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Synthesis of Aminopropylamino Derivatives of Betulinic and Oleanolic Acids

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Abstract—Triterpenoids with the following amine fragments in C3 and C28 positions were synthesized on the basis of betulinic and oleanolic acids: (3-aminopropoxy)-, 3-acetyl-(3-aminopropyl)amino-, 6-[bis(3-aminopropyl)amino]hexylamino-, and (3-aminopropyl)-4-aminophenylsulfonyl-4-phenylamino. Amide of betulonic acid with 4,4'-diaminodiphenylsulfonic substituent was shown to exhibit no antitumor effect, but to have a pronounced anti-inflammatory activity.

Keywords: triterpenoids, amides, cyanoethylation, aminopropylamino derivatives, anti-inflammatory activity
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INTRODUCTION

Derivatives of betulinic acid with long-chain substituents in position C28 are known as effective antiviral agents [1, 2]. In general, syntheses of amides and ureides with amino acids, diamines, and peptides are described [3]. The triterpenoid structures with branched fragments in their side chains are of interest, for example, dioxalates of 3,28-polyethyleniminobetulin [4] and hydroxyl/amino derivatives of betulinic acid (esters with 2,3-dihydroxy-2-hydroxymethylpropane, 2-amino-2,3-dihydroxymethylpropane, *N*-(1,1-bis(hydroxymethyl)-2-hydroxyethyl)formamide, and others). The antitumor activity of these derivatives is several times higher than that of betulinic acid [5, 6]. Aminopropoxy derivatives of betulin, erythrodiol, uvaol, and oleantriol were synthesized by cyanoethylation of hydroxyl groups of the triterpenoids and subsequent reduction of the cyanoethyl fragments, and high antitumor activity of these compounds towards large number of human tumor cell lines in vitro was shown [7]. Derivatives of lupane and ursane with various substituents in positions C3 and C28 are also promising modifiers of biological reactions with antioxidative, anti-inflammatory, and cytoprotective activity [8–10].

One of the new approaches to the introduction of long-chain branched substituents in position C28 of triterpenic acids involves synthesis of amides, interaction of their terminal amino groups with acrylonitrile, and subsequent reduction of cyanoethyl derivatives with hydrogen. Cyanoethylation of amines with the subsequent reduction of the nitrile group is known to result in the preparation of mono-*N*- or bis-*N*-propylamines depending on the structure and reaction conditions [11].

RESULTS AND DISCUSSION

We applied the aforementioned approach to the synthesis of a new group of triterpenoids which contained various alkanepolyamine fragments. Amides of betulonic and oleanonic acids with aliphatic and aromatic diamines were used as starting compounds (Schemes 1 and 2). Exhaustive cyanoethylation of the terminal NH_2-group with the formation of bis-*N*-propionitrile (**III**) was observed during the interaction of amide of betulonic acid (**V**) with acrylonitrile in dioxane. Products of biscyanoethylation were found even at equimolar amounts of the diamine and acrylonitrile. The monosubstituted derivative (**VI**) was formed from amide (**V**), probably due to steric factors. As it was previously reported [12, 13], we found no products of the cyanoethylation of the triterpenic skeleton of compounds (**II**) and (**V**). Resonances from the nitrile groups were identified in the ^{13}C NMR spectra at δ 118.6 and 119.4 ppm. The resonances from C7' and C10' of bisnitrile (**III**) and C1' of nitrile (**VI**) were observed in the region of δ 49.7–49.9 ppm, whereas the resonances from C8' and C11' of nitrile (**III**) and C2' of derivative (**VI**) were found at δ 12.2, 12.3, and 17.3 ppm, respectively.

