]

In Vivo Testing. PAF-acether, 4 and 7 were tested by the intravenous (iv) route in pentobarbitone (30 mg/kg, ip) in anesthetized guinea pigs. The number of free circulating platelets and the bronchial resistance to inflation (or bronchoconstriction) were recorded as described previously.[9] PAF-acether was injected at 30–70 ng/kg whereas the ether derivatives 4 and 7 were injected iv in variable concentrations followed by PAF-acether. This procedure allows measurement of intrinsic activity of a given product and evaluation of potential antagonism of PAF-acether effects. Serotonin (5HT) was used as a standard bronchoconstrictor agent (1–2 μg/kg).

B. 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 carried out on silica gel (Merck, particle size 0.063–0.200 mm), without any special treatment. Melting points were determined on a hot stage microscope (Reichert Thermovar). The structures of all compounds were confirmed by IR (Pye-Unicam SP3-200) and ^{1}H NMR (Varian EM 360 or Brücker 250 MHz) in $CDCl_3$ or CD_3OD with Me_4Si as an internal standard.[10] Chemical-ionization mass spectra were obtained at 220 °C on a modified AEI spectrometer.[11] Elemental analyses[11] are indicated only by symbols of elements and are consistent with the proposed structures.

1-*O*-Octadecyl-2-*O*-benzyl-3-*O*-[γ-(dimethylamino)-propyl]glycerol (2). 1-*O*-Octadecyl-2-*O*-benzylglycerol (1; 4.6 mmol) and KH (1 g, 35% suspension in paraffin oil) in 20 mL of DMF were stirred for 30 min at room temperature. A solution of γ-(dimethylamino)propyl methanesulfonate (14.5 mmol) freshly prepared was added dropwise and the mixture was stirred overnight at room temperature. The DMF was evaporated in vacuo and the residue, diluted with water, was extracted with $CHCl_3$ (emulsions were broken, if necessary, by adding small amounts of MeOH). The organic layer was washed with water until pH 7 was reached, dried (Na_2SO_4), and evaporated in vacuo to give an oily residue. Chromatography on a silica gel column using from 5–20% MeOH in $CHCl_3$ as eluents yielded pure 2 (52%): mp 77 °C; R_f 0.49 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v); IR (film) 3090–2870 (CH), 2820, 2800, 2770 (NCH_3), 1100 large (CO) cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.88 (t, 3 H, CH_3), 1.26 (large s, 30 H, $(CH_2)_{15}$), 1.57 (m, 2 H, $OCH_2CH_2C_{16}$), 1.76 (q, 2 H, $OCH_2CH_2CH_2N$), 2.24 (s, 6 H, $N(CH_3)_2$), 2.35 (t, 2 H, CH_2N), 3.38–3.57 (m, 8 H, CH_2O), 3.72 (q, 1 H, CHO), 4.70 (s, 2 H, $CH_2C_6H_5$), 7.33 (m, 5 H, C_6H_5). Anal. ($C_{33}H_{61}NO_3$) C, H, N.

1-*O*-Octadecyl-3-*O*-[γ-(dimethylamino)propyl]glycerol (3). A mixture of 2 (1 mmol) and Pd/C, 10% in 20 mL of absolute EtOH, was hydrogenated at 50 °C for 12 h (20 psi). After filtration on paper, the catalyst was washed several times with MeOH and $CHCl_3$. The solvents were removed, and the residue was purified on a silica gel column with use of MeOH/$CHCl_3$ (10:90, v/v) as eluent for eluting the remaining triether 2 and then with use of MeOH/$CHCl_3$ (15:85, v/v) for the "lyso" derivative 3 (yield 30%): R_f 0.26 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v); IR (film) 3360 (OH), 1110 (COC) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3 H, CH_3), 1.20 (large s, 30 H, $(CH_2)_{15}$), 1.50 (q, 2 H, $OCH_2CH_2C_{16}$), 1.80 (q, 2 H, $OCH_2CH_2CH_2N$), 2.36 (s, 6 H, $N(CH_3)_2$), 2.58 (t, 2 H, CH_2N), 2.85 (m, 1 H, OH), 3.34–3.60 (m, 8 H, CH_2O), 3.88 (q, 1 H, CHOH).

1-*O*-Octadecyl-2-*O*-acetyl-3-*O*-[γ-(dimethylamino)-propyl]glycerol Hydrochloride (4). A mixture of 3 (0.3 mmol) and CH_3COCl (0.1 mL) in 5 mL of anhydrous ethanol-free $CHCl_3$ was stirred overnight at room temperature. The disparition of 3 was checked by TLC ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v). Evaporation of $CHCl_3$ and an excess of CH_3COCl in vacuo gave the final product 4 (yield 95%): R_f 0.37 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v); IR (Nujol) 2460 (NH), 1725 (C=O), 1240 (COC ester), 1015 (COC ether) cm^{-1}; ^{1}H NMR (CD_3OD) δ 0.90 (t, 3 H, CH_3), 1.29 (large s, 30 H, $(CH_2)_{15}$), 1.56 (m, 2 H, $OCH_2CH_2C_{16}$),

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(10) ^{1}H NMR spectra were performed by O. Convert, Laboratoire de Chimie Organique Structurale, Université P. et M. Curie, 75230 Paris Cedex 05, France.
(11) Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique (CNRS), Gif sur Yvette, France.

2.01 (q, 2 H, $OCH_2CH_2CH_2N^+$), 2.09 (s, 3 H, CH_3CO), 2.91 (s, 6 H, $^+N(CH_3)_2$), 3.24 (t, 2 H, $OCH_2CH_2CH_2N^+$), 3.46 (m, 2 H, OCH_2C_{17}), 3.55 (d, 2 H, CH_2OC_{18}), 3.62 (m, 4 H, $CH_2O + CH_2N^+$), 5.17 (q, 1 H, CHOCO); mass spectrum, m/z 472 ($MH^+ - HCl$). Anal. ($C_{28}H_{58}NO_4Cl \cdot 2H_2O$) C, H, N, Cl.

