ELSEVIER

Chemistry and Physics of Lipids
81 (1996) 35–43

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

On the synthesis of platelet-activating factor via acetylation of 1-alkyl-*sn*-glycero-3-phosphocholine. Formation of structural isomer of PAF in the presence of bases

V.V. Chupin*, O.V. Ostapenko, V.N. Klykov, M.V. Anikin, G.A. Serebrennikova

M.V. Lomonosov State Academy of Fine Chemical Technology, 86 pr. Vernadskogo Moscow 117571, Russia

Received 19 June 1995; revised 30 January 1996; accepted 31 January 1996

Abstract

It has been shown that platelet-activating factor (PAF) specimens prepared via acetylation of 1-alkyl-*sn*-glycero-3-phosphocholine (lyso-PAF) with acetic anhydride are heterogeneous. The contaminated compound was isolated and identified to be the structural isomer of PAF, 1-alkyl-3-acetyl-*sn*-glycero-2-phosphocholine (iso-PAF). It appeared, that iso-PAF is formed when performing the reaction in the presence of organic bases, but not under acid catalysis. The mechanism of iso-PAF formation is discussed.

Keywords: Platelet-activating factor; Positional isomer; Lyso-platelet-activating factor; Acetylation

1. Introduction

Lipid platelet-activating factor (PAF, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is known to be a potent immunobiological regulator possessing a wide range of biological effects [1,2]. Extensive biological investigation of the PAF functions has spurred the elaboration of synthetic ways to preparation of PAF and its analogs. Since its structure was determined in the late seventies [3,4], a good number of studies have been devoted to the synthesis of PAF [5–16]. The reported methods of PAF production involve means which are conventional for phospholipid chemistry. These are based on either: (i) phosphorylation of 1-alkyl-2-acetyl-*sn*-glycerols [7,8,10,16]; or (ii) acetylation of 1-alkyl-*sn*-glycero-3-phosphocholines (lyso-PAF) [5,6,9,11–16].

In our laboratory we tried to adopt an efficient and reliable synthetic route of PAF preparation. It was obvious that the former of the above approaches can lead to the mixture of positional isomers of PAF, as the acetyl residue can migrate to the position 3 of glycerol during phosphorylation [17]. We focused therefore on the latter approach since it has not been reported so far to give positional isomers. Nevertheless after thorough elaboration of the acetylation under standard conditions, we found that carrying out the reaction in the presence of bases leads to contamination of the desired PAF with its positional

* Corresponding author. Tel.: +7 (095) 437 1925.

PII S0009-3084(96)02530-3

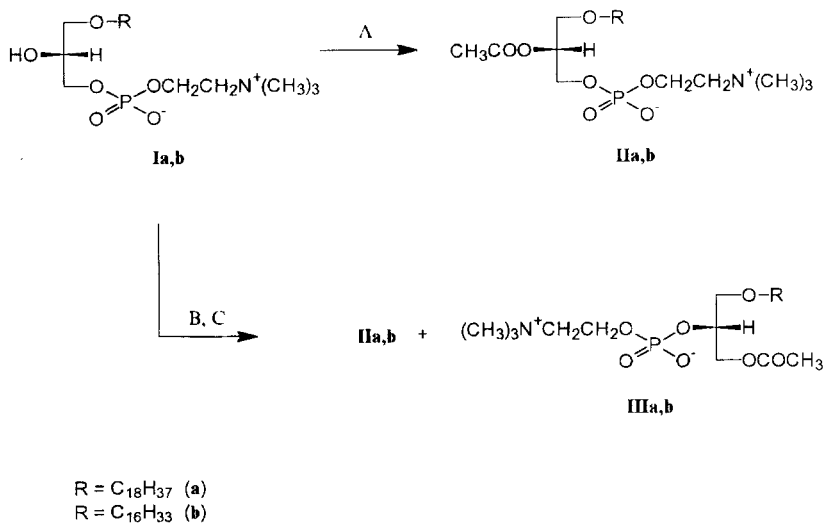

Fig. 1. Acetylation of lyso-PAF. A: $(CH_3CO)_2O/HClO_4$; B: $(CH_3CO)_2O/(C_2H_5)_3N$; C: $(CH_3CO)_2O$/DMAP.

isomer, 1-alkyl-3-acetyl-*sn*-glycero-2-phosphocholine (iso-PAF). In the present paper we discuss the possible mechanism of iso-PAF formation[1].

2. Materials and methods

1-*O*-Octadecyl-*sn*-glycero-3-phosphocholine (**Ia**) and 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (**Ib**) were prepared by the treatment of corresponding 1-*O*-alkyl-2-*O*-palmitoyl-*rac*-glycero-3-phosphocholines with phospholipase A_2 in conditions described in Section 2.3. Whole venom of viper *Echis multisqu amatus* was used as phospholipase A_2 preparation. 1-*O*-Octadecyl-*rac*-glycerol was obtained by standard synthesis [19] and choline *p*-toluenesulfonate was synthesized in accordance with Ref. [20]. 1-*O*-Octadecyl-3-*O*-lauroyl-*rac*-glycerol was obtained by acylation of 1-*O*-octadecyl-*rac*-glycerol with lauroyl chloride. Preparative chromatographic separations were done on silica gel L 40/100 (Chemapol). TLC was carried out on Silufol UV-240 plates (Kavalier). The mobile phase was chloroform/methanol/water (65:25:4 v/v) unless otherwise indicated. Spots were visualized by heating over a flame, or by using a molybdenum spray [21] (for phosphorus-containing compounds).