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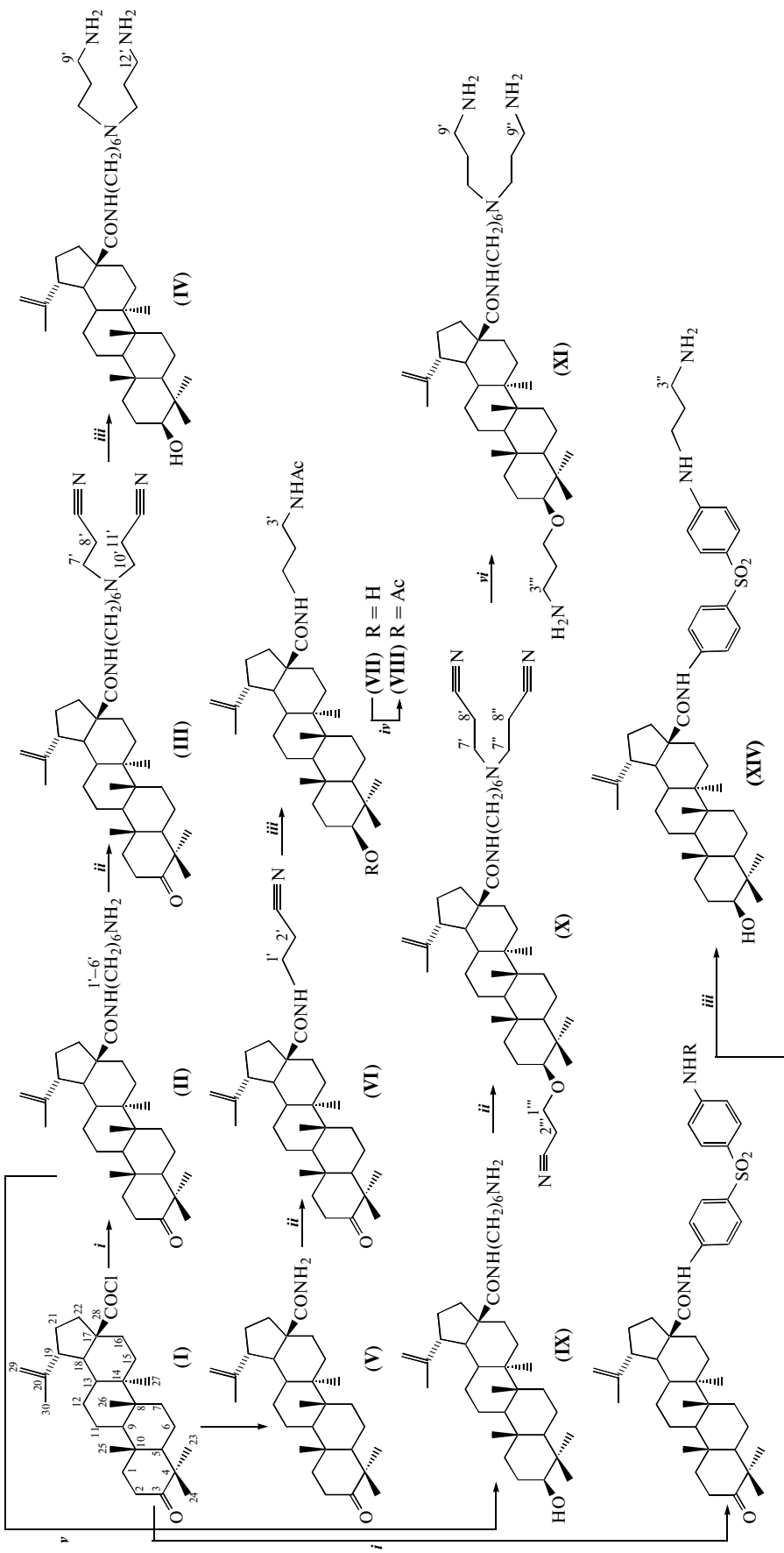
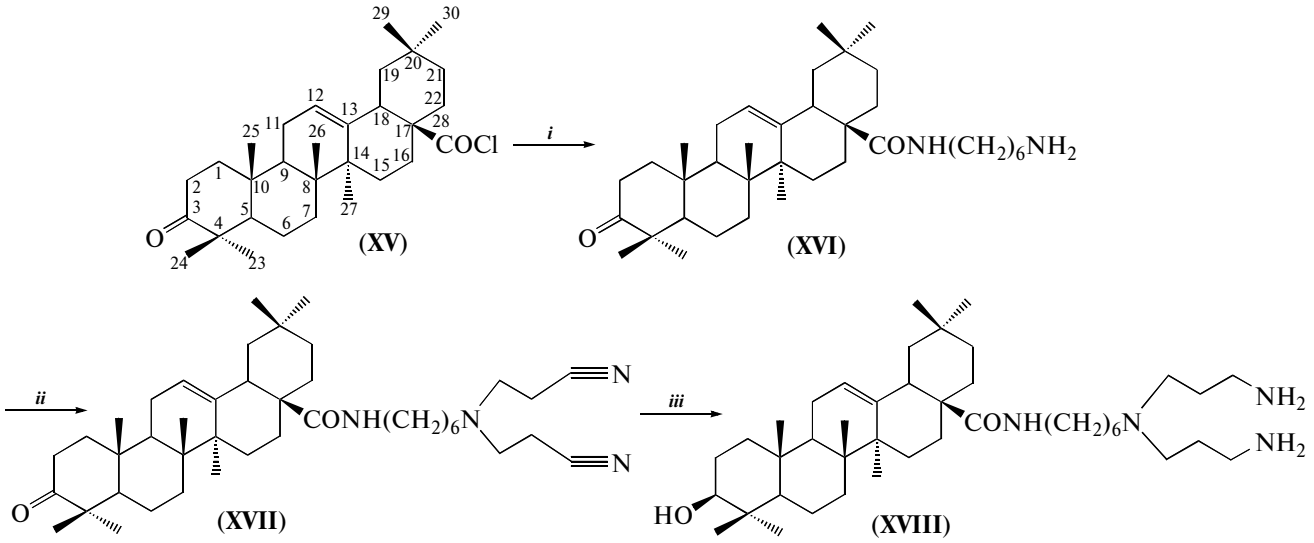


Reaction conditions: ***i***, $NH_2(CH_2)_6NH_2$, 4,4'-diaminodiphenylsulfone, Et_3N, chloroform; ***ii***, CH_2=CH-CN, 40% KOH, 1,4-dioxane; ***iii***, $LiAlH_4$, THF; ***iv***, AC_2O, pyridine; ***v***, $NaBH_4$, isopropanol; ***vi***, H_2, Ni-Raney, MeOH; ***vii***, CH_2=CH-CN, $Cu(OAC)_2$, 1,4-dioxane

Scheme 1.



Reaction conditions: ***i***, $NH_2(CH_2)_6NH_2$, Et_3N, chloroform; ***ii***, CH_2=CHCN, 1,4-dioxane, 40% KOH; ***iii***, $LiAlH_4$, THF.

Scheme 2.

Bisamino derivative (**IV**) was prepared by reduction of compound (**III**) by lithium aluminum hydride in boiling THF with a yield of 74%. The CON-bond was not reduced under the reaction conditions, possible owing to the bulk substituent at the C17 atom. Prolonged reduction (for 20 h) of amide (**III**) by lithium aluminum hydride did not affect the amide bond, although the amide of acetyl betulinic acid was converted into the corresponding amine under similar conditions [14]. At the same time, the stability of amides of glycyrrhetic acid towards the reduction by lithium aluminum hydride is well known [15]. The type of triterpenic backbone and steric factors were probably important for every concrete case. Resonances from CN-groups at δ 118.4 and 119.4 were absent in the ^{13}C NMR spectrum of compound (**IV**), but resonances from the C9' and C12' aminomethylene groups were observed at δ 39.2 ppm. The product of the reduction of compound (**VI**) was characterized as its *O*,*N*-diacetate (**VIII**).

Exhaustive cyanoethylation of amide of oleanonic acid (**XVI**) to [6-bis(2-cyanoethyl)amino)hexylamino]olean-12-ene (**XVII**) with its subsequent reduction to 3β-hydroxy-28-{6-[bis(3-aminopropyl)amino]hexylamino}olean-12-ene (**XVIII**) was performed according to a similar scheme (Scheme 2). Structures of compounds (**XVII**) and (**XVIII**) were confirmed by their NMR and IR spectra.

Sequential cyanoethylation of amide of betulinic acid (**IX**) and catalytic hydrogen reduction of tricyano derivative (**X**) result in the formation of lupane trisamino derivative (**XI**). The broadened resonances from aminomethylene groups are observed in its ^1H NMR spectrum at δ 2.64–2.88 ppm (H3''') and 3.08–3.34 ppm (H1'''). Steroid compounds of this type exhibit an antimicrobial activity [16].

