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Synthesis and Biological Activity of Novel Acetylene Betulonic Acid Derivatives

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Abstract

Acetylene derivatives of betulonic acid amide were synthesized. Their hepatoprotective and anti-inflammatory activity was investigated. In the series of betulonic acid derivatives, two compounds with a substantial hepatoprotective and anti-inflammatory effect were revealed.

Key words: triterpenoids, betulonic acid, aryl acetylenes, hepatoprotective activity, anti-inflammatory activity

INTRODUCTION

Synthetic transformations of triterpenoids represent one of most intensely developing fields of organic chemistry, which is connected with a great demand for this series of compounds used as medicinal preparation with broad spectrum of action. It is known that betulonic acid amides exhibit manifold biological activity such as antiviral, antineoplastic, immunostimulating [1] and antioxidative [2] types of activities.

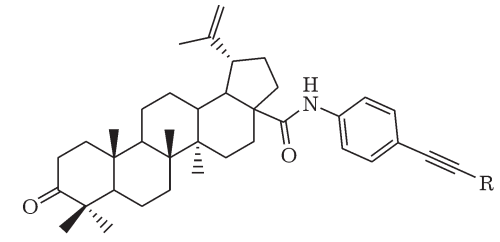
The directed synthetic modification of triterpenoids allows one to essentially enhance, and in some cases to change their biological activity.

Earlier we demonstrated the outlook of the modification of triterpenoids via introducing acetylene residues into their structure. Obtaining similar derivatives and their pharmacological properties available from the literature was not discussed till now. Among the acetylene derivatives of betulonic acid amides we have synthesized [3], efficient anti-inflammatory and hepatoprotective agents were revealed.

In the present work, a series of obtained earlier acetylenyl betulonic acid amides was added with novel derivatives **1–5** with various substituents in a *para*-position of the benzene ring.

These substituents include: 2-ethinylpyridyl **1**; 1-[5-hydroxy-5-methylbutadiine-1,3-yl)] **2**; *N*-methyl-*N*-[(1*S*,2*S*)-2(methylamino)-1-phenyl-1-hydroxypropyl] propargine-1-yl **3**; *N*-pyrrolidine-propargine-1-yl **4**, and its open-ring form *N*,*N*-diethylaminopropargine-1-yl **5**.

Acetylene derivatives **1**, **2** and **5** were synthesized according to the techniques we described earlier [4]. Aminoacetylenes **3** and **4**



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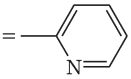

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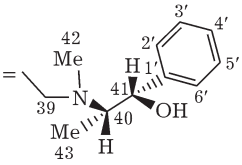

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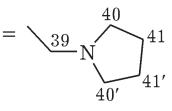

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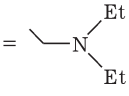
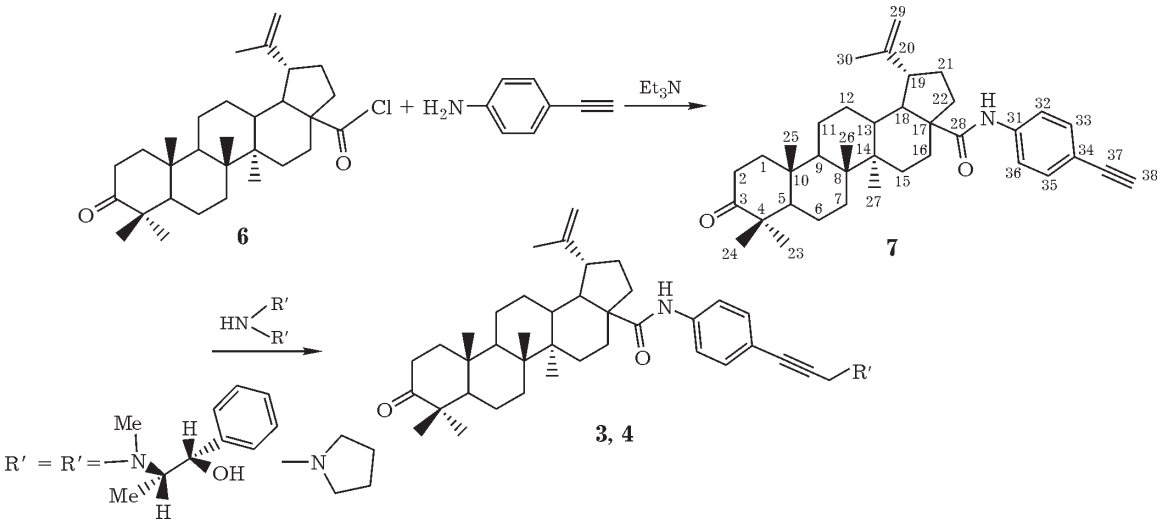




Scheme 1.

were obtained *via* aminoalkylation of ethinyl betulonic acid amide **7**, synthesized basing on betulonic acid chlor-anhydride **6** and 4-aminophenylacetylene in the presence of triethylamine [4] (Scheme 1).