^{1}H-NMR spectra were taken on a Bruker MSL-200 spectrometer, ^{13}C- and ^{31}P-NMR spectra were recorded on a Bruker MSL-300 instrument. Chemical shifts are given in p.p.m. relative to tetramethylsilane in ^{1}H- and ^{13}C-NMR spectra, and relative to 85% phosphoric acid in ^{31}P-NMR spectra. Samples were dissolved in $CDCl_3$/CD_3OD/D_2O (1:1:0.15, v/v) unless otherwise indicated. In obtaining the ^{31}P-NMR spectra with no decoupling, the free induction decays were subjected to Gaussian multiplication.

Optical rotations were measured on a Perkin-Elmer 241 MC spectropolarimeter at 20°C.

2.1. 1-O-Octadecyl-2-O-acetyl-sn-glycero-3-phosphocholine (IIa) and 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine (IIb)

2.1.1. Acetylation under acid catalysis

Acetic anhydride (0.6 ml, 6.4 mmol) was added to a stirring solution of 1-*O*-octadecyl-*sn*-glycero-3-phosphocholine (98 mg, 0.19 mmol) in 3 ml of chloroform. Perchloric acid (57%, 0.3 ml) was then added dropwise, the mixture was stirred at room temperature for 1 min and cooled to 0°C.

[1] We have reported preliminary data of this study in Ref. [18].

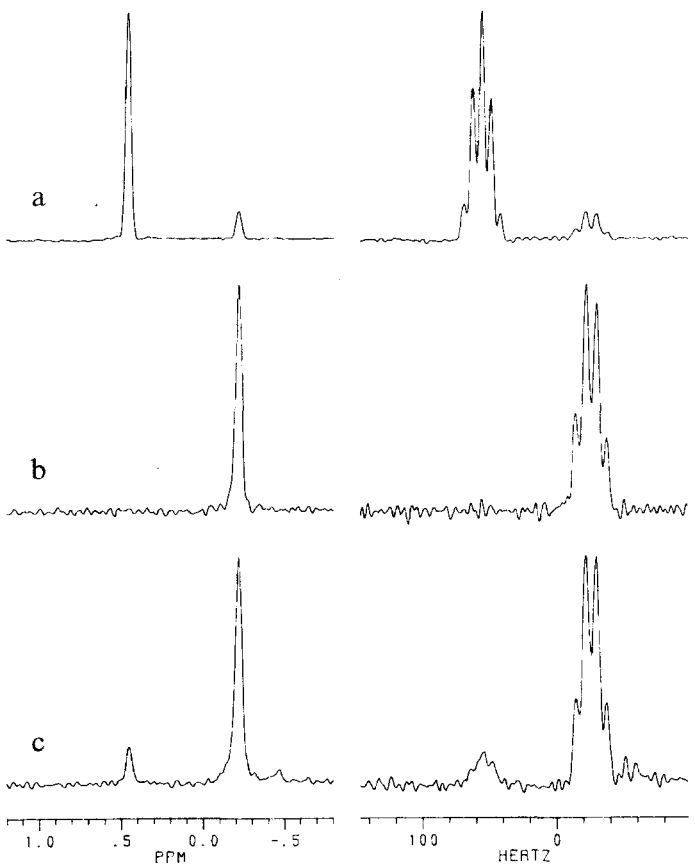

Fig. 2. ^{31}P-NMR spectra (121.5 MHz) of (a) PAF preparation obtained via acetylation of lyso-PAF **Ia** in the presence of $(C_2H_5)_3N$, (b) iso-PAF **IIIa** synthesized as described in Section 2.5.1, and (c) the side product of lyso-PAF **Ia** acetylation (see Section 2.1.2) isolated in accordance with Section 2.5.2. Proton-decoupled spectra and spectra with no decoupling are shown on the left and on the right, respectively.