We used amide of betulonic acid with 4,4'-diaminodiphenylsulfonic substituent (**XII**) as one more compound for the formation of a branched derivative (Scheme 1). Note that amide of deoxycholic acid with 4,4'-diaminodiphenylsulfonic substituent was tested as an anticancer agent [17]. Therefore, we synthesized the analogous derivative of betulonic acid in order to study the effect of 4,4'-diaminodiphenylsulfonic substituent on the antitumor and anti-inflammatory activity.

Aromatic amines are known to be cyanoethylated only after heating to 100–180°C for many hours in the presence of salts of Cu, Zn, Co, or Ni [18]. Cyanoethylation of amide (**XII**) to derivative (**XIII**) according to the method [19] required the presence of $Cu(OAc)_2$. Reduction of compound (**XIII**) by lithium aluminum hydride in boiling THF yielded 56% of lupene derivative (**XIV**) with the unreduced sulfogroup. Resonances from the H2'' and H1'' protons of the aminomethylene groups were found in the ^1H NMR spectrum of compound (**XIV**) in the area of δ 1.21–2.00 and 3.60–3.75 ppm as multiplets.

The antitumor properties of 4,4'-diaminodiphenylsulfonic amide of betulonic acid (**XII**) were examined on female mice of the C57BL/6 line with the intramuscularly transplanted Lewis lung carcinoma (2×10^6 cells). Compound (**XII**) was daily introduced on the cancer visualization stage from the 10th to 16th day after the cancer transplantation at a dose of 50 mg/kg (see Experimental). The mixture of antitumor agents that imitated polychemotherapy (PCT) served as a standard of the antitumor activity [20]. The

Table 1. Dynamics of growth of the Lewis lung carcinoma in the C57BL/6 mice in the course of treatment with compound (**XII**)

Group	The cancer volume (V, cm^3; %) in the course of the treatment with agent (**XII**)							
	10 day[1]		12 day		14 day		17 day	
	V, cm^3	V, %	V, cm^3	V, %	V, cm^3	V, %	V, cm^3	V, %
Control	1.33 ± 0.07	0	2.05 ± 0.13	54	2.78 ± 0.18	109	4.67 ± 0.22	251
PCT	1.12 ± 0.08	0	1.42 ± 0.10**	27	1.74 ± 0.13***	55	3.08 ± 0.18***	175
(**XII**)	1.02 ± 0.05**	0	1.66 ± 0.09*	63	2.51±0.13###	146	4.16 ± 0.17###	308

*** $p \le 0.001$; ** $p \le 0.01$ – relative to the control group;
$p \le 0.001$; ## $p \le 0.01$ – relative to the PCT group;
[1] 1 days after the cancer transplantation.

Table 2. Values of the edema index for a leg of mice with the induced inflammation

Group	Dose, mg/kg	Edema index, %	Relative edema index, %
Control	–	19.80 ± 1.58	100
(**XII**)	100	15.15 ± 1.18*	75
Betulonic acid	100	14.54 ± 1.40*	73
Indomethacin	20	14.17 ± 2.57	72

* $P < 0.05$ differences with the control are significant.

control group was treated with a water-tween mixture. Volumes of tumor nodes were measured during the treatment with compound (**XII**). The dynamics of the growth of transplants was presented as a percentage of their starting sizes before the administration of the agents (Table 1).

As follows from Table 1, compound (**XII**) exhibited no antitumor effect: the average sizes of the transplants of experimental and control groups did not significantly differ. We noted a negative tendency to advanced growth of the transplants in the experimental group in comparison with the control group (taking into account the differences between the groups before the administration of the compound). At the end of the experiment, sizes of the cancer in mice that were treated with compound (**XII**) were 1.2 times higher than those in the control group, whereas they were 1.4 times lower in the PCT group. Previously, we demonstrated that betulonic acid that was administered to mice with the Lewis lung carcinoma according to the same scheme exhibited no antitumor activity [21]. Thus, the introduction of 4,4'-diaminodiphenylsulfonic fragment in betulonic acid did not result in the appearance of the target activity.

Anti-inflammatory properties of compound (**XII**) were studied on a model of inflammation which was induced by the subplanar administration of histamine (see Experimental). The results are given in Table 2. Compound (**XII**) was shown to exhibit a significant anti-inflammatory effect; the leg edema was decreased by 25% in comparison with the control. The pronouncement of this effect corresponded to that of indomethacin (the well-known non-steroid anti-inflammatory agent) (28%) that was administered in the effective dose. No considerable differences in the anti-inflammatory activity were found between betulonic acid and its derivative (**XII**). This fact pointed to the leading role of the triterpene backbone in the realization of this activity.

Thus, triterpenoids with the following amine fragments: (3-aminopropooxy)-, 3-acetyl-(3-aminopropyl)amino-, 6-[bis(3-aminopropyl)amino]hexylamino-, and (3-aminopropyl)-4-aminophenylsulfonyl-4-phenylamino in positions C3 and C28, were synthesized on the basis of betulinic and oleanolic acids. No antitumor effect was shown to appear after the attachment of 4,4'-diaminodiphenylsulfonic substituent to betulonic acid, but this derivative preserved the anti-inflammatory activity specific for betulonic acid.

EXPERIMENTAL

IR spectra were recorded on an IRPrestige-21 Shimadzu spectrophotometer (Japan) in a paste with Vaseline oil. The absorption band of valence oscillations of the nitrile group in IR spectra of compounds (**III**) and (**VI**) was observed in the region of 2260 cm^{-1}. The ^1H and ^{13}C NMR spectra (δ, ppm) were recorded on a Bruker AM-300 spectrometer (300.13 and 75.47 MHz, respectively) in deuterochloroform (if otherwise indicated) with trimethyl silane as an internal standard. Melting points were determined on a Boetius device. Optical absorption was measured on a Perkin-Elmer

241 MC polarimeter in a 1-decimeter tube. TLC was carried out on Sorbfil plates (ZAO Sorbpolimer, Russia) in the chloroform–ethyl acetate system (40 : 1). Substances were detected by treatment with 10% H_2SO_4 (heating for 2–3 min at 100–120°C). The element analysis was performed on a EuruEA-3000 CHNS-analyzer using acetanilide as a main standard. The column chromatography was carried out on neutral Al_2O_3 (Reakhim, Russia). Oleanolic acid was isolated as described in [22] and oxidized to oleanonic acid by the Jones reagent in acetone. Betulonic acid was synthesized from betulin as described in [23]. Chlorides of betulonic acid (**I**) and oleanonic acid (**XV**) were prepared by treatment with oxalyl chloride in chloroform [24]. The Raney Nickel catalyst was prepared according to [25].

