SYNTHESIS OF AN ANALOGUE OF THE PHOSPHOLIPID PLATELET ACTIVATION FACTOR FROM A NATURAL ETHANOLAMINE PLASMALOGEN

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UDC 615.273.015.4:612.111.7

The synthesis has been performed of an analogue of the phospholipid platelet activation factor – 1-O-(alk-1'-enyl)-2-acetyl-sn-glycero-3-phospho-N-(N'-acetylglycylethanolamine). In concentrations of 10^{-7}-10^{-6} M, the analogues so obtained inhibited the aggregation of human platelets stimulated by the phospholipid activation factor.

The phospholipid platelet activation factor (PAF) – 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine – is an endogenous lipid bioregulator with a broad spectrum of biological activity [1-3]. At the present time, an intensive search is being made for chemical compounds that are antagonists of PTA, blocking its action by binding with the specific PAF receptors. Some antagonists of PAF have been isolated from such natural materials as the leaves of Ginkgo biloba L. (ginkgolide B or BN 52021) [3] and of Piper futokadsurae (kadsurenone) [4]. A number of synthetic compounds (CV-3988, Ro19-3704, U66985) exhibiting the properties of PAF antagonists have been obtained by modifying the molecular structure of PAF [5-7].

In this paper we describe the chemical synthesis of an analogue of PAF having the structure of O-[1-O-(alk-1'-enyl)-2-acetyl-sn-glycero-3-phospho]-N-(N'-acetylglycyl)ethanolamine (V) from natural bovine ethanolamine plasmalogen.

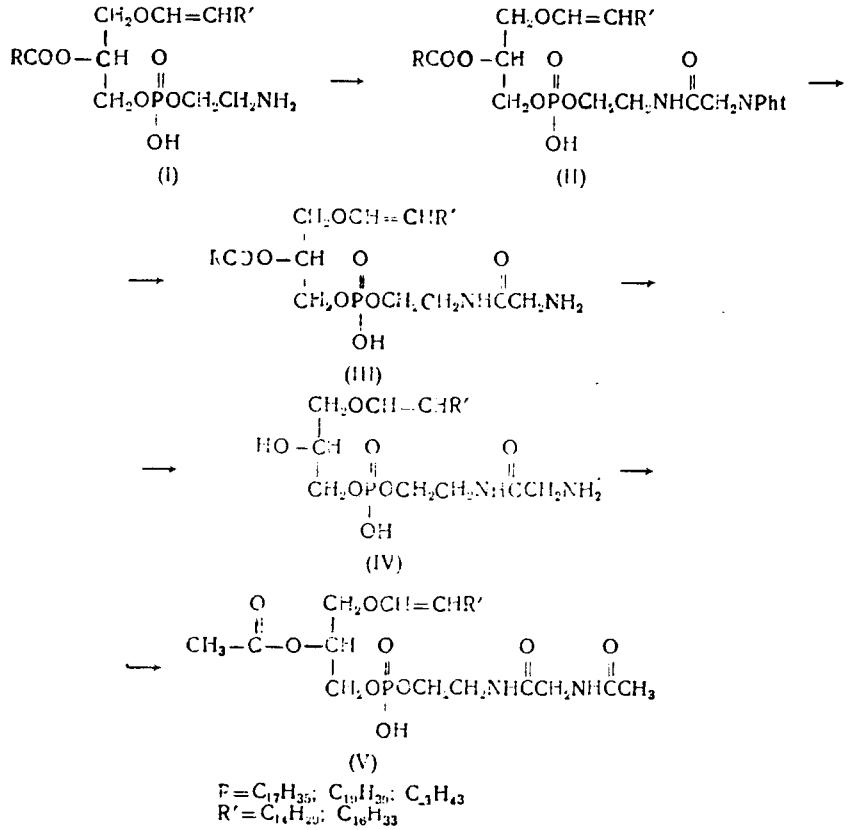
To synthesize the PAF analogue (V) we took the ethanolamine plasmalogens (I) isolated from cattle brain and containing in position 1 C-14 and C-16 residues in a ratio of 1:2.3, and in position 2 C-18, C-20, and C-22 acyl residues in a ratio of 2.4:1:1.3 [8]. In the first stage of the synthesis the ethanolamine plasmalogens were condensed with N-phthaloylglycine in the presence of dicyclohexylcarbodiimide. When the ethanolamine plasmalogen derivative (II) was treated with aqueous hydrazine hydrate, quantitative elimination of the phthaloyl protection took place with the formation of compound (III), containing a glycine residue in the polar part of the molecule. The formation of compound (III) was confirmed by IR spectroscopy (the appearance of amide (I) and amide (II) bands), by elementary analysis for phosphorus and nitrogen (N/P ratio 2.03 (g-atom/g-atom)), and also by the results of the specific detection of a free amino group of compound (III) with a solution of ninhydrin.