Ice water (10.8 ml), chloroform (11.7 ml), and methanol (12 ml) were added, the organic layer was separated and washed twice with 10 ml of methanol/water (10:9 v/v). Solvents were removed under reduced pressure, and the residue was chromatographed on silica gel eluting the desired product with chloroform/methanol/water (65:25:3 v/v). Yield 93 mg (88%). TLC $R_f = 0.23$. $[\alpha]D^{20} = -1.2°$ (c 1.50, chloroform). ^{13}C-NMR: 14.2 ($CH_2\underline{C}H_3$); 21.0 ($CO\underline{C}H_3$); 23.0, 26.3, 29.7, 29.9, 30.0, 32.3 ($(\underline{C}H_2)_{16}CH_3$); 54.4 (JCN 3.7, $N(\underline{C}H_3)_3$); 59.6 (JCP 4.5, $PO\underline{C}H_2CH_2$); 64.6 (JCP 5.3, $\underline{C}H_2OP$); 66.7 ($\underline{C}H_2N$); 69.5 (C1 Gro); 72.2 ($O\underline{C}H_2CH_2$); 72.5 (JCP 8.3, C2 Gro); 172.1 ($\underline{C}O$). ^{1}H-NMR: 0.86 (3H, m, CH2C$\underline{H}$3); 1.26 (30H, s, $(C\underline{H}_2)_{15}CH_3$); 1.55 (2H, m, $OCH_2C\underline{H}_2$); 2.08 (3H, s, $CO\underline{CH}_3$); 3.20 (9H, s, $N(C\underline{H}_3)_3$); 3.41 (1H, dt, Jd 9.9, Jt 6.8) and 3.45 (1H, dt, Jd 9.9, Jt 6.8) ($OC\underline{H}_2CH_2$); 3.58 (2H, d, J 5.0, $C\underline{H}_2OCH_2CH_2$); 3.59 (2H, m, $C\underline{H}_2N$); 3.95 (2H, m, $C\underline{H}_2OPOCH_2CH_2$); 4.22 (2H, m, $POC\underline{H}_2CH_2$); 5.11 (1H, m, $C\underline{H}OCOCH_3$).

2.1.2. Acetylation in the presence of triethylamine

Lyso-PAF **Ia** (1.05 g, 2.06 mmol), triethylamine (7.14 ml, 51.5 mmol), and acetic anhydride (1.93 ml, 20.5 mmol) were combined in 60 ml of chloroform. The mixture was heated to reflux for 8 h, cooled, diluted with 200 ml of chloroform, and washed with 80 ml of water. The organic layer

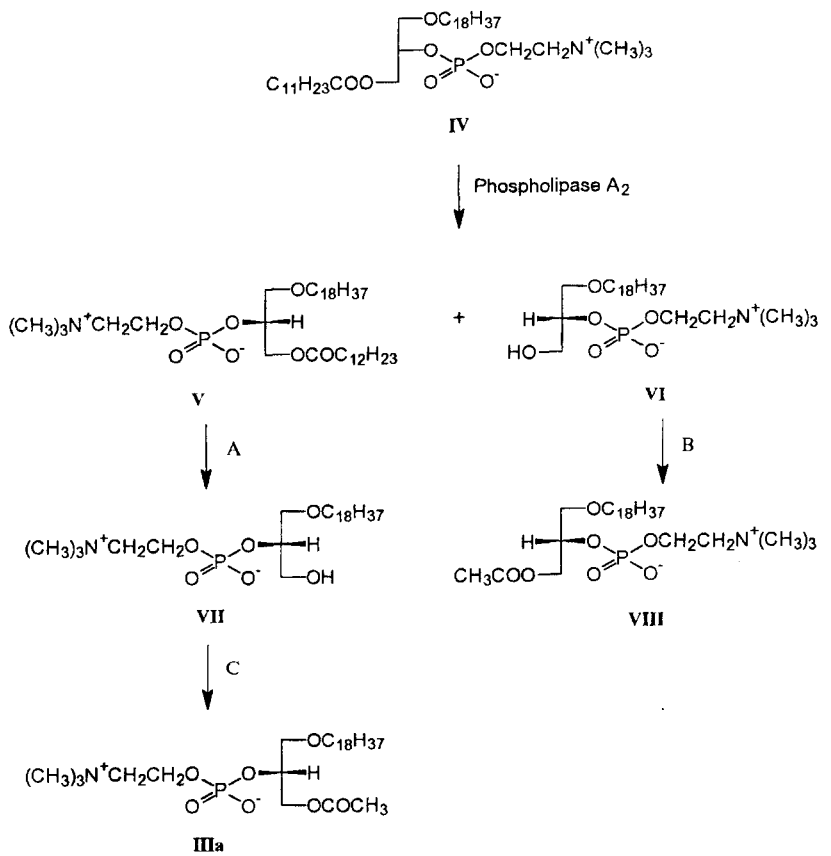

Fig. 3. Synthesis of iso-PAF enantiomers **IIIa** and **VIII**. A: $C_{11}H_{23}COCl/C_5H_5N/CHCl_3$; B: (i) $POCl_3/(C_2H_5)_3N/CHCl_3$, (ii) choline *p*-toluenesulfonate/C_5H_5N, (iii) H_2O; C: CH_3ONa/CH_3OH; D: $(CH_3CO)_2O/HClO_4$.

was evaporated under vacuum, and the residue was purified on a silica gel column eluting the compound **IIa** with chloroform/methanol/water (65:25:3 v/v). Yield 677 mg (60%). TLC $R_f = 0.23$.

2.1.3. Acetylation in the presence of 4-dimethylaminopyridine

A mixture of 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (290 mg, 0.60 mmol), acetic anhydride (0.66 ml, 7.0 mmol), 4-dimethylaminopyridine (DMAP) (35 mg, 0.29 mmol), anhydrous benzene (10 ml) and anhydrous acetonitrile (7 ml) was stirred at 70°C until the lyso-PAF was completely dissolved (about 15 min). The solution was evaporated under reduced pressure to dryness, and DMAP was removed by sublimation at 60°C (1 Torr, 1 h). PAF **IIb** was purified by silica gel column chromatography eluting with chloroform/methanol/water (65:25:4 v/v). Yield 303 mg (95%). TLC $R_f = 0.23$.

