SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIHYPERTENSIVE ACTIVITY OF SOME 1-O-ALKYLGLYCERO-3-PHOSPHOCHOLINE DERIVATIVES

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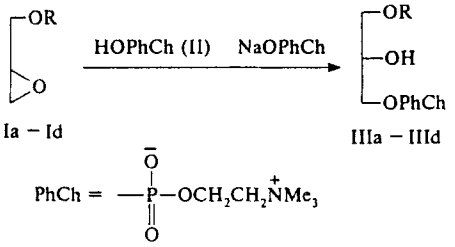
Thrombocyte activation factor (TAF) exhibits a broad spectrum of biological activity, in particular, the ability to reduce the systemic arterial pressure.

In our search for new antihypertensive preparations, we have synthesized a series of glycerophosphorylcholines (IV – IX) belonging to the class of TAF analogs. The initial compounds were 1-O-alkylglycero-3-phosphocholines (III), obtained using a modified procedure reported previously for 1-O-hexadecylglycero-3-phosphocholine (IIIa) [1].

Below we demonstrate that this method can be used to obtain the other derivatives III, including those containing polyfluoroalkyl radicals.



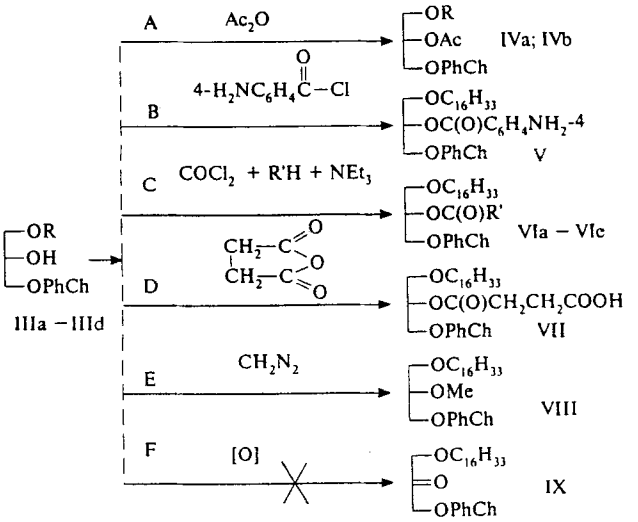
IIIa: R = $C_{16}H_{33}$; IIIb: R = $C_{18}H_{37}$; IIIc: R = $CH_2(CF_2)_6H$; IIIc: R = $CH_2CF_2(OCF_2CF_2)_3OCF_3$.

The procedure was essentially as follows: a mixture of reagents was dissolved in a 5 – 10-fold excess of methanol and boiled for 20 h. Then the target product III was isolated by liquid chromatography.

The course of the conversion was monitored by TLC. The yield of glycerophosphocholines IIIa – IIId varied within 30 – 60%.

The resulting glycerophosphocholines IIIa – IIId were used in the reactions described below. Processes A, B, and C constitute a group of related reactions, of which the last has not been reported so far. This reaction is carried out in an anhydrous organic solvent at – (20 – 40)°C, without separating the intermediate products of the interaction between III and phosgen. The products VId and VIe were obtained using the corresponding presynthesized amides of chlorocarbonic acid. Reaction D can be considered as a special case of reaction A.

1-O-Alkyl-2-O-methylglycero-3-phosphocholine (VIII) was obtained by pathway D in the presence of a catalyst (fluoboric acid).



IVa: R = $CH_2(CF_2)_6H$;
IVb: R = $CH_2CF_2(OCF_2CF_2)_3OCF_3$;
VIa: R' = OMe;
VIb: R' = OEt;
VIc: R' = NH_2;
VId: R' = NMe_2;
VIe: R' = 

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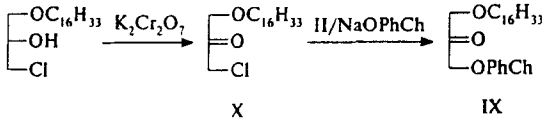
Attempts to synthesize compound IX via pathway F (i.e., by oxidation of IIIa with sodium or potassium bichromate in sulfuric acid, or with chromic anhydride in acetic acid and pyridine) were unsuccessful. This compound was obtained in insignificant yield using the following scheme:



X IX

Involving phosphorylation of compound X in boiling acetonitrile.

Some physicochemical characteristics and yields of the synthesized compounds are listed in Table 1.