To eliminate the diacyl forms and to obtain the lyso derivative (IV), the phospholipid (III) was deacylated with a solution of alkali. The marked decrease in R_f for compound (IV) as compared with compound (III) showed the considerable change in the polarity of compound (IV) that is usually observed on the deacylation of phospholipids. The presence of a glycine residue in compound (IV) was confirmed by the results of elementary analysis (the N/P ratio (g-atom/g-atom) for compound (IV) was 2.05).

The acetylation of the lyso derivative (IV) with acetic anhydride in the presence of triethylamine gave the PAF analogue (V). IR spectroscopy, showing the presence of an ester bond in compound (V) and also the considerable change in R_f value for (V) as compared with (IV), indicated the introduction of an acetyl residue at the second position of the glycerol skeleton of the molecule. The absence of specific coloring with the ninhydrin solution on chromatography indicated that the compound contained no free amino group. This showed the introduction of the acetyl residue at the free amino group of compound (IV) on acetylation. The disappearance of the specific color reaction with ninhydrin on the introduction of an acetyl residue at an amino group with the formation of an amide bond was con-

Scientific-Research Institute of Biomedical Technology, USSR Ministry of Health, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 325-330, May-June, 1990. Original article submitted June 26, 1988; revision submitted July 4, 1989.

0009-3130/90/2603-0268$12.50



firmed by the authors' results on the absence of this reaction for N-acetylglycine, in contrast to glycine, which is in harmony with literature information on the use of ninhydrin for the determination of free amino group in lipids, proteins, and amino acids [8-10].

The fact that the formation of compound (V) was not the result of the splitting out of glycine residue from compound (IV) and subsequent acetylation of the free amino group of the phosphatidylethanolamine was confirmed, in the first place, by a comparison of the chromatographic mobilities of compound (V) and of the model compound O-[1-O-(alk-1'-enyl)-2-acetyl-sn-glycero-3-phospho]-N-acetylethanolamine obtained by treating O-[1-O-(alk-1'-enyl)-lysoglycero-3-phospho]ethanolamine with acetic anhydride, which showed that these compounds had different R_f values (0.62 and 0.45, respectively), and in the second place by the results of elementary analysis which showed that the ratio of the number of nitrogen atoms in compound (V) to the number of phosphorus atoms was 2.05, corresponding to the elementary composition of the structure of compound (V) shown in the scheme.

The existence of a plasmalogen structure in compound (V) was confirmed by the results of acid hydrolysis combined with two-dimensional chromatography performed by Vas'kovskii's method [11] and the results of IR spectroscopy, showing the presence in compound (V) of an aldehydogenic bond. Analysis of the alkenyl residues in compound (V), performed by the GLC method, revealed the presence of C-14 and C-16 alkenyl residues in a ratio of 1:2.1 in position, which corresponds to literature information for the composition of the alkenyl chain of the initial bovine ethanolamine plasmalogen [8].