2.2. 1-O-Octadecyl-3-O-lauroyl-rac-glycero-2-phosphocholine (IV)

1-*O*-Octadecyl-3-*O*-lauroyl-*rac*-glycerol (1.31 g, 2.5 mmol) was treated by phosphoryl chloride (0.28 ml, 3.1 mmol) in the presence of triethylamine (0.43 ml, 3.1 mmol) and subsequently by choline *p*-toluenesulfonate (1.5 g, 5.5 mmol) under conditions described in [22]. Yield 1.72 g (55%). TLC $R_f = 0.31$. ^{13}C-NMR: 14.3 ($CH_2\underline{C}H_3$); 23.1, 25.4, 26.8, 29.6, 29.7, 29.9, 30.1, 32.3

(($\underline{C}H_2)_{16}CH_3$ and ($\underline{C}H_2)_9CH_3$); 34.6 (CO$\underline{C}H_2$); 54.6 (J_{CN} 3.3, N($\underline{C}H_3)_3$); 59.7 (J_{CP} 4.6, PO$\underline{C}$H2CH2); 63.8 (J_{CP} 4.2, C3 Gro); 67.0 ($\underline{C}H_2$N); 70.2 (J_{CP} 4.6, C1 Gro); 72.3 (O$\underline{C}H_2CH_2$); 72.6 (J_{CP} 5.5, C2 Gro); 174.8 ($\underline{C}$O). ^{1}H-NMR: 0.86 (6H, m, 2$CH_2C\underline{H}_3$); 1.24 (46H, s, ($C\underline{H}_2)_{15}CH_3$ and ($C\underline{H}_2)_8CH_3$); 1.54 (4H, m, $OCH_2C\underline{H}_2$ and $OCOCH_2C\underline{H}_2$); 2.31 (2H, t, J 7.5, OCOC$\underline{H}_2$); 3.18 (9H, s, N($C\underline{H}_3)_3$); 3.44 (2H, t, J 6.6, $CH_2OC\underline{H}_2CH_2$); 3.55–3.62 (4H, m, $C\underline{H}_2OCH_2CH_2$ and $C\underline{H}_2$N); 4.17–4.44 (5H, m, C$\underline{H}$OPOC$\underline{H}_2$ and C$\underline{H}_2$OCO).

2.3. 1-O-Octadecyl-3-O-lauroyl-sn-glycero-2-phosphocholine (V) and 3-O-octadecyl-sn-glycero-2-phosphocholine (VI)

A solution of phospholipid (**IV**) (940 mg) in 60 ml of chloroform was combined with a solution of viper venom (100 mg) in 44 ml of Tris–HCl buffer (pH 8) containing 25 mM $CaCl_2$. The mixture was stirred at 35°C for 15 h, extracted with 150 ml of chloroform/methanol (2:1 v/v), and organic layer was evaporated under reduced pressure. The resulting mixture of compounds **V** and **VI** was chromatographed on a silica gel column. Lipid **V** was eluted with chloroform/methanol/water (65:25:2 v/v) and lyso-derivative **VI** with chloroform/methanol/water (65:25:4 v/v). Phospholipid **V** was treated once more with 50 mg of viper venom for 9 h and purified as above yielding 343 mg (73%). TLC $R_f = 0.31$. ^{13}C- and ^{1}H-NMR spectra were identical to those of compound *V*. Lyso-derivative **VI** was obtained in 306 mg (88%) yield. TLC $R_f = 0.15$. ^{13}C-NMR: 14.2 ($CH_2C\underline{H}_3$); 23.0, 26.4, 29.7, 30.0, 32.3 (($\underline{C}H_2)_{16}CH_3$); 54.4 ($J_{CN}$ 2.9 N($\underline{C}H_3)_3$); 59.6 (J_{CP} 5.2, PO$\underline{C}H_2CH_2$); 63.3 (J_{CP} 3.5, C3 Gro); 66.9 ($\underline{C}H_2$N); 70.9 (J_{CP} 5.1, C1 Gro); 72.2 (O$\underline{C}H_2CH_2$); 76.3 (J_{CP} 6.3, C2 Gro). ^{1}H-NMR: 0.86 (3H, m, $CH_2C\underline{H}_3$); 1.26 (30H, s, ($C\underline{H}_2)_{15}CH_3$); 1.55 (2H, m, $OCH_2C\underline{H}_2$); 3.20 (9H, s, N($C\underline{H}_3)_3$); 3.46 (2H, t, J 6.8, $CH_2OC\underline{H}_2CH_2$); 3.52–3.65 (4H, m, $C\underline{H}_2OCH_2CH_2$ and $C\underline{H}_2$N); 3.66 (1H, dd, J 6.0 and 12.0) and 3.74 (1H, ddd, J_{PH} 1.0, 3.8 and 12.0) ($C\underline{H}_2$OH); 4.17–4.36 (3H, m, C$\underline{H}$OPOC$\underline{H}_2$).