Initial amidoacetylene **7** was entered into reaction with (+)-pseudoephedrine, pyrrolidine and paraformaldehyde in the presence of CuCl, which resulted in the formation of corresponding Mannich bases **3** and **4** with the yield of 82 and 72 %, respectively. The structure of newly obtained compounds was confirmed by analytical and spectral data.

The pharmacological properties of compounds **1–5** were investigated in outbred mice *via* determining hepatoprotective, antioxidative and anti-inflammatory activity according to methodological recommendations [6]. As reference preparations, we used an antioxidative agent dihydroquercetin (99.9 % purity) and nonsteroid anti-inflammatory preparation indometacin (the substance from Fluka, BioChemika).

RESULTS AND DISCUSSION

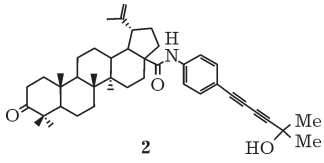
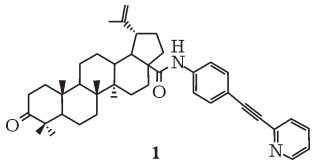
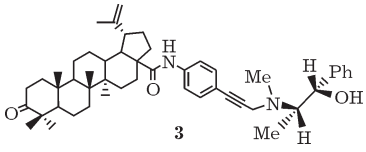
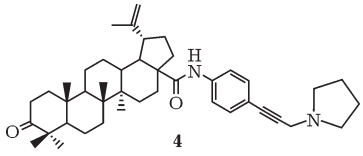
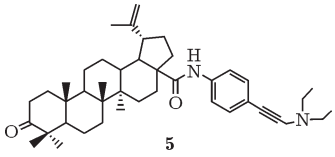
The hepatoprotective effect was determined from decreasing the content of cytolysis and cholestasis markers in blood serum, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP). The antioxidative properties were estimated according to decreasing the concentration of peroxide oxidation products (TBAPC) determined from the reaction with thiobarbituric acid [7].

The biochemical analysis of blood serum (Table 1) demonstrated that among the compounds tested there are two agents, exhibiting a significant hepatoprotective effect. So, compound **3** reduces the activity of both transaminase by 1.4 times ($p < 0.05$) with respect to reference, and agent **5** to a similar extent reduces the activity of AST. Both compounds compare well with dihydroquercetin in the intensity of the hepatoprotective effect, whereas agent **3** is even better, since it reliably reduces the activity of ALT, the marker hepatocyte cytolysis. Under the conditions of this experiment, none of compounds tested lowered the activity of AP, which indicates the absence of anti-cholestatic properties therein. Besides the anticytolytic action, agent **5** demonstrates also antioxidative effect reducing the concentration of TBAPC in blood by 1.4 times extent comparing to the reference ($p < 0.05$). The greatest antioxidative effect among the derivatives betulonic acid is exhibited by compound **1**: the level of TBAPC decreased by 1.6 times comparing to the reference, whereas the reference demonstrated a 1.4-fold decrease.

Studies on anti-inflammatory action in the model of histamine paw hypostasis have demonstrated that among the compounds synthesized, the three agents exhibit statistically reliable effect (Table 2). The maximum intensity of the effect was observed for the group of

TABLE 1

Effect of betulonic acid derivatives on the biochemical parameters of blood serum in mice with CCl_4 hepatitis

Groups	Dose, mg/kg	AP, units/L	ALT, units/L	ACT, units/L	TBARC, μmol/L
Reference group	–	897.44±105.63	144±13.63	142.13±10.88	2.59±0.32
 **2**	50	(1429.38±83.7)*	149.33±21.22	154.89±8.48	2.08±0.16
 **1**	50	1208.29±69.72	139.89±14.88	141.78±12.74	(1.58±0.15)*
 **3**	50	920±55.99	(103.13±12.83)*#	(101.5±11.48)*	(2.34±0.15)#
 **4**	50	(1060.33±77.17)#	131.67±15.57	114.63±8.76	(2.51±0.22)#
 **5**	50	856.44±68.78	141.89±17.79	(104.63±11.76)*	(1.79±0.18)*
Dihydroquercetin	100	892.11±58.69	139.11±7.53	(98.25±6.79)*	(1.81±0.16)*

*$p < 0.05$ with respect to reference group.