[3-(1-*O*-Octadecyl-2-*O*-benzylglycero-3)propyl]trimethylammonium Iodide (5). Compound **2** (1.37 mmol) and MeI (13.7 mmol) in 10 mL of dry acetone were stirred overnight at room temperature. Acetone and the excess of MeI were evaporated, and the residue was purified on a silica gel column using $CHCl_3$/MeOH (90:10, v/v) as eluent to yield **5** (48%): R_f 0.29 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v); ^1H NMR (80 MHz, CD_3OD) δ 0.82 (t, 3 H, CH_3), 1.22 (large s, 32 H, $(CH_2)_{16}$), 2.00 (m, 2 H, $OCH_2CH_2CH_2N^+$), 3.05 (s, 9 H, $^+N(CH_3)_3$), 3.27–3.75 (m, 11 H, $CH_2O + CHO + CH_2N^+$), 4.61 (s, 2 H, $CH_2C_6H_5$), 7.27 (large s, 5 H, C_6H_5).

[3-(1-*O*-Octadecylglycero-3)propyl]trimethylammonium Chloride (6). A solution of 0.1–0.2 mol of HCl in 40 mL of MeOH was added to **5** (1.5 mmol). MeOH and MeI were distillated under normal pressure. This operation was repeated twice. The remaining residue was chromatographed on a silica gel column using $CHCl_3$/MeOH (80:20, v/v) as eluent. This purification yielded pure **6** (40%): R_f 0.12 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v).

[3-(1-*O*-Octadecyl-2-*O*-acetylglycero-3)propyl]trimethylammonium Chloride (7). Compound **6** was acetylated in the same manner as described above for **4** and gave **7** in 100% yield: R_f 0.13 ($CHCl_3$/MeOH/H_2O, 80:20:2, v/v/v); IR (film) 3400, 1640 (H_2O), 1730 (C=O), 1260 (COC ester), 1020 (COC ether) cm^{-1}; ^1H NMR (CD_3OD) δ 0.81 (t, 3 H, CH_3), 1.21 (large s, 30 H, $(CH_2)_{15}$), 1.45 (m, 2 H, $OCH_2CH_2C_{16}$), 1.95 (m + s, 5 H, $OCH_2CH_2CH_2N^+ + CH_3CO$), 3.05 (s, 9 H, $^+N(CH_3)_3$), 3.26–3.56 (m, 10 H, $CH_2O + CH_2N^+$), 5.04 (q, 1 H, CHOCO); mass spectrum, m/z 472 ($MH^+ - CH_3Cl$). Anal. ($C_{29}H_{60}NO_4Cl \cdot 2H_2O$) C, H, N, Cl.

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Improved Synthesis of 2′-Deoxyformycin A and Studies of Its in Vitro Activity against Mouse Lymphoma of T-Cell Origin

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7-Amino-3-(2′-deoxy-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (2′-deoxyformycin A) was synthesized from formycin A by a sequence consisting of (i) 3′,5′-cyclosilylation with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, (ii) 2′-acylation with phenoxythiocarbonyl chloride and 4-(*N*,*N*-dimethylamino)pyridine, (iii) N-trimethylsilylation with hexamethyldisilazane, (iv) reduction of the 2′-*O*-phenoxythiocarbonyl group with tri-*n*-butyltin hydride, and (v) desilylation with tetra-*n*-butylammonium fluoride. 2′-Deoxyformycin A was a potent inhibitor of the in vitro growth of S49 lymphoma, a murine tumor of T-cell origin. The IC_{50} of 2′-deoxyformycin A against S49 cells was 10–15 μM, whereas that of 2′-deoxyadenosine (dAdo) under the same conditions (72-h incubation in medium containing heat-inactivated horse serum) was 180 μM. In the presence of 10 μM *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) to block intracellular adenosine deaminase (ADA) activity, 2′-deoxyformycin A and dAdo both gave IC_{50}'s of 5–10 μM. When assayed against a mutant S49 subline lacking adenosine kinase (AK) or a subline with a combined deletion of AK and deoxycytidine kinase (dCK), 2′-deoxyformycin A in combination with 10 μM EHNA was inactive at concentrations of up to 50 μM. Similar lack of activity against kinase-deficient cells was shown by formycin A. Thus, phosphorylation of 2′-deoxyformycin A appears to be required for biological activity and is probably catalyzed by AK rather than dCK. 2′-Deoxyformycin A and related 2′-deoxyribo-C-nucleoside analogues of the purine type may be of interest as potential T-cell specific cytotoxic agents.

Formycin A (7-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (1) is a C-nucleoside antibiotic with biochemical and biological properties that reflect its ability to mimic adenosine in being incorporated into RNA.[1] The resultant production of altered RNA species is lethal to cells. For a number of years, clinical enthusiasm for 1 was dampened by the fact that it is hydrolyzed readily by adenosine deaminase (ADA) to the less active 7(6*H*)-oxo derivative, formycin B.[2] Recently, interest in the potential of 1 as an anticancer agent has been rekindled by the finding that, in combination with an ADA inhibitor, this compound is superior to adenine arabinoside (*ara*-A) in prolonging the life of mice with L1210 leukemia.[3] Moderate antiviral activity is also observed.[4] Moreover, it has been demonstrated in mammalian cells[5,6] and in *Leishmania*[7,8] that the adenylosuccinate synthetase/lyase system provides a pathway for the metabolic regeneration of

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