Table 1
Optical rotation values of iso-PAF preparations

Compound	[α]*D* (°)
1-*O*-Acetyl-3-*O*-octadecyl-*sn*-glycero-2-phosphocholine (**VIII**)	+9.1 (c 1.5, chloroform/methanol 1:1 v/v)[a] +8.4 (c 1.5, chloroform)[a] +10.0 (c 5, chloroform/methanol 1:1 v/v)[b]
1-*O*-Octadecyl-3-*O*-acetyl-*sn*-glycero-2-phosphocholine (**IIIa**)	−9.3 (c 1, chloroform/methanol 1:1 v/v)[c] −8.3 (c 1.3, chloroform)[c] −7.1 (c 1, chloroform)[d] −9.96 (c 5, chloroform-methanol 1:1)[b]

[a] Prepared as described in Section 2.6.
[b] From Ref. [16].
[c] Prepared as described in Section 2.5.1.
[d] Isolated as described in Section 2.5.2.

2.4. 1-O-Octadecyl-sn-glycero-2-phosphocholine (VII)

To a stirring solution of phospholipid **V** (343 mg) in 37 ml of chloroform/methanol/water (1:1:0.3 v/v) 0.2 N solution of CH_3ONa in methanol was added dropwise to pH 9. This was stirred for 5 h keeping pH constant by adding small portions of the CH_3ONa solution. The mixture was neutralized with dilute hydrochloric acid to pH 7, chloroform (50 ml) was added, the organic layer was washed with 25 ml of water and evaporated in vacuum. The residue was purified on a silica gel column. Lyso-derivative **VII** was eluted with chloroform/methanol/water (65:25:4 v/v). Yield 227 mg (90%). ^{13}C- and ^{1}H-NMR spectra were identical to those of compound **VI**.

2.5. 1-O-Octadecyl-3-O-acetyl-sn-glycero-2-phosphocholine (IIIa)

2.5.1. Acetylation of compound VII

1-*O*-Octadecyl-*sn*-glycero-2-phosphocholine (175 mg, 0.34 mmol) was treated with acetic anhy-

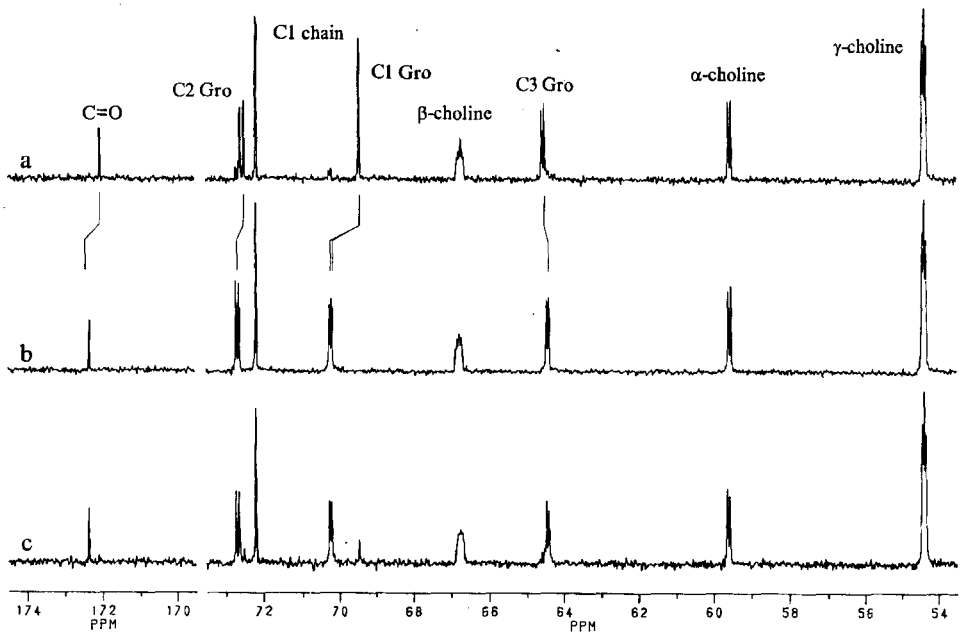

Fig. 4. Fragments of 75.8 MHz ^{13}C-NMR spectra of (a) PAF preparation obtained via acetylation of lyso-PAF **Ia** in the presence of $(C_2H_5)_3N$, (b) iso-PAF **IIIa** synthesized as shown in Fig. 3, and (c) the side product of lyso-PAF **Ia** acetylation (see Section 2.1.2) isolated in accordance with Section 2.5.2. Some amounts of iso-PAF and PAF are seen in spectra a and c, respectively.