6-Aminohexylamide of betulonic (II) and oleanonic (XVI) acids. Diaminohexane (0.16 g, 1 mmol) and, then, triethyl amine (0.3 mL, dropwise) were added to the solution of chloride of betulonic or oleanonic acids (0.46 g, 1 mmol) in anhydrous $CHCl_3$ (30 mL). The reaction mixture was boiled for 8 h, washed with 5% HCl (2 × 50 mL) and cold water (50 mL), dried over $CaCl_2$, and evaporated in vacuum. The residue was fractionated on a column with Al_2O_3 that was eluted with benzene and chloroform.

3,28-Dioxo-28-(6-aminohexylamino)lup-20(29)-ene (II). The yield of compound (**II**) was 0.48 g (87%); mp 188–190°C. $[\alpha]_D^{20}$ −36° (*c* 0.07, $CHCl_3$); ^{1}H NMR: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5s, $5CH_3$), 1.20–2.13 (32 H, CH_2, CH, H2', H3', H4', H5', NH_2), 1.67 (3 H, s, H30), 2.32–2.58 (5 H, m, H13, H16, H6'), 3.03–3.32 (3 H, m, H19, H1'), 4.58 and 4.72 (2 H, both broadened s, H29), 5.75–5.88 (1 H, m, CONH); ^{13}C NMR: 14.5, 15.9, 16.1, 19.1, 19.4, 20.9, 21.4, 23.5, 25.5, 25.9 (C4'), 26.5 (C2'), 26.6 (C3'), 29.3, 29.7, 30.8, 33.7, 34.1, 36.8, 37.7, 38.4, 39.6, 40.6, 42.4 (C5'), 45.8 (C1'), 46.6, 47.3 (C6'), 49.3, 49.9, 50.0, 54.9, 55.5, 109.3 (C29), 150.9 (C20), 176.1 (C28), 218.2 (C3).

Found, %: C 77.84, H 10.62, N, 4.72. Calc. for $C_{36}H_{60}N_2O_2$ (*M* 552.881), %: C 78.21, H 10.94, N 5.07.

3,28-Dioxo-28-(6-aminohexylamino)olean-12-ene (XVI). The yield of compound (**XVI**) 0.33 g (62%); R_f 0.42; mp 150–152°C. $[\alpha]_D^{20}$ +21° (*c* 1.02, $CHCl_3$); ^{1}H NMR : 0.78, 0.89, 0.93, 1.01, 1.03, 1.11, 1.14 (21 H, 7s, CH_3), 1.20–2.12 (32 H, CH_2, CH, H2', H3', H4', H5', NH_2), 2.42–2.62 (2 H, m, H6'), 2.74–2.84 (1 H, m, H11), 2.93–3.03 (2 H, m, H1'), 5.18–5.34 (1 H, broadened s, H12), 5.78–5.88 (1 H, m, CONH); ^{13}C NMR: 14.0, 15.0, 16.8, 17.9, 19.5, 21.4, 22.6, 23.5, 24.8, 25.5 (C3'), 26.4 (C2'), 26.5 (C4'), 27.1, 29.6, 30.6, 31.6, 32.6, 32.9, 34.0, 36.6, 39.1 (C5'), 39.3, 42.1, 42.3, 45.8 (C1'), 46.2, 46.7, 47.3 (C6'), 49.9, 51.3, 55.2, 122.3 (C12), 145.1 (C14), 177.7 (C28), 217.2 (C3).

Found, %: C 77.79, H 10.67, N 4.81. Calc. for $C_{36}H_{60}N_2O_2$ (*M* 552.881), %: C 78.21, H 10.94, N 5.07.

Technique of the synthesis of compounds (III), (VI), and (XVII). The mixture of amide (**II**), (**V**), or (**XVI**) (1 mmol) acrylonitrile (8 mL), and 40% KOH (0.3 mL) in dioxane (15 mL) was stirred for 14 h at 20°C and poured into ice (50 g) with HCl (5 mL). The precipitate was filtered, washed with water to pH 7, dried in air, and fractionated on a column with Al_2O_3 that was subsequently eluted with benzene and chloroform.

3,28-Dioxo-28-{6-[bis(2-cyanoethyl)amino]hexylamino}lup-20(29)-ene (III). The yield of compound (**III**) 0.37 g (74%); R_f 0.32; mp 143–145°C. $[\alpha]_D^{20}$ +12° (*c* 0.02, CH_3COCH_3); ^{1}H NMR: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5s, $5CH_3$), 1.20–2.00 (29 H, CH_2, CH, H2', H3', H4', H5'), 1.69 (3 H, s, H30), 2.08–2.34 (5 H, m, H13, H16, H6'), 2.49–2.68 (4 H, m, H7', H10'), 3.04–3.27 (7 H, m, H19, H1', H8', H11'), 4.62 and 4.75 (2 H, both broadened s, H29), 5.57–5.68 (1H, m, CONH); ^{13}C NMR: 12.2 (C8'), 12.3 (C11'), 14.4, 15.4, 17.5, 19.4, 20.1, 21.5, 21.7, 22.9, 25.5 (C3'), 25.9, 29.2 (C2'), 29.4 (C5'), 29.7 (C4'), 30.7, 32.2, 32.7, 33.6, 36.7, 37.3, 37.5, 38.4, 38.5, 40.5, 42.5, 46.0 (C1'), 46.5, 48.0, 49.8 (C7'), 49.9 (C10'), 50.0, 51.1, 51.6 (C6'), 55.0, 55.4, 109.4 (C29), 118.6 (C9'), 119.4 (C12'), 150.7 (C20), 175.9 (C28), 218.6 (C3).

