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SYNTHESIS OF SOME DERIVATIVES OF GLYCEROPHOSPHOCHOLINES AND THEIR INFLUENCE ON THE CYTOTOXIC ACTIVITY OF PLATELETS

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At the present time great significance in processes of oncogenesis is attributed to platelets, which possess spontaneous cytotoxic activity against malignantly transformed cells [1, 6]. As is well known, platelet activation is modulated by numerous factors, among which platelet activation factor (PAF) is of special significance [2].

Considering the specific properties of PAF in the activation of platelets, as well as the existing information on the antitumor activity of glycerophosphocholine derivatives of PAF, in this work we studied the possibility of enhancing the spontaneous antitumor cytotoxicity of platelets using 1-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) and 1-hexadecyl-sn-glycero-3-phosphocholine (lyso-PAF).

A racemate of PAF (VI) was synthesized using 2-chloro-2-oxo-1,3,2-dioxaphospholane according to schemes A and B, which made it possible to reduce the number of steps of the synthesis in comparison with the previously described methods [3, 5]. The starting material in both schemes is chymyl alcohol (I). According to scheme A, first 1-hexadecyl-2-acetylglycerol (IV) was produced from it, using the method of silyl protection; the phosphorylation of (IV) under mild conditions (−10°C) yields the corresponding dioxaphospholane derivative (V), which can be converted further to VI by the well-known method of [7]. According to scheme B, I first is selectively phosphorylated in the 3-position of the glycerol skeleton and then, after acetylation in the 2-position, VI is obtained.

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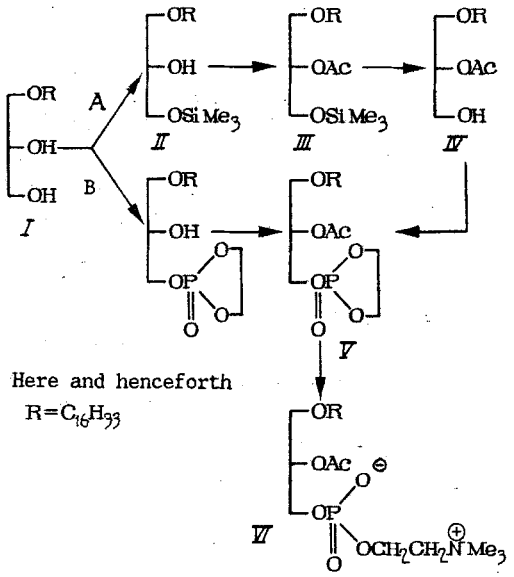
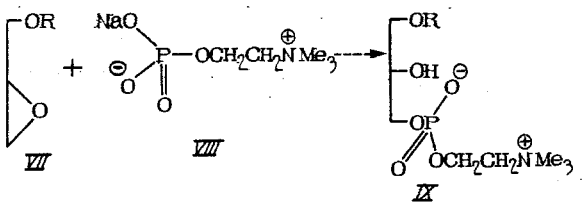


The racemate of lyso-PAF (IX) was synthesized from hexadecyldiglycidyl ester and choline phosphate according to the scheme described in [4]. It was shown that the use of the sodium salt of choline phosphate (instead of the potassium acetate catalyst) significantly increases the yield of the end product and decreases the polymer impurities.



The PAF and lyso-PAF obtained were purified by TLC or liquid chromatography. Their structure was demonstrated by PMR methods.

EXPERIMENTAL (CHEMICAL)

The PMR spectra were recorded on the EM-390 (Varian), working frequency 90 mHz and FT-80A (Varian), working frequency 80 mHz, external standard TMS; the ^{31}T NMR spectra were recorded on the HA-100D (Varian), working frequency 40.5 mHz and FT-80A (Varian), working frequency 32 mHz. The external standard was 85% H_3PO_4.

1-Hexadecyl-3-trimethylsilylglycerol (II). To a solution of 2 g (6 mmoles) 1-hexadecylglycerol I and 0.6 g (6 mmoles) Et_3N in 90 ml of toluene, a solution of 0.63 g (6 mmoles) Me_3SiCl was added at −10°C with mixing; the reaction mixture was exposed for 2 h, filtered, the solvent was removed under vacuum, the residue was dissolved in hexane and filtered, and the hexane was removed under vacuum. Yield 1.1 g (92%) II. PMR spectrum, δ, ppm: 0.0 (9H, s, $SiMe_3$), 0.85 (3H, t, CH_3), 1.3 (28H, s, $14CH_2$), 2.9 (1H, s, OH), 3.4-4.0 (7H, m, CHO, $3CH_2O$).

1-Hexadecyl-2-acetyl-3-trimethylsilylglycerol (III). To a solution of 2.1 g (6 mmoles) (II) and 0.6 g (6 mmoles) Et_3N in 100 ml of toluene, a solution of 0.45 g (6 mmoles) acetyl chloride in 10 ml of toluene was added dropwise with mixing at −10°C. The reaction mixture was exposed at −10°C for 2 h, then at room temperature for 1 h, then filtered; the solvent was removed under vacuum. Yield 2.55 g (95%) III. PMR spectrum, δ, ppm: 0.0 (9H, s, $SiMe_3$), 0.85 (3H, t, CH_3), 1.25 (28H, s, $14CH_2$), 2.1 (3H, s, $COCH_3$), 3.5-4.0 (7H, m, CHO, $3CH_2O$).