EXPERIMENTAL CHEMICAL PART

The ^1H and ^{19}F NMR spectra were measured on a Varian EM-390 spectrometer, operated at a working frequency of 90 MHz (for protons) or 84 MHz (for the ^{19}F nuclei). The chemical shifts were determined using tetramethylsilane (^1H signal) or Freon 11 (^{19}F signal) as the internal standards.

The course of the reactions was monitored and the purity of products was checked by TLC on Silufol plates eluted with a chloroform – methanol – water (65 : 35 : 6) mixture and developed using iodine vapor or Vas'kovskii reagent. The data of elemental analyses agreed with the results of analytical calculations.

1-O-Alkylglycero-3-phosphocholines (IIIa – IIId). To a solution of 20 mmole of IIa – IId in 30 ml of methanol was added 25 mmole of compound II and 10 mmole of a sodium

TABLE 1. Pysicochemical Properties and Yields of 1-O-Alkylglycero-3-Phosphocholines (IIIa – IIId) and Their Derivatives (IV – IX)

Compound	Yield, %;	M.p., °C	R_f	Empirical formula	IR spectrum, $\nu_{C=O}$, cm^{-1}	NMR chemical shifts, δ, ppm	
						^1H	^{19}F
IIIa	73.4	215	0.22	$C_{24}H_{52}NO_6P$	–	0.9 (t, 3H, CH_3), 1.26 (s, 28H, $14CH_2$), 3.35 [(s, 9H, $N^+(CH_3)_3$)], 3.5 – 4.2 (m, 12H, CHO, CH_2O, CH_2N, OH).	–
IIIb	71.8	245	0.24	$C_{26}H_{56}NO_6P$	–	0.9 (t, 3H, CH_3), 1.28 (s, 32H, $16CH_2$), 3.4 [(s, 9H, $N^+(CH_3)_3$)], 3.6 – 4.2 (m, 12H, CHO, CH_2O, CH_2N, OH).	–
IIIc	62.7	183	0.12	$C_{15}H_{22}F_{12}NO_6P$	–	3.0 [(s, 9H, $N^+(CH_3)_3$)], 3.05 – 4.25 (m, 12H, CHO, CH_2O, CH_2N, OH), 6.32 (tt, 1H, CF_2H, J_{HCF} 51 Hz, J_{HCCF} 4.5 Hz).	119.6 (s, 2F), 121.7 (s, 2F), 123.3 (s, 4F), 129.1 (m, 2F), 137.1 (d, 2F, CF_2H, J_{HCF} 51 Hz).
IIId	31.8	176	0.13	$C_{17}H_{21}F_{17}NO_{10}P$	–	3.2 [(s, 9H, $N^+(CH_3)_3$)], 3.0 – 4.5 (m, 12H, CHO, CH_2O, CH_2N, OH).	77.1 (m, 2F, CF_2O), 84.7 (m, 10F), 88.0 (kb, 2F, J_{FCCF} 9.9 Hz), 59.1 (t, 3F, CF_3, J_{FCCF} 9.9 Hz).
IVa	55.2	–	0.23	$C_{17}H_{24}F_{12}NO_7P$	–	2.1 (s, 3H, $COCH_3$), 3.0 [(s, 9H, $N^+(CH_3)_3$)], 3.05 – 4.25 (m, 10H, CH_2O, CH_2N), 5.2 (m, 1H, CHO), 6.32 (tt, 1H, CF_2H, J_{HCF} 51 Hz, J_{HCCF} 4.5 Hz).	119.6 (s, 2F), 121.7 (s, 2F), 123.3 (s, 4F), 129.1 (m, 2F), 137.1 (d, 2F, CF_2H, J_{HCF} 5.1 Hz).
IVb	41.1	–	0.21	$C_{19}H_{23}F_{17}NO_{11}P$	–	2.2 (s, 3H, $COCH_3$), 3.0 – 4.5 (m, 10H, CH_2O, CH_2N), 3.2 [(s, 9H, $N^+(CH_3)_3$)], 5.2 (m, 1H, OCH).	77.2 (m, 2F), 84.7 (m, 10F), 88.1 (kb, 2F, J_{FCCF} 9.9 Hz), 59.1 (t, 3F, CF_3).
V	42.4	165	0.31	$C_{31}H_{57}N_2O_7P$	1703	0.9 (t, 3H, CH_3), 1.22 (m, 26H, $13CH_2$), 1.9 (m, 2H, OCH_2CH_2), 3.24 [(s, 9H, $N^+(CH_3)_3$)], 3.4 – 4.5 (m, 13H, OCH_2, NH_2, NCH_2, NCH, OCH), 7.03 (d, 4H, C_6H_4, J_{HCCH} 9 Hz).	
VIa	42.8	–	0.14	$C_{26}H_{54}NO_8P$	1739	–	–
VIb	54.7	–	0.16	$C_{27}H_{56}NO_8P$	1746	–	–
VIc	48.5	–	0.33	$C_{25}H_{33}N_2O_7P$	1722	–	–
VId	22.4	–	0.38	$C_{27}H_{57}N_2O_7P$	1732	–	–
VIe	35.8	–	0.43	$C_{29}H_{59}N_2O_8P$	1728	–	–
VII	47.8	186	0.28	$C_{28}H_{56}NO_9P$	1731	–	–
VIII	50.5	270	0.12	$C_{25}H_{54}NO_6P$	–	0.9 (t, 3H, CH_3), 1.27 (s, 28H, $14CH_2$), 3.4 [(s, 9H, $N^+(CH_3)_3$)], 3.48 (s, 3H, OCH_3), 3.2 – 4.5 (m, 11H, OCH_2, NCH_2, OCH).	–
IX	18.1	168	0.12	$C_{24}H_{50}NO_6P$	1740	–	–
X	27.7	37	0.73	$C_{19}H_{37}ClO_2$	1743	0.9 (t, 3H, CH_3), 1.26 (s, 28H, $14CH_3$), 3.4 – 4.5 (m, 6H, CH_2Cl, OCH_2).	