Found, %: C 76.12, H 9.72, N 8.12. Calc. for $C_{42}H_{66}N_4O_2$ (*M* 659.008), %: C 76.55, H 10.09, N 8.50.

3,28-Dioxo-28-[(2-cyanoethyl)amino]lup-20(29)-ene (VI). The yield of compound (**VI**) 0.34 g (68%); R_f 0.43; mp 94–96°C. $[\alpha]_D^{20}$ +24° (*c* 1.0, CH_3Cl); ^{1}H NMR: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5s, CH_3), 1.18–2.03 (21 H, CH_2, CH_3), 1.69 (3 H, s, H30), 2.18–2.67 (5 H, m H13, H16, H2'), 3.01–3.18 (1 H, m, H19), 3.42–3.53 (2 H, m, H1'), 4.62 and 4.78 (2 H, both broadened s, H29), 5.57–5.68 (1 H, m, CONH); ^{13}C NMR: 14.2, 15.3, 17.3 (C2'), 18.6, 19.3, 20.0, 21.6, 22.8, 25.4, 29.2, 29.8, 30.1, 30.4, 32.2, 32.6, 33.5, 36.6, 37.0, 37.3, 38.0, 40.4, 42.5, 46.5, 47.8, 49.7 (C1'), 50.9, 51.4, 55.4, 109.4 (C29), 119.1 (C3'), 150.2 (C20), 178.4 (C28), 217.9 (C3).

Found, %: C 77.82, H 9.56, N 5.24. Calc. for $C_{33}H_{50}N_2O_2$ (*M* 506.769), %: C 78.21, H 9.94, N 5.53.

3,28-Dioxo-28-{6-[bis(2-cyanoethyl)amino]hexylamino}olean-12-ene (XVII). The yield of compound (**XVII**) 0.50 g (78%); R_f 0.31; mp 86–88°C; $[\alpha]_D^{20}$ +34° (*c* 1.1, $CHCl_3$); ^{1}H NMR: 0.78, 0.89, 0.93, 1.01, 1.03, 1.11, 1.14 (21 H, 7s, CH_3), 1.20–2.12 (30 H, CH_2, CH, H2', H3', H4', H5'), 2.14–2.26 (2 H, m,

H6'), 2.49–2.68 (4 H, m, H7', H9'), 2.74–2.84 (1 H, m, H11), 3.03–3.28 (6 H, m, H1', H8', H10'), 5.18–5.34 (1 H, broadened s, H12), 5.88–5.98 (1 H, m, CONH); ^{13}C NMR: 12.0 (C8'), 12.3 (C11'), 14.0, 15.0, 16.8, 17.9, 19.5, 21.4, 22.6, 23.5, 24.8, 25.5 (C3'), 26.4 (C2', C4'), 27.1, 29.6, 27.2, 30.6, 31.6, 32.6, 32.9, 34.0, 36.6, 39.1 (C5'), 39.3, 42.1, 42.3, 45.8 (C1'), 46.2, 46.7, 47.3 (C6'), 47.9 (C7', C10'), 48.1, 51.3, 55.2, 118.4 (C9'), 119.4 (C12'), 121.8 (C12), 144.8 (C14), 177.5 (C28), 218.1 (C3).

Found, %: C 76.13, H 9.64, N 8.17. Calc. for $C_{42}H_{66}N_4O_2$ (*M* 659.008), %: C 76.55, H 10.09, N 8.50.

Synthesis of compounds (IV) and (XVIII). $LiAlH_4$ (467.4 mg, 12.3 mmol) was added to the solution of compound (**III**) or (**XVII**) (1 mmol) in anhydrous tetrahydrofuran with intensive stirring. The reaction mixture was boiled for 3 h and diluted with water (3 mL). The precipitate of $Al(OH)_3$ was filtered off, and the water layer was washed with chloroform (3 × 60 mL), dried over $CaCl_2$, and evaporated in vacuum. The residue was fractionated on a column with SiO_2 that was subsequently eluted with chloroform and methanol.

3β-Hydroxy-28-oxo-28-{6-[bis(3-aminopropyl)amino]hexylamino}lup-20(29)-ene (IV). The yield of compound (**IV**) 0.49 g (74%); R_f 0.13; mp 250–252°C. $[\alpha]_D^{20}$ +9° (*c* 0.51, CH_3OH); ^{1}H NMR (CD_3OD): 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5s CH_3), 1.18–2.03 (38 H, CH_2, CH_3, H2', H3', H4', H5', H8', H11', 2 NH_2), 1.69 (3 H, s, H30), 2.18–2.48 (5 H, m H13, H16, H6'), 2.46–2.67 (4 H, m H7', H10'), 3.05–3.28 (2 H, m, H19, H3), 3.31–3.48 (6 H, m, H1', H9', H12'), 4.62 and 4.78 (2 H, both broadened s, H29), 5.52–5.73 (1 H, m, CONH); ^{13}C NMR (CD_3OD): 14.4, 15.4, 17.6, 19.3, 20.1, 21.8, 22.9, 23.6, 25.4 (C3'), 25.6, 28.9 (C5'), 29.2 (C2'), 29.9 (C4'), 30.1, 30.3, 30.7, 32.3, 32.7, 33.5, 34.2 (C8', C11'), 35.2, 36.7, 37.3, 37.7, 38.3, 39.2 (C9'), 39.2 (C12'), 40.5, 42.6, 46.4 (C1'), 46.5, 48.1, 49.7 (C7', C10'), 51.1, 51.7 (C6'), 55.7, 77.3 (C3), 109.8 (C29), 150.7 (C20), 176.8 (C28).

Found, %: C 74.82, H 11.26, N 7.94. Calc. for $C_{42}H_{78}N_4O_2$ (*M* 671.013), %: C 75.37, H 11.71, N 8.35.