1-Hexadecyl-2-acetylglycerol (IV). To a solution of 2.58 g (6 mmoles) III in 30 ml of methanol we added 0.34 g (6 mmoles) ammonium bifluoride; the reaction mixture was exposed for 3 h with mixing, the solvent was removed under vacuum, the residue was dissolved in chloroform, and reprecipitation with acetone yielded 1.13 g (52%) IV, R_f 0.8 (Silufol, ether–hexane, 4:1; developer morphine). PMR spectrum, δ, ppm: 0.9 (3H, t, CH_3), 1.3 (28H, s, $14CH_2$), 2.1 (3H, s, $COCH_3$), 3.4-4.1 (7H, m, CHO, $3CH_2O$).

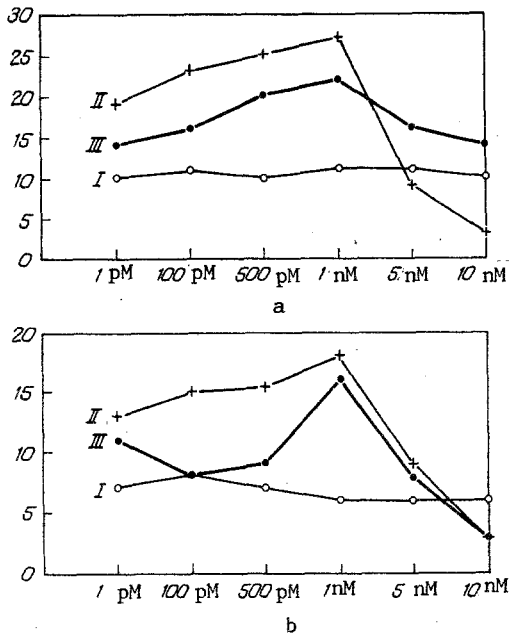


Fig. 1. Comparative analysis of the cytotoxic effect of donor and patient platelets in the case of incubation with PAF and lyso-PAF (% cytotoxicity). Donor (a) and patient (b) platelets were cultured in a 50:1 ratio with Na_2CrO_4-labeled cells of pulmonary adenocarcinoma for 18 h in the presence of PAF (II) and lyso-PAF (III) in the concentration range from 10 pM to 10 nM. Both PAF and lyso-PAF enhanced the initial cytotoxicity (I) of platelets; the maximum enhancement is at a concentration of 1 nM.

1-Hexadecyl-2-acetylglycero-3-(2-oxo-1,3,2-dioxa)phospholane (V). A. To a solution of 1.75 g (4.5 mmoles) IV and 0.46 g (4.5 mmoles) Et_3N in 25 ml of toluene in an atmosphere of argon, 0.65 g (4.5 mmoles) 2-chloro-2-oxo-1,3,2-dioxaphospholane in 5 ml of toluene was added dropwise with mixing at −10°C. The reaction mixture was exposed for 3 h at room temperature, filtered, and a toluene solution of V was obtained. ^{31}P NMR, δ, ppm: −18.7. PMR spectrum of V, δ, ppm: 0.9 (3H, t, CH_3), 1.28 (28H, s, $14CH_2$), 2.06 (3H, s, $COCH_3$), 3.0-4.5 (11H, m, CHO, $5CH_2O$) B. To a solution of 2 g (6 mmoles) I and 0.6 g (6 mmoles) Et_3N in 80 ml of toluene, a solution of 0.83 g (6 mmoles) 2-chloro-2-oxo-1,3,2-dioxaphospholane in 10 ml of toluene was added dropwise with mixing in an atmosphere of argon at 0°C; the reaction mixture was exposed for 2 h at 0°C, then for 1 h at 20°C. A solution of V with the indices cited above was obtained.

1-Hexadecyl-2-acetylglycero-3-phosphocholine (VI). To a toluene solution of V we added Et_3N (fourfold excess); the reaction mixture was exposed in a sealed ampule at 60-70°C for 48 h, the solvent was removed under vacuum, and unpurified VI was isolated with a yield of 80%. From 2.5 g unpurified VI, TLC (silica gel L 40/100, $CHCl_3$–MeOH–water, 65:35:8) yielded 0.76 g VI, R_f 0.42, ^{31}P NMR, δ, ppm 1.3; PMR spectrum, δ, ppm: 0.9 (3H, t, CH_3), 1.24 (28H, s, $14CH_2$), 2.1 (3H, s, $COCH_3$), 3.35 (9H, s, NMe_3), 3.5-4.2 (11H, m, CHO, $5CH_2O$).