dride (1.1 ml, 11.7 mmol) in the presence of 57% perchloric acid (0.5 ml) as described above under 2.1.1 to yield 121 mg (64%) of iso-PAF **IIIa**. TLC $R_f = 0.23$. ^{13}C-NMR: 14.2 ($CH_2\underline{C}H_3$); 20.8 ($CO\underline{C}H_3$); 23.0, 26.4, 29.7, 29.9, 30.0, 32.3 ($(\underline{C}H_2)_{16}CH_3$); 54.4 ($J_{CN}$ 3.2, $N(\underline{C}H_3)_3$); 59.6 (J_{CP} 4.6, $PO\underline{C}H_2CH_2$); 64.4 (J_{CP} 4.3, C3 Gro); 66.7 ($\underline{C}H_2N$); 70.3 (J_{CP} 4.4, C1 Gro); 72.2 ($OCH_2\underline{C}H_2$); 72.7 (J_{CP} 5.5, C2 Gro); 172.4 ($\underline{C}O$). ^{1}H-NMR δ 0.86 (3H, m, $CH_2C\underline{H}_3$); 1.24 (30H, s, $(C\underline{H}_2)_{15}CH_3$); 1.54 (2H, m, $OCH_2C\underline{H}_2$); 2.06 (3H, s, $CO\underline{CH}_3$); 3.20 (9H, s, $N(C\underline{H}_3)_3$); 3.45 (2H, t, J 6.6, $CH_2O\underline{CH}_2CH_2$); 3.56–3.63 (4H, m, $C\underline{H}_2OCH_2CH_2$ and $C\underline{H}2N$); 4.13–4.51 (5H, m, $C\underline{H}OPOC\underline{H}_2$ and $C\underline{H}_2OCOCH_2$).

2.5.2. Isolation from the products of lyso-PAF acetylation

The PAF specimen (580 mg) obtained via acetylation of compound **Ia** in the presence of triethylamine was treated with 50 mg of viper venom for 9 h as described in Section 2.3. After chromatographic separation of the products, compound **IIIa** was obtained in 50 mg yield. TLC $R_f = 0.23$.

2.6. 1-O-Acetyl-3-O-octadecyl-sn-glycero-2-phosphocholine (VIII)

3-*O*-Octadecyl-*sn*-glycero-2-phosphocholine (98 mg, 0.19 mmol) was acetylated by acetic anhydride (0.6 ml, 6.4 mmol) in the presence of 57% perchloric acid (0.3 ml) as described Section 2.1.1. Yield 92 mg (87%). TLC $R_f = 0.23$. ^{13}C- and ^{1}H-NMR spectra of compound **VIII** were identical to those of its enantiomer **IIIa**.

3. Results and discussion

As it is known from literature, lyso-PAFs have been prepared both by total synthesis from opti-

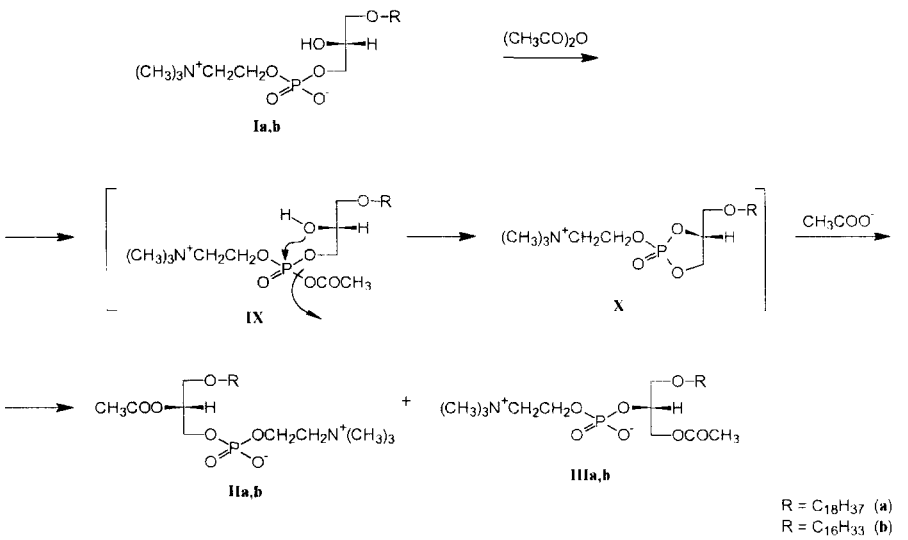

Fig. 5. Possible mechanism of iso-PAF formation in the acetylation of lyso-PAF with acetic anhydride in the presence of bases.

cally active starting materials [5,6,12–16], and via stereospecific hydrolysis of racemic 1-alkyl-2-acylglycerophosphocholines with phospholipase A_2 [9,11,23]. To prepare lyso-PAFs **Ia** and **Ib** in this study we have used the more simple second way. It was evident from ^{31}P-NMR data that lyso-PAFs obtained in this manner are not contaminated with their structural isomers having the phosphocholine groups at C2 of glycerol. These isomers have other chemical shifts than those of lyso-PAFs, so they must be seen in ^{31}P-NMR spectra [24,25].

The key stage of the PAF synthesis is reaction of acetylation of lyso-PAF (see Fig. 1). For the most part, the acetylation is carried out with acetic anhydride in the presence of organic bases [5,6,9,13–16]. As well, we tried to do the acetylation in the presence of catalytic amounts of perchloric acid. This reaction has been described previously in acetylation of 1-alkyl-*sn*-glycero-3-phosphoethan-olamine [26].