3β-Hydroxy-28-oxo-28-{6-[bis(3-aminopropyl)amino]hexylamino}olean-12-ene (XVIII). The yield of compound (**XVIII**) 0.41 g (62%); R_f 0.22; mp 125–127°C. $[\alpha]_D^{20}$ +67° (*c* 0.48, CH_3OH); ^{1}H NMR (CD_3OD): 0.78, 0.89, 0.93, 1.01, 1.03, 1.11, 1.14 (21 H, 7s, CH_3), 1.20–2.03 (41 H, CH_2, CH, H2', H3', H4', H5', H8', H11', 2NH_2), 2.14–2.26 (2 H, m, H6'), 2.49–2.68 (4 H, m, H7', H10'), 2.74–2.84 (1 H, m, H11), 3.09–3.25 (7 H, m, H3, H1', H9', H12'), 5.28–5.44 (1 H, broadened s, H12), 5.89–5.98 (1 H, m, CONH); ^{13}C NMR (CD_3OD): 14.0, 15.0, 16.8, 17.9, 19.5, 20.1, 21.4, 22.6, 23.5, 24.2, 24.8, 25.3 (C3'), 25.6, 27.1, 28.7 (C5'), 29.1 (C2'), 29.6 (C4'), 30.1, 30.6, 31.6, 32.6, 32.9, 34.0, 34.2 (C8'), 34.4 (C11'), 36.6, 39.1 (C9'), 39.3 (C12'), 42.1, 42.3, 45.8 (C1'), 46.2, 46.7, 47.3 (C6'), 47.9 (C7', C10'), 51.3, 55.2, 77.8 (C3), 121.8 (C12), 144.8 (C14), 177.5 (C28).

Found, %: C 74.63, H 11.45, N 7.89. Calc. for $C_{42}H_{78}N_4O_2$ (*M* 671.103), %: C 75.17, H 11.71, N 8.35.

3β-Acetoxy-28-oxo-28-[3-acetyl-(3-aminopropyl)-amino]lup-20(29)-ene (VIII). Compound (**VIII**) was prepared from compound (**VI**) similarly to compound (**IV**). Further, the reaction mixture was boiled for 8 h with acetic anhydride (1.3 mL) and poured into 5% solution of HCl (100 mL). The precipitate was filtered, washed with water, dried, and recrystallized from ethanol. The yield of compound (**VIII**) 0.42 g (70%); R_f 0.34; mp 128–130°C. $[\alpha]_D^{20}$ +11° (*c* 0.13, $CHCl_3$); ^{1}H NMR: 0.86, 0.92, 0.96, 1.04, 1.13 (15 H, 5s, CH_3), 1.18–2.03 (25 H, CH_2, CH, H2', NH), 1.69 (3 H, s, H30), 2.08 and 2.31 (6 H, 2s, 2 Ac), 2.18–2.71 (3 H, m, H13, H16), 3.23–3.53 (5 H, m, H19, H3', H1'), 4.42–4.45 (1 H, m, H3), 4.62 and 4.78 (2 H, both broadened s, H29); ^{13}C NMR: 14.2, 15.3, 17.4, 18.6, 19.3, 20.0, 21.6, 22.8, 25.4, 29.8, 30.1, 30.4, 32.2, 32.6, 33.5, 34.3 (C2'), 36.6, 37.0, 37.3, 38.0, 39.7 (C3'), 40.4, 42.5, 43.4 (C1'), 45.7, 46.2, 46.5, 47.8, 50.9, 51.4, 55.4, 80.8 (C3), 109.4 (C29), 150.2 (C20), 170.4 ($CH_3\underline{C}O$), 172.4 (NH-$\underline{C}OCH_3$), 178.4 (C28).

Found, %: C 74.07, H 9.85, N 4.26. Calc. for $C_{37}H_{60}N_2O_2$ (*M* 569.912), %: C 74.45, H 10.13, N 4.69.

3β-Hydroxy-28-oxo-28-(6-aminohexylamino)lup-20(29)-ene (IX). $NaBH_4$ (120 mg, 3.12 mmol) was added to the solution of compound (**II**) (0.66 g, 1 mmol) in isopropanol (20 mL) for 10 min. The reaction mixture was kept for 2 h and diluted with 30% solution of HCl (72 mL). The precipitate was filtered, washed with water, dried, and recrystallized from ethanol. The yield of compound (**IX**) 0.40 g (80%); R_f 0.18; mp 68–70°C. $[\alpha]_D^{20}$ +4° (*c* 0.38, $CHCl_3$); ^{1}H NMR: 0.76, 0.82, 0.92, 0.96, 1.04 (15 H, 5s, CH_3), 1.12–2.13 (32 H, CH_2, CH, H2', H3', H4', H5', NH_2), 1.67 (3 H, s, H30), 2.39–2.57 (5 H, m, H13, H16, H6'), 3.03–3.38 (4 H, m, H3, H19), 4.58 and 4.72 (2 H, both broadened s, H29), 5.75–5.88 (1 H, m, CONH); ^{13}C NMR: 14.6, 15.3, 16.1, 18.2, 19.4, 20.9, 25.6, 25.9 (C3'), 27.4, 27.9 (C2'), 28.0 (C4'), 28.1 (C5'), 28.2, 28.3, 29.4, 29.7, 30.9, 33.8, 34.4, 37.2, 37.6, 38.4, 38.7 (C1'), 38.8 (C6'), 39.6, 40.7, 42.4, 46.6, 50.1, 50.6, 55.4, 55.5, 78.9 (C3), 109.2 (C29), 150.9 (C20), 176.1 (C28).

Found, %: C 77.64, H 10.88, N 4.72. Calc. for $C_{36}H_{62}N_2O_2$ (*M* 554.897), %: C 77.92, H 11.26, N 5.05.