The presence of a phosphate group in compound (V) was confirmed by the results of elementary analysis for phosphorus and a positive reaction in the detection of phosphates with the Vas'kovskii reagent [8], but also by the results of IR spectroscopy (P—O—C band).

Thus, on the basis of the analysis performed it is possible to conclude that compound (V) had the structure of O-[1-O-(alk-1'-enyl)-2-acetyl-sn-glycero-3-phospho]-N-(N'-acetylglycyl)ethanolamine (V).

A study of the biological activity of compound (V) in the human platelet aggregation test showed that it was a potential PAF antagonist. It was found that the preliminary treatment of platelet-enriched plasma with compound (V) in concentrations of 10^{-7}-10^{-6} M inhibited the aggregation of the platelets on the subsequent action of PAF to the extent of 70-90%.

The results of the study of the biological activity of compound (V) confirmed the conclusion drawn previously [5, 7] that the properties of PAF antagonists are exhibited by structural analogues of it modified in the polar part of the molecule. However, the complete chemical synthesis of PAF analogues modified in the polar part of the molecule is, as a rule, laborious and includes 10-13 stages [6].

We have performed the chemical synthesis of a PAF analogue from natural bovine ethanolamine plasmalogens which include four chemical stages. The PAF analogue obtained by this method contains C-14 and C-16 alkenyl residues. An advantage of this method of synthesis is the considerable decrease in the number of chemical stages as compared with the total synthesis of PAF analogues [6], and also industrial applicability, since the raw material it uses is bovine ethanolamine plasmalogen, the output of which has recently been started up in the Khar'ov Enterprise for the Production of Bacterial Preparations of the USSR Ministry of the Medical and Biological Industry.

EXPERIMENTAL

In the synthesis we used glycine from Reanal (Hungary), dicyclohexylcarbodiimide from Fluka (Switzerland), and phthaloylglycine obtained by treating glycine with phthalic anhydride [12].

The ethanolamine plasmalogens were isolated from cattle brain by the procedure of [8]. The PAF was synthesized from beef heart choline plasmalogens [13].

For column chromatography we used silica gel L 40/100 μm from Chemapol (Czechoslovakia). Thin-layer chromatography was conducted on the silica gel Kieselgel $60F_{254}$ from Merck (FRG), in the following solvent system: chloroform–methanol–water (65:25:4). The spots were detected with iodine, the Vas'kovskii reagent, and a solution of ninhydrin in n-butanol. IR spectra were taken on a Perkin-Elmer model 682 instrument.

Amounts of plasmalogens were determined by thin-layer chromatography combined with acid hydrolysis [11], lipid phosphorus by Vas'kovskii's method [14], and nitrogen by the Kjeldahl method [10]. Alkenyl residues were analyzed by the GLC method on a Tsvet-530 chromatograph using a known procedure [15].

O-[1-O-(Alk-1'-enyl)-2-O-acyl-sn-glycero-3-phospho]-N-(N'-phthaloylglycyl)ethanolamine (II). To 200 mg of bovine ethanolamine plasmalogens (I) that had been dried in vacuum over P_2O_5 and dissolved in 16 ml of chloroform were added 120 mg of N-phthaloylglycine and 123 mg of dicyclohexylcarbodiimide. The reaction mixture was purged with nitrogen and was kept at room temperature for 48 h. After purification of the reaction mixture by column chromatography on silica gel, 137 mg of a yellow amorphous chromatographically pure substance (II) was obtained with R_f 0.75 in the chloroform–MeOH–water (65:25:4) system (for compound (I), R_f 0.68). IR spectrum (cm^{-1}): λ_{max}^{KBr} 2920 (CH_2, CH), 1775 (C=O, Pht), 1725 (C=O, ester), 1645, 1580 (CONH), 1240 (–C–O, aldehydogenic bond), 1060 (P–O–C).