Surprisingly, carrying out the reaction under acid catalysis and in the presence of bases gave noticeable different results (see Fig. 2). Acid-catalyzed reaction led to the preparation having single signal in its ^{31}P-NMR spectrum. The multiplicity of the signal indicates that the phosphate fragment is linked with phosphodiester bond to two methylene groups (quintet). In contrast, two signals arised in ^{31}P-NMR spectra of PAF preparations obtained by acetylation in the presence of triethylamine (Fig. 2a) or DMAP. Here, the reaction gives apparently structural isomer of PAF (iso-PAF) having phosphocholine group at C2 of the glycerol residue (see Fig. 1). It is supported by multiplicity of the less intense signal (quartet) showing that the phosphodiester bond links methyne and methylene groups. It is necessary to note that in the presence of DMAP it was markedly less iso-PAF formed.

Since it is impossible to make structural conclusions basing only on ^{31}P-NMR data, we decided to synthesize the positional isomer of PAF **IIIa**, isolate the side product from the reaction of acetylation of lyso-PAF, and compare their characteristics to confirm the structure of the side product and to determine its absolute configuration.

In order to obtain optically active iso-PAF, we treated the racemic mixture **IV** with phos-

pholipase A_2 (Fig. 3). It has been shown that phospholipase A_2 hydrolyzes 1,3-diacylglycero-2-phosphocholines in a stereospecific manner producing optically active lyso-derivatives, 3-*O*-acyl-*sn*-glycero-2-phosphocholines, from 1,3-di-*O*-acylglycero-2-phosphocholines [27,28], and 3-*O*-benzyl-*sn*-glycero-2-phosphocholine from 1-*O*-stearoyl-3-*O*-benzyl-*rac*-glycero-2-phosphocholine [29].

We found that the enzyme is stereospecific in respect to structural isomers of alkylacylphosphatidylcholines as well. As a result of enzymic hydrolysis of compound **IV**, a mixture of non-hydrolyzable enantiomer **V** and lyso-form **VI** of hydrolyzable enantiomer was formed. Compound **V** was deacylated by alkaline hydrolysis to provide lyso-lipid **VII**. Lysophosphatidylcholines **VI** and **VII** were treated with acetic anhydride in the presence of perchloric acid leading to both enantiomers of iso-PAF.

Table 1 shows that iso-PAF **VIII**, that is the one hydrolyzable by phospholipase A_2, exhibits a positive [α] value, whereas enantiomer **IIIa** is negative in [α] value. Stereo configurations shown in Fig. 3 were assigned to iso-PAFs **VIII** and **IIIa** basing on the data of Hirth and Barner [16], who have demonstrated that 1-*O*-acetyl-3-*O*-octadecyl-*sn*-glycero-2-phosphocholine and 3-*O*-acetyl-1-*O*-octadecyl-*sn*-glycero-2-phosphocholine prepared by asymmetric synthesis are of positive and negative [α] values, respectively (see Table 1). As evident from Fig. 2, compounds **VIII**, **IIIa**, and the side product formed in acetylation of lyso-PAF were identical in chemical shifts and multiplicities of their ^{31}P-NMR signals. Further structural information can be derived from ^{13}C-NMR spectra. As it is seen in Fig. 4a,b, signals of C1 and C2 of glycerol are displaced downfield upon the phosphocholine residue changes its position from primary to secondary glycerol function. In addition, C1 resonance becomes splitted into a doublet by spin-spin interaction with phosphorus. Signal of C3 glycerol atom is shifted slightly upfield. It is remarkable that minor peaks seen in the top spectrum coinside in chemical shifts with signals in spectrum of iso-PAF.

To make final conclusion on the structure of the side product formed in the acetylation reaction, we needed to separate it from PAF. We tried to do this by TLC or HPLC, but we could not isolate iso-PAF neither on silica gel with chloroform/methanol/water mobile phases nor on C-18 or C-8 hydrophobic sorbents with acetonitrile/water eluents. Fortunately, it turned out that, in contrast to PAF, the side product can not be hydrolized by phospholipase A_2. This enabled us to isolate it. For this purpose, the preparation of PAF obtained via acetylation of lyso-PAF in the presence of triethylamine was treated with phospholipase A_2. The lyso-PAF formed and the intact side product were resolved by column chromatography on silica gel.

The isolated side product was found to be identical with synthesized iso-PAF **IIIa** basing on ^{31}P- and ^{13}C-NMR data (Fig. 2b,c, Fig. 4). Positions of the signals of protons at C2 and C3 atoms of glycerol in ^{1}H-NMR spectra of iso-PAF and isolated side product were also the same, supporting that these compounds were identical. Taking into account that phospholipase A_2 does not hydrolyze the side product, and basing on the [α] value of the latter, we could assign its structure to be 1-*O*-octadecyl-3-*O*-acetyl-*sn*-glycero-2-phosphocholine.