3β-(2-Cyanoethoxy)-28-oxo-28-{6-[bis(2-cyanoethyl)amino]hexylamino}lup-20(29)-ene (X). was prepared from compound (**IX**) similarly to compound (**III**). The yield of compound (**X**) 0.41 g (74%); R_f 0.57; mp 149–151°C; $[\alpha]_D^{20}$ +13° (*c* 1.0, $CHCl_3$); ^{1}H NMR: 0.76, 0.82, 0.92, 0.96, 1.04 (15 H, 5s, CH_3), 1.12–2.03 (30 H, CH_2, CH, H2', H3', H4', H5'), 1.67 (3 H, s, H30), 2.39–2.51 (5 H, m, H13, H16, H6'), 2.54–2.62 (4 H, m, H8', H8''), 2.64–2.68 (4 H, m, H7', H7''), 3.08–3.34 (5 H, m, H19, H1', H2'''), 3.69–3.78 (2 H, m, H1'''), 4.58 and 4.72 (2 H, both broadened s, H29), 5.75–5.88 (1 H, m, CONH); ^{13}C NMR: 14.6, 15.3, 16.1 (C8', C8''), 18.2, 19.2 (C2'''), 19.5, 20.9, 25.6, 25.9 (C3'), 26.5 (C2'), 27.9 (C4', C5'), 28.0, 28.3, 29.4, 29.7, 30.9, 33.8, 34.4, 37.1, 37.2, 37.6, 38.4, 38.7 (C1'), 39.6, 40.7, 42.4, 46.7, 50.1, 50.6, 55.4, 55.5, 55.8 (C6'), 55.9 (C7'), 56.0 (C7''), 65.9 (C1'''), 67.0, 87.7 (C3), 109.2 (C29), 117.3 (C3'''), 118.2 (C9'), 118.3 (C9''), 150.9 (C20), 176.1 (C28).

Found, %: C 75.11, H 9.63, N 9.25. Calc. for $C_{45}H_{71}N_5O_2$ (*M* 714.087), %: C 75.69, H 10.02, N 9.81.

3β-(3-Aminopropoxy)-28-oxo-28-{6-[bis(3-aminopropyl)amino]hexylamino}lup-20(29)-ene (XI). Compound (**X**) (1 mmol) was dissolved in methanol (30 mL), and Ni-Raney (0.96 g) was added to the solution. The reaction mixture was kept in an autoclave at 100 atmospheres for 8 h. The catalyst was filtered off and the filtrate was diluted with water. The precipitate was washed with water to pH 7, dried, and recrystallized from ethanol. The yield of compound (**XI**) 0.41 g (88%); R_f 0.07; mp 208–210°C. $[\alpha]_D^{20}$ −5° (*c* 0.45, CH_3OH); ^{1}H NMR (CD_3OD): 0.82, 0.90, 0.92, 0.96, 1.04 (15 H, 5s, CH_3), 1.12–2.03 (47 H, CH_2, CH, H2', H3', H4', H5', H8', H8'', H2''', $3NH_2$), 2.14–2.19 (4 H, m, H7', H7''), 2.39–2.51 (5 H, m, H13, H16, H6'), 2.64–2.88 (6 H, m, H9', H9'', H3'''), 3.08–3.34 (5 H, m, H19, H1', H1'''), 4.62 and 4.78 (2 H, both broadened s, H29), 5.75–5.88 (1 H, m, CONH); ^{13}C NMR (CD_3OD): 14.2, 14.7, 14.8, 16.9, 19.8, 21.5, 21.7, 22.2, 22.4, 24.7, 25.9 (C3'), 26.1, 26.7, 27.9 (C2'), 28.0 (C4'), 28.1 (C5'), 28.7 (C20), 31.9 (C8'), 32.0 (C8''), 33.4, 36.0 (C2'''), 37.1, 37.4, 37.6, 38.6 (C1'), 38.7 (C9'), 38.8 (C9''), 38.9 (C3'''), 39.6, 41.3, 42.8, 46.7, 48.5, 48.6, 49.3, 54.3, 54.6, 55.7 (C7', C7''), 58.6 (C6'), 65.4 (C1'''), 86.2 (C3), 109.2 (C29), 150.9 (C20), 176.1 (C28).

Found, %: C 73.85, H 11.31, N 9.27. Calc. for $C_{45}H_{85}N_5O_2$ (*M* 728.198), %: C 74.22, H 11.76, N 9.62.

3,28-Dioxo-28-(4-aminophenylsulfonyl-4-phenylamino)lup-20(29)-ene (XII). 4,4'-Diaminodiphenylsulfone (1 mmol) was added to the solution of chloride of betulonic acid (**I**) (0.47 g, 1 mmol) in anhydrous $CHCl_3$ (30 mL), then, triethyl amine (3 mL) was added dropwise. The reaction mixture was boiled for 8 h with a backflow condenser, washed with 5% HCl (2 × 50 mL) and cold water (1 × 50 mL), and dried over $CaCl_2$. The solvent was evaporated in vacuum, and the residue was fractionated on a column with Al_2O_3 that was eluted with chloroform. The yield of compound (**XII**) 0.40 g (85%); R_f 0.30. mp 114–115°C. $[\alpha]_D^{20}$ −19° (*c* 0.05, $CHCl_3$); ^{1}H NMR: 0.81, 0.94, 0.97, 0.98, 1.02 (15 H, 5s, CH_3), 1.21–2.00 (24 H, CH_2, CH, NH, NH_2), 1.69 (3 H, s, H30), 2.28–2.55 (3 H, m, H13, H16), 3.02–3.18 (1 H, m, H19), 4.58 and 4.71 (2 H, both broadened s, H29), 6.40–6.58 (2 H, m, H9', H10'), 7.52–7.98 (6 H, m, H2', H3', H5', H6', H8', H11'); ^{13}C NMR: 11.8, 14.7, 16.1, 19.5, 20.0, 21.2, 21.9, 26.0, 26.8, 30.0, 31.1, 33.7, 34.0, 34.5, 37.3, 37.9, 38.1, 40.0, 41.1, 43.0, 46.8, 47.6, 50.3, 50.5, 55.3, 57.2, 109.7 (C20), 114.4 (C2', C9'), 120.1 (C6', C10'), 128.4 (C8'), 128.7 (C11'), 128.8 (C3'), 129.8 (C5'), 136.4 (C7'), 137.6 (C4'), 143.7 (C1'), 151.1 (C29), 151.9 (C12'), 175.5 (C28), 218.0 (C3).

Found, %: C 71.62, H 7.78, N 3.63, S 4.17. Calc. for $C_{42}H_{56}N_2O_5S$ (*M* 700.973), %: C 72.06, H 8.20, N 4.07, S 4.60.