#$p < 0.05$ with respect to dihydroquercetin.

mice, after introducing the agent **3**, which resulted in a much more considerable decrease in hypostasis comparing to that for reference groups of mice and the group that received indometacin (by 1.9 and 1.4 times, respectively). A considerable activity was demonstrated also by the agent **5** caused a 1.6-fold decrease of hypostasis with respect to reference group, whereas similar decrease in the case of indometacin amounted to 1.4 times. It was demonstrated that agent **2** on anti-inflammatory activity is highly competitive with indometacin.

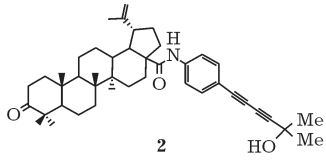
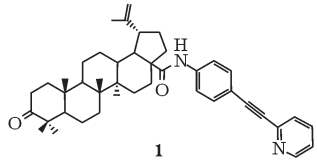
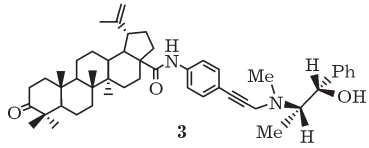
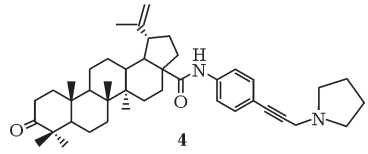
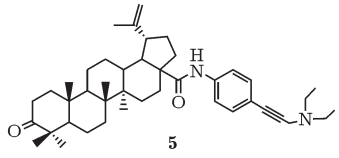
EXPERIMENTAL

Chemistry

Melting point values were determined using a Koffler apparatus. IR spectra were registered using a Bruker Vector 22 spectrometer with KBr pellets. High resolution mass spectra were obtained on a Thermo Electron Corporation DFS mass spectrometer. The elemental analysis was performed on a Carlo Erba model 1106 CHN analyzer. NMR spectra were registered using a

TABLE 2

Effect of compounds on mice paw edema indices caused by subplanar histamine introduction

Agents	Dose, mg/kg	Inflammation index, %	Edema size with respect to reference, %
Reference	–	22.1±2.6	100
 **2**	20	(15.9±1.1)*	71.9
 **1**	20	19.7±1.9	89.1
 **3**	20	(11.8±1.4)*#	53.4
 **4**	20	18.1±1.9	81.9
 **5**	20	(13.7±1.4)*	62.0
Indometacin	20	16.8±0.9	76.0

*$p < 0.05$ with respect to reference group.

#$p < 0.05$ with respect to indometacin.

Bruker AV-300 spectrometer (300.13 MHz (^{1}H) and 75.47 MHz (^{13}C)), Bruker AV-400 spectrometer (400.13 MHz (^{1}H) and 100.61 MHz (^{13}C)) for the solutions of substances in $CDCl_3$. Chemical shift values were measured against residual $CHCl_3$ signals in the solvent (δ_H 7.24 ppm and δ_C 76.90 ppm). The multiplicity of NMR signals for ^{13}C spectra was determined in a J-modulation mode (JMOD). The assignment of ^{1}H and ^{13}C signals in NMR spectra for a polycyclic skeleton of compounds **3**, **4** was performed *via* the comparison with the corresponding spectra of betulonic acid as a key compound [5]. Owing to difficulties in assigning all signals in ^{1}H NMR spectra, for the mentioned compounds, data concerning characteristic signals are presented. The main part of protons in the triterpenoid skeleton exhibits resonance within the range of 2.7–0.8 ppm.