We suppose a mechanism of iso-PAF formation in the acetylation of lyso-PAF in the presence of bases (see Fig. 5). Along with acetylation of lyso-PAF hydroxy function, formation of mixed anhydride **IX** can occur in an excess of acetic anhydride. This is followed by intramolecular nucleophilic substitution of acetate at phosphorus atom to give phospholane **X**. Phospholanes are known to react easily with nucleophiles, which occur with opening of the cyclophosphate ring [30]. Apparently, acetate ions, accumulated in the reaction, act on the phospholan **X** as the nucleophiles resulting in the formation of iso-PAF **IIIa,b** in parallel with PAF **IIa,b**. Absolute stereo configuration of the C2 atom of glycerol is not changed in this case.

Acknowledgements

This work was supported in part by the Russian Foundation for Fundamental Research, project No 94-03-08166.

References

[1] Platelet-Activating Factor and Related Lipid Mediators (1987) in: F. Snyder (Ed.), Plenum Press, New York, London.

[2] P. Braquet, L. Touqui, T.Y. Shen and B.B. Vargaftig (1987) Pharmacol. Rev. 39, 97–145.

[3] C.A. Demopoulos, N.R. Pinckard and D.J. Hanahan (1979) J. Biol. Chem. 254, 9355–9358.

[4] J. Benveniste, M. Tence, P. Varenne, J. Bidault, C. Boullet and J. Polonsky (1979) C.R. Acad. Sci. Paris 289, 1037–1040.

[5] M. Ohno, K. Fujita, H. Nakai, S. Kobayashi, K. Inoue and S. Nojima (1985) Chem. Pharm. Bull. 33, 572–582.

[6] K. Fujita, H. Nakai, S. Kobayashi, K. Inoue, S. Nojima and M. Ohno (1982) Tetrahedron Lett. 23, 3507–3510.

[7] C.A.A. van Boeckel, G.A. van der Marel, P. Westerduin and J.H. van Boom (1982) Synthesis 5, 399–402.

[8] J.-J. Godfroid, F. Heymans, E. Michel, C. Redeuilh, E. Steiner and J. Benveniste (1980) FEBS Lett. 116, 161–164.

[9] H.-P. Kertscher and G. Ostermann (1986) Pharmazie 41, 596.

[10] A. Kumar and V. Bhakuni (1993) Tetrahedron Lett. 34, 3463–3466.

[11] J.R. Surles, R.L. Wykle, J.T. O'Flaherty, W.L. Salzer, M.J. Thomas, F. Snyder and C. Piantadosi (1985) J. Med. Chem. 28, 73–78.

[12] T. Tsuri and S. Kamata (1985) Tetrahedron Lett. 26, 5195–5198.

[13] P.N. Guivisdalsky and R. Bittman (1989) J. Org. Chem. 54, 4643–4648.

[14] N. Nakamura, H. Miyazaki, N. Ohkawa, T. Oshima and H. Koike (1990) Tetrahedron Lett. 31, 699–702.

[15] F. Heymans, E. Michel, M.-C. Borrel, B. Wichrowski, J.-J. Godfroid, O. Convert, E. Coeffier, M. Tence and J. Benveniste (1981) Biochim. Biophys. Acta 666, 230–237.

[16] G. Hirth and R. Barner (1982) Helv. Chim. Acta 65, 1059–1084.

[17] P. Bett-Bobillo, A. Bienvenue, C. Broquet and L. Maurin (1985) Chem. Phys. Lipids 37, 215–226.

[18] V.V. Chupin, O.V. Ostapenko, V.N. Klykov, M.V. Anikin, and G.A. Serebrennikova (1993) Bioorgan. Khim. 19, 1111–1121.

[19] B. Palameta and M. Kates (1966) Biochemistry 5, 618–625.

[20] A.F. Rosenthal (1966) J. Lipid Res. 7, 779–785.

[21] J.C. Dittmer and R.L. Lester (1964) J. Lipid Res. 5, 126–127.

[22] H. Brockerhoff and N.K.N. Ayengar (1979) Lipids 14, 88–89.

[23] M.P. Murari, R. Murari, S. Parthasarathy, C.A. Guy, V.V. Kumar and B. Malewicz (1990) Lipids 25, 606–612.

[24] A. Pluckthun and E.A. Dennis (1982) Biochemistry 21, 1743–1750.

[25] M. Tence, E. Coeffier, J. Polonsky and J. Benveniste (1983) Biochim. Biophys. Acta 755, 526–530.

[26] R. Kumar, S.T. Weintraub, L.M. McManus, R.N. Pinckard and D.J. Hanahan (1984) J. Lipid Res. 25, 198–208.

[27] L.L.M. Van Deenen and G.H. de Haas (1963) Biochim. Biophys. Acta 70, 538–553.

[28] G.H. De Haas and L.L.M. van Deenen (1964) Biochim. Biophys. Acta 84, 469–471.

[29] G.H. De Haas and L.L.M. van Deenen (1965) Biochim. Biophys. Acta 106, 315–325.

[30] V.V. Chupin, E.V. Malina, I.V. Revenko, I.A. Vasilenko, G.A. Serebrennikova and R.P. Evstigneeva (1984) Bioorg. Khim. 10, 970–974.