1-Hexadecylglycero-3-phosphocholine (IX). To a solution of 6 g (20 mmoles) hexadecylglycidyl ester VII in 10 ml of MeOH we added 3.7 g (20 mmoles) choline phosphate and 2 g (10 mmoles) of the sodium salt of choline phosphate VIII; the reaction mixture was boiled for 20 h, cooled to room temperature, hydrochloric acid was added to a neutral pH, and the mixture was separated on a chromatographic column (length 300 mm, diameter 25 mm, silica gel L 100/250, eluent MeOH). Yield 6.9 g (71.6%) IX, R_f 0.22 (Silufol, eluent $CHCl_3$–MeOH–water, 65:35:6, developer iodine or Vas'kovskii's reagent). PMR spectrum, δ, ppm: 0.9 (3H, t, CH_3), 1.26 (28H, s, $14CH_2$), 3.35 (9H, s, $\overset{+}{N}Me_3$), 3.5-4.2 (11H, CHO, $4CH_2O$, CH_2N). Elemental analysis corresponds to the gross formula $C_{24}H_{52}NPO_6$.

EXPERIMENTAL (BIOLOGICAL)

The cytotoxicity of the platelets of 70 cancer patients with various localizations of the tumor process (ages from 30 to 70): lung cancer in 24 patients, breast cancer in 16 patients, stomach cancer in 10, and Kaposi's sarcoma in 20 patients, was studied. The cytotoxicity of the platelets of 30 donors was investigated as a control.

Portions of 10 ml of peripheral venous blood were drawn from the patients examined and the donors of the control group into test tubes with heparin (15 units/ml). Platelet-enriched plasma was produced by centrifuging the heparinized blood at 200g (10 min at 4°C); then the plasma was centrifuged at 1600g (20 min at 4°C). The platelet pellet obtained was resuspended in 1 ml of medium (RPMI-1640 medium, Gibco, Flow) containing 5-10% inactivated human serum (2-3)·10 L-glutamine (Gibco), 5 mM HEPES (Gibco), (3-5)·10 M 2-mercaptoethanol (Merck), 100 units penicillin, and 100 μg streptomycin per ml of medium. The number of platelets was counted in a Goryaev chamber. There were no extraneous mononuclear peripheral blood cells in the platelet suspension obtained.

The following lines of tumor target cells were used in the work: ACL – a continuous monolayer culture of pulmonary adenocarcinoma cells (E. A. Timofeevskaya et al., 1983).

On the day before the experiment, the tumor target cells were labeled with 25-50 μCi $Na_2^{51}CrO_4$ (Medical Radioactive Preparations Factory) on a water bath at 37°C. After 45-60 min, the cells were washed three times with RPMI-1640 medium and introduced in a concentration of $2 \cdot 10^4$ cells per well into 96-well plates (Falcon) with a flat bottom. Platelets were added to the target cells in ratios 100:1, 50:1, 25:1, and 12:1 in a total volume of 0.2 ml of medium per well. They were incubated at 37°C in an atmosphere with 5% CO_2 in a medium (RPMI-1640, Gibco, Flow) containing from 10 nM to 10 pM PAF. After 18 h of incubation, the supernatant liquid was cautiously collected from each well into plastic ampules for determination of the radioactivity in a gamma spectrometer.

Figure 1 (a, b) shows a comparative analysis of the cytotoxic effect of the platelets of donors and cancer patients, stimulated by PAF and its precursor lyso-PAF. Incubation of donor and patient platelets with PAF and its analog lyso-PAF in the concentration range from 10 pM to 1.0 nM led to an enhancement of the platelet cytotoxicity. When the donor platelets were activated with PAF (see Fig. 1a), the initial cytotoxicity, an average of 13.8 ± 3.8%, was increased to 26.1 ± 2.1%, whereas when the platelets were activated with lyso-PAF it was increased to 21.2 ± 2.2%. A comparison of the cytotoxic activation of the patient platelets (see Fig. 1b) by PAF and lyso-PAF revealed that in the case of incubation with PAF the average initial platelet cytotoxicity was increased from 6.4 ± 1.2% to 18.1 ± 2.40%, whereas in the case of incubation with lyso-PAF it was increased to 15.6 ± 2.60%. The action of PAF and lyso-PAF is most effective in concentrations of 500 pM to 1 nM. Further increasing the concentration both of PAF and of lyso-PAF causes a decrease in the cytotoxic effect of the platelets. One of the peculiarities of the action of PAF is its ability to enhance the cytotoxic effect of platelets possessing low activity to a greater degree. The cytotoxic effect of platelets was increased by a factor of 4.02 ± 0.5, both for donors and for cancer patients, in the case of incubation with PAF when their initial cytotoxicity did not exceed 10%. Killer activity of the platelets exceeding 10% was increased by a factor of 1.3 ± 0.2 when they were incubated with PAF.

Thus, PAF and its lyso-form (lyso-PAF) are capable of activation the antitumor killer activity of platelets; a greater degree of activation is observed in cases when there is a low initial platelet cytotoxicity.

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