***N*-(3-oxo-20(29)-lupen-28-oyl)-4-{*N*-methyl-*N*-[(1*S*,2*S*)-2-(methylamino)-1-phenyl-1-hydroxypropyl]-propargine-1-yl)} aniline (3).** To 184 mg (1.1 mmol) of (+)-pseudoephedrine was added 33 mg (1.1 mmol) of paraformaldehyde in 5 mL dioxane at a room temperature. The mixture was stirred at 70 °C during 2–2.5 h till a complete dissolution of paraformaldehyde. A mixture consisting of 300 mg (0.54 mmol) of

acetylene **7**, 184 mg (0.54 mmol) of di(*N*-(1*S*,2*S*)-2(methylamino)-phenyl-1-hydroxypropyl) methane, 15 mg (0.15 mmol) of CuCl in 10 mL of dioxane was stirred in a flow of argon for 3.5 h at 80–85 °C. After completing the synthesis we washed the reaction mixture with aqueous ammonia. The organic layer was dried using Na_2SO_4, filtered through the layer of Al_2O_3 (1×1.5 cm). The solvent was removed under vacuum, the residue was triturated with hexane, and then it was filtered to obtain 300 mg (82 %) of compounds **3**, m.p. 110–112 °C (benzene). High resolution mass spectrum, found: *m/z* 729.499 [$M-H^+$]. $C_{43}H_{60}N_2O_2$. Calculated: M = 729.4977. ^{1}H NMR, δ, ppm (*J*, Hz): 0.85 (3H, m, Me-43), 0.90 (3H, s, Me-25), 0.95 (3H, s, Me-24), 0.99 (6H, s, Me-26, 27), 1.04 (3H, s, Me-23), 1.68 (3H, s, Me-30), 2.41 (3H, s, Me-42), 2.87 (1H, m, 40), 3.14 (1H, dt, 19, J_1 = 4, J_2 = 11), 3.59 (2H, m, CH_2-39), 4.23 (1H, d, 41), 4.59 (1H, s, 29), 4.73 (1H, s, 29), 7.25–7.45 (9H, m, 32, 36, 33, 35, 2′, 3′, 4′, 5′, 6′); ^{13}C NMR, δ, ppm: 8.17 (C-43), 14.43 (C-27), 15.80 (C-26), 15.85 (C-25), 19.38 (C-30), 19.48 (C-6), 20.91 (C-24), 21.31 (C-11), 25.47 (C-12), 26.42 (C-23), 29.45 (C-21), 30.62 (C-15), 33.55 (C-16), 33.69 (C-7), 34.03 (C-2), 35.21 (C-42), 36.80 (C-22), 37.49 (C-13), 37.95 (C-10), 39.51 (C-1), 40.57 (C-8), 42.47 (C-14), 44.69 (C-39), 46.30 (C-19), 47.22 (C-4), 49.89 (C-9), 50.06 (C-18), 54.92 (C-5), 56.47 (C-17), 64.62 (C-40), 74.82 (C-41), 84.36 (C-38), 85.28 (C-37), 109.49 (C-29), 118.23 (C-34), 119.47 (C-32, 36), 123.28 (C-2′, 6′), 127.65 (C-4′), 128.13 (C-3′, 5′), 132.15 (C-33, 35), 138.08 (C-31), 141.68 (C-1′), 150.43 (C-20), 174.33 (C-28), 218.03 (C-3). IR spectrum (KBr, ν, cm^{-1}): 1703 (C=O); 2227 (C≡C). Found, %: C 80.76, H 9.21, N 4.51. $C_{49}H_{65}N_2O_3$. Calculated, %: C 80.50, H 9.10, N 3.83.