3,28-Dioxo-28-[(2-cyanoethyl)-4-aminophenylsulfonyl-4-phenylamino]lup-20(29)-ene (XIII). The mixture of amide (**XII**) (0.48 g, 1 mmol), acrylonitrile (4 mL), and $Cu(OAc)_2$ (0.2 g) in dioxane (15 mL) was boiled for 20 h at 90°C, cooled, and poured onto ice (50 g) with HCl (5 mL). The precipitate was filtered, washed with water to pH 7, dried in air, and fractionated on a column with Al_2O_3 that was subsequently eluted with benzene and chloroform. The yield of compound (**XIII**) 0.56 g (80%); R_f 0.43; mp 188–190°C. $[\alpha]_D^{20}$ +2° (*c* 0.55, $CHCl_3$); ^{1}H NMR: 0.81, 0.94, 0.97, 0.98, 1.02 (15 H, 5s, CH_3), 1.21–2.00 (22 H, CH_2, CH, NH), 1.69 (3 H, s, H30), 2.48–2.68 (5 H, m, H13, H16, H2''), 2.98–3.18 (1 H, m, H19), 3.61–3.74 (2 H, m, H1''), 4.53 and 4.69 (2 H, both broadened s, H29), 6.51–6.65 (1 H, m, CONH), 6.40–6.58 (2 H, m, H9', H10'), 7.52–7.98 (6 H, m, H2', H3', H5', H6', H8', H11'); ^{13}C NMR: 11.7, 14.7, 16.1, 19.5, 20.0, 21.2, 21.4 (C2''), 21.9, 26.0, 26.8, 30.0, 31.1, 33.7, 34.0, 34.5, 37.3, 37.9, 38.1, 40.0, 41.1, 43.0, 45.7 (C1''), 46.8, 47.6, 50.3, 50.5, 55.3, 57.2, 109.7 (C20), 114.4 (C2', C9'), 118.6 (C3''), 120.1 (C6', C10'), 128.4 (C8'), 128.7 (C11'), 128.8 (C3'), 129.8 (C5'), 136.4 (C7'), 137.6 (C4'), 143.7 (C1'), 151.1 (C29), 151.9 (C12'), 175.5 (C28), 218.0 (C3).

Found, %: C 72.96, H 7.64, N 5.23, S 4.02. Calc. for $C_{45}H_{59}N_3O_4S$ (*M* 738.037), %: C 73.23, H 8.06, N 5.69, S 4.34.

3β-Hydroxy-28-oxo-28-[(3-aminopropyl)-4-aminophenylsulfonyl-4-phenylamino]lup-20(29)-ene (XIV) was prepared from compound (**XIII**) as described for compound (**IV**). The yield of compound (**XIV**) 0.53 g

(72%); R_f 0.19; mp 196–198°C. $[\alpha]_D^{20}$ −8° (*c* 0.06, CH_3OH); ^1H NMR (CD_3OD): 0.81, 0.94, 0.97, 0.98, 1.02 (15 H, 5s, CH_3), 1.21–2.00 (26 H, CH_2, CH, H2'', NH_2, NH), 1.69 (3 H, s, H30), 2.47–2.74 (5 H, m, H13, H16, H3''), 2.98–3.20 (1 H, m, H19), 3.60–3.75 (2 H, m, H1''), 4.54 and 4.67 (1 H, both broadened s, H29), 6.52–6.63 (1 H, m, CONH), 6.40–6.58 (2 H, m, H9', H10'), 7.52–7.98 (6 H, m, H2', H3', H5', H6', H8', H11'); ^{13}C NMR (CD_3OD): 11.7, 14.7, 16.1, 19.5, 20.0, 21.2, 21.9, 26.0, 26.8, 29.1 (C2''), 30.0, 31.1, 33.7, 34.0, 34.5, 37.3, 37.9, 38.1, 40.0, 41.1, 42.4 (C3''), 43.0, 46.0 (C1''), 46.8, 47.6, 50.3, 50.5, 55.3, 57.2, 78.4 (C3), 109.8 (C20), 114.6 (C2'), 114.7 (C9'), 120.2 (C6', C10'), 128.6 (C8'), 128.9 (C11'), 129.0 (C3'), 130.1 (C5'), 136.1 (C7'), 137.2 (C4'), 143.4 (C1'), 151.2 (C29), 151.6 (C12'), 175.7 (C28).

Found, %: C 72.46, H 8.11, N 5.21, S 3.87. Calc. for $C_{45}H_{63}N_3O_4S$ (*M* 742.069), %: C 72.84, H 8.56, N 5.66, S 4.32.

Studies of the antitumor activity on mice with the transplanted Lewis lung carcinoma. The experiment was performed on female mice of the C57BL/6 line (20–25 g body weight). The mice were kept under the standard vivarium conditions in a natural light regime and the usual consumption of nutrition and water. The mice were treated according to the European convention for the humane treatment of laboratory animals. Cells of the Lewis lung carcinoma were intramuscularly transplanted (2×10^6 cells in 0.1 mL of physiological solution). Compound (**XII**) was daily administered to the experimental group of mice at a dose of 50 mg/kg in an aqueous suspension with Tween from 10th to 16th day after the transplantation. The total dose was 350 mg/kg. The following mixture of PCT-imitated antitumor agents was once administered to the comparison group: doxorubicine (4 mg/kg), cyclophosphan (50 mg/kg), vincristine (0.4 mg/kg), and prednisolone (5 mg/kg) [20]. The water-Tween mixture was administered to the control group in the equivalent volume. Every group consisted of 11–14 animals.

The volumes of tumor nodes were measured by a bar in three mutually perpendicular directions before and in the course of the administration of compound (**XII**).

Studies of the anti-inflammatory activity on the model of histamine-induced inflammation. The inflammatory activity was determined according to a decrease in edema of a mouse leg. The edema was induced by the subplanar administration of 0.1% solution of histamine. The water-Tween suspension of compound (**XII**) was administered in a stomach at a dose of 100 mg/kg one hour before the phlogogene administration. The indomethacin reference agent (Fluka) was administered in the same way at a dose of 20 mg/kg. Betulonic acid was administered to a separate group of mice at a dose of 100 mg/kg. The control animals received the water-Tween mixture in the equivalent volume. Every group involved 8 mice. The value of edema was determined 5 h after the histamine introduction from the difference in the masses of the inflamed and the healthy leg. The inflammatory index was calculated for every animal as a ratio of this difference to the mass of the healthy leg. The results were processed using the STATISTIKA 6 software. The differences with $p < 0.05$ were considered as significant. The edema value was calculated for every experimental group relatively to the control (100%).

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