O-[1-O-(Alk-1'-enyl)-2-O-acyl-sn-glycero-3-phospho]-N-glycylethanolamine (III). A solution of 98 mg of the phospholipid (II) in 20 ml of 98% ethanol was treated with 0.05 ml of a 25% solution of hydrazine, and the mixture was boiled under reflux for 2 h. Glacial acetic acid (0.16 ml) was added to the cooled reaction mixture and the precipitate that deposited was separated off by centrifugation. After purification on silica gel, 91 mg of the yellowish amorphous chromatographically pure substance (III) was obtained with R_f 0.7 in the chloroform–MeOH–water (65:25:4) system which, in contrast to compound (II), was revealed with a ninhydrin solution. IR spectrum (cm^{-1}): λ_{max}^{KBr} 2920 (CH_2, CH), 1740 (C=O), 2860, 1575 ($-NH_3^+$), 1680, 1525 (CONH), 1220 (C–O, aldehydogenic bond), 1050 (P–O–C).

O-[1-O-(Alk-1'-enyl)-sn-glycero-3-phospho]-N-glycylethanolamine (IV). A solution of 91 mg of the phospholipid (III) in 2.5 ml of chloroform was treated with 5 ml of 0.33 N NaOH in methanol and the mixture was stirred at room temperature for 4 h. Compound (IV) was isolated from the reaction mixture by extraction after the addition of chloroform and water to the system. This gave 27 mg of phospholipid (IV) in the form of a yellowish amorphous substance with R_f 0.26 in the chloroform–methanol–water (65:25:4) system. IR spectrum (cm^{-1}): λ_{max}^{KBr} 2920 (CH_2, CH), 1680, 1525 (NHCO), 1230 (C–O, aldehydogenic bond), 1050 (P–O–C).

O-[1-O-(Alk-1'-enyl)-2-O-acetyl-sn-glycero-3-phospho]-N-(N'-acetylglycyl)ethanolamine (V). A solution of 27 mg of phospholipid (IV) in 1.5 ml of chloroform was treated with 0.09 ml of acetic anhydride and 0.12 ml of triethylamine. The reaction mixture was heated at

60°C. The compound (V) was purified on a column with elution by a stepwise gradient of the following solvent systems: chloroform–methanol (9:1), (8:2), (7:3), (1:1), and (2:8), and then with methanol and with chloroform–methanol–water (1:2:0.8). This gave 8 mg of the chromatographically pure substance (V) in the form of an amorphous colorless mass with R_f 0.62 in the chloroform–MeOH–water (64:25:4) system. IR spectrum (cm^{-1}): λ_{max}^{KBr} 2920 (CH_2, CH), 1745 (C=O), 1680, 1525 (NHCO), 1230 (C–O, aldehydogenic bond), 1050 (P–O–C).

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ISOLATION OF PREPARATIONS WITH A HIGH CONTENT OF EICOSAPENTAENOIC ACID FROM THE RED MARINE ALGA *Palmaria stenogona*

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UDC 577.115.3:582.26

The composition is given of the fatty acids of the red marine alga *Palmaria stenogona*. The amount of eicosapentaenoic acid (EPA) in the alga was 69.9-77.0% of the total fatty acids. The procedure for isolating EPA of 80-90% purity from the alga is described which includes the saponification of the algal lipids, the selective extraction of the unsaponifiable lipids, the isolation of the free fatty acids, and their separation by the method of crystallizing inclusion complexes of the fatty acids with urea.

Eicosapentaenoic acid, a representative of the higher polyenoic acids of the linolenic acid series, is attracting the attention of researchers by its biological activity. It is an essential fatty acid for some species of marine fish and invertebrates and can be used as one of the components of the diet of the animals in mariculture [1, 2]. In higher plants it stimulates the synthesis of phytoalexins possessing fungicidal and bactericidal proper-

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Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 330-333, May-June, 1990. Original article submitted July 13, 1989; revision submitted December 12, 1989.

0009-3130/90/2603-0271$12.50