***N*-(3-oxo-20(29)-lupen-28-oyl)-4-(*N*-pyrrolidinopropargyl-1-in) aniline (4).** To 4.00 g (56 mmol) of pyrrolidine was added 1.68 (56 mmol) paraformaldehyde in 15 mL of dioxane at a room temperature. The mixture was stirred under 70 °C during 2–2.5 h up to a complete dissolution of paraformaldehyde. A mixture of 287 mg (0.52 mmol) of acetylene **7**, 82 mg (0.53 mmol) of di(N-pyrrolidino)methane, 14 mg (0.14 mmol) of CuCl in 10 mL of dioxane was stirred in a flow of argon at 80–85 °C during 1 h. After completing the synthesis, the reaction mixture was washed by aqueous ammonia. The organic layer was dried using Na_2SO_4, filtered through the layer of Al_2O_3 (1 × 1.5 cm). The solvent was removed in vacuum, whereas the remainder was triturated with hexane. The precipitate was filtered, with obtaining 230 mg (72 %) of Mannich base **4**, m.p. 135–137 °C (benzene). High resolution mass spectrum, found: *m/z* 636.4636 [M]$^+$. $C_{43}H_{60}N_2O_2$. Calculated: M = 636.4649. ^{1}H NMR, δ, ppm (*J*, Hz): 0.90 (3H, s, Me-25), 0.95 (3H, s, Me-24), 0.98 (6H, s, Me-26, 27), 1.04 (3H, s, Me-23), 1.67 (3H, s, Me-30), 1.81 (4H, m, 40, 40′), 2.66 (4H, m, 41, 41′), 3.13 (1H, dt, 19, J_1 = 4, J_2 = 11), 3.59 (2H, s, CH_2-39), 4.59 (1H, s, 29), 4.73 (1H, s, 29), 7.37 (4H, m, 32, 36, 33, 35); ^{13}C NMR, δ, ppm: 14.11 (C-27), 15.49 (C-26), 15.51 (C-25), 19.05 (C-30), 19.15 (C-6), 20.59 (C-24), 20.98 (C-11), 23.33 (C-41, 41′), 25.14 (C-12), 26.09 (C-23), 29.12 (C-21), 30.29 (C-15), 33.21 (C-16), 33.38 (C-7), 33.72 (C-2), 36.47 (C-22), 37.15 (C-13), 37.65 (C-10), 39.19 (C-1), 40.23 (C-8), 42.14 (C-14), 43.47 (C-39), 45.98 (C-19), 46.91 (C-4), 49.56 (C-9), 49.72 (C-18), 52.33 (C-40, 40′), 54.58 (C-5), 56.13 (C-17), 83.54 (C-38), 84.41 (C-37), 109.18 (C-29), 118.17 (C-34), 119.08 (C-32, 36), 131.96 (C-33, 35), 137.55 (C-31), 150.12 (C-20), 173.98 (C-28), 217.79 (C-3). IR spectrum (KBr, ν, $см^{-1}$): 1697 (C=O); 2211 (C≡C). Found, %: C 80.95, H 9.57, N 4.26. $C_{43}H_{60}N_2O_2$. Calculated, %: C 81.08, H 9.49, N 4.40.

Biology

In the experiments we used outbred mice males with the body mass of 22–25 g, received from the *vivarium* of the Institute of Cytology and Genetics, SB RAS (Novosibirsk). During the experiments animals were kept under standard conditions, received granulated fodder and water *ad libitum*; all the manipulations were performed in accordance with the Convention for humane laboratory animal handling.

CCl_4 hepatitis model. Acute toxic hepatitis was produced *via* single introduction of 25 % CCl_4 solution in sunflower-seed oil into mice stomach [6]. The agents under testing were introduced intragastrally in the form of water-Tween emulsion an hour before reproducing the hepatitis at a dose of 50 mg/kg. The reference compound, such as antioxidative agent dihyd-

roquercetin [(2*R*,3*R*)-3,5,7,3′,4′-pentahydroxyflavanone] (99 % purity) was introduced into stomach at an effective dose of 100 mg/kg. The Animals of the reference group received the water-Tween emulsion in an equivalent dose. Each group consisted of not less than 10 individuals. In 1 day in the mice blood serum we determined AP, ALT, ACT, activity using standard reagent kits (Biocon, Olvex Diagnosticum). The level TBA reactive compounds (TBARC) was determined *via* commonly known method [7].

Histamine induced inflammation model. The inflammatory edema was caused in 72 mice *via* introducing 0.1 % histamine aqueous solution into the aponeurosis of back paw in the amount of 0.05 mL. The agents under testing were introduced intragastrally in the form of water-Tween emulsion an hour before introducing the flogogen at a dose of 20 mg/kg. In a similar manner, we introduced the reference preparation indometacin (Fluka) at a same dose. The animals of the reference group were introduced with an equivalent amount of water with Tween. In 5 h after introducing the flogogen the animals were killed *via* craniocervical dislocation, back paws were cut off below ankle joint and with determining the mass of each paw. The inflammation index was calculated on the base of these values as a ratio of the difference between the mass of healthy and inflamed paw to the mass of healthy paw. The anti-inflammatory effect was estimated from the reduction of the edema index for the animals of experimental group comparing to the reference group.

The results were processed by means of Statistica 6.0 software package. The differences were considered reliable for the probability value $p < 0.05$.

CONCLUSION

Thus, in the series of betulonic acid acetylene derivatives we revealed two compounds with a complex action such as compound **3**, exhibiting pronounced hepatoprotective and anti-inflammatory action, and agent **5**, exerting considerable antioxidative, hepatoprotective and anti-inflammatory effects. Moreover, prospective compounds were revealed with a high level of anti-inflammatory activity (agent **2**) and antioxidative activity (agent **1**).

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