salt of II. The reaction mass was boiled for 20 h, cooled to the room temperature, neutralized with 20% HCl, and partitioned on a chromatographic column (length, 300 mm; diameter, 25 mm) filled with an L 100/250 silica gel and eluted with methanol. The yields and characteristics of IIIa – IIId are given in Table 1.

1-O-Perfluoroalkyl-2-O-acetylglycero-3-phosphocholine (IVa and IVb). A solution of 4 mmole IIIc (or IIId), 40 mmole triethylamine, and 10 mmole acetic anhydride in 15 ml chloroform was boiled for 6 h and cooled. The solvent was evaporated and the residue was partitioned on a chromatographic column as described for the previous synthesis.

1-O-Hexadecyl-2-O-(4-aminobenzoyl)glycero-3-phosphocholine (V). A mixture of 1.73 g (3.6 mmole) IIIa, 2.8 g (18 mmole) 4-aminobenzoyl chloride, and 1.8 g (18 mmole) triethylamine in 15 ml of chloroform was boiled for 5 h. Then the solvent was evaporated in vacuum (at 1 Torr) and the residue was partitioned on a chromatographic column as described above.

1-O-Hexadecyl-2-O-alkoxycarbonylglycero-3-phosphocholine (VIa and VIb). Phosgen (500 mmole) was condensed into a mixture of 5 mmole IIIa and 20 mmole triethylamine in 15 ml of chloroform cooled to – 20°C, and the reaction mass was stirred at this temperature for 1 h. On continuing the stirring, the mixture was slowly heated to room temperature and the excess phosgen was evaporated at a reduced pressure (100 – 150 Torr). To the residue, cooled to – 10°C, was added 100 mmole of the corresponding alcohol, and the reaction mixture was heated to room temperature and stored for 12 h. Then the low-volatile components were evaporated in vacuum (1 – 2 Torr) and the residue was partitioned on a chromatographic column as described above.

1-O-Hexadecyl-2-O-aminocarbonylglycero-3-phosphocholine (VIc). Phosgen (1.2 g, 12 mmole) was condensed at – 20°C into a mixture of 0.24 g (0.5 mmole) IIIa and 0.30 g (3 mmole) triethylamine in 15 ml of chloroform. Then the reaction mixture was heated to 10°C and kept at this temperature for 2 h. The excess phosgen was removed, the residue at – 40°C was bubbled with excess ammonia (2 g) and carefully heated to room temperature, and the reaction mass was allowed to stand overnight. Then the solvent was evaporated in vacuum (1 Torr) and the residue was partitioned on a chromatographic column as described above.

1-O-Hexadecyl-2-O-(N,N-dimethylaminocarbonyl)glycero-3-phosphocholine (VId). To a mixture of 0.3 g (0.6 mmole) IIIa with 12 ml of chloroform at 0°C were sequentially added 1 g (10 mmole) triethylamine and 0.54 g (5 mmole) of the N,N-dimethylamide of chlorocarbonic acid. The reaction mass was boiled for 6 h, the solvent was evaporated in vacuum (at 1 Torr), and the residue was treated as indicated above.

1-O-Hexadecyl-2-O-(morpholinocarbonyl)glycero-3-phosphocholine (VIe). Obtained similarly to VId using a mixture of 0.3 g (0.6 mmole) IIIa, 1 g (10 mmole) triethylamine, and 0.75 g (5 mmole) of the morpholide of chlorocarbonic acid. Yield of compound VIe, 0.17 g (49.8%).

1-O-Hexadecyl-2-O-3-(carboxypropanoyl)glycero-3-phosphocholine (VII). To 25 ml of methylene chloride were sequentially added compound IIIa (0.73 g, 1.5 mmole), triethylamine (1 g, 10 mmole), and succinic anhydride (1.6 g, 16 mmole), and the mixture was boiled for 4 h. Then the solvent was evaporated in vacuum (1 Torr) and the residue was partitioned on a chromatographic column as described above.

1-O-Hexadecyl-2-O-methylglycero-3-phosphocholine (VIII). To 0.72 g (1.5 mmole) of compound IIIa in 25 ml of methylene chloride were added 3 drops of fluoboric acid and, on stirring, a solution of diazomethane in methylene chloride was carefully introduced dropwise until the intensive nitrogen evolution ceased (in approximately 30 min). Then the reaction mixture was allowed to stand for 3 h at room temperature, the solvent was evaporated, and the residue partitioned on a chromatographic column to obtain 0.37 g (50.5%) of compound VIII; m.p., 269 – 270°C (reported: m.p., 270°C [2]).

1-Chloro-3-hexadecylhydroxypropan-2-one (X). To 11.25 g (34 mmole) of 1-chloro-3-hexadecylhydroxy-2-propanol in 120 ml of acetone at (– 10) – 0°C was added dropwise a mixture of 9 g of potassium bichromate in 25 ml of 55% sulfuric acid, and the reaction mass was heated to room temperature and stirred for 8 h. Then 10 ml of isopropyl alcohol was added and the mixture was stirred for another 8 h. The acetone solution was separated, the solvent was evaporated in vacuum (1 Torr), and the residue partitioned on a chromatographic column eluted with a $CCl_4 - CHCl_3$ (3 : 1) mixture.

3-O-Hexadecylhydroxy-2-oxopropyl phosphocholine ester (IX). A mixture of 1.3 g (4 mmole) of compound X in 10 ml acetonitrile, 0.8 g (4 mmole) of a sodium salt of II, and 0.18 g (1 mmole) of compound II was boiled for 20 h. Then the solvent was evaporated in vacuum (1 Torr) and the residue was partitioned on a chromatographic column.

EXPERIMENTAL PHARMACOLOGICAL PART

The antihypertensive activity of synthesized compounds was studied by intravenous injections to 180 – 200 g rats with spontaneous hypertension. The systemic arterial pressure was measured by an electronic manometer in the femoral artery and recorded on a Nihon Kohden RM-86 polygraph (Japan). The effective dose (ED_{50}) producing a 50% decrease in the arterial pressure was determined from the dose – effect plots [3]. Each dose was studied on a group of 6 test animals.

The acute toxicity (LD_{50}) was determined by a single intravenous injections to Guinea pigs weighing 200 – 250 g.

It was established that LD_{50} values of the synthesized compounds ranged from 0.3 to 5.0 mg/kg. The less toxic compounds VIb, VIe, and VIII ($LD_{50} > 1.0$ mg/kg) exhibited a pronounced antihypertensive effect (Table 2). At a medium dose, these TAF derivatives decreased the arterial pressure 3 – 5 sec after the injection, with the effect lasting about 20 min.

TABLE 2. Antihypertensive Activity of Some 1-O-Alkylglycero-3-Phosphocholine Derivatives

Compound	ED_{50}, mg / kg
VIe	0.082 ± 0.12
VIb	0.12 ± 0.02
VIII	0.27 ± 0.11

Thus, some of the new 1-O-alkylglycero-3-phosphocholine derivatives synthesized in this work possess pronounced antihypertensive activity.

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