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CHEMISTRY

Synthesis of the First Acetylene Derivatives of Betulonic Acid

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Modification of natural compounds with the aim of searching for efficient therapeutic agents is one of the main trends of the promising research direction appeared at the interface between fine organic synthesis and medicinal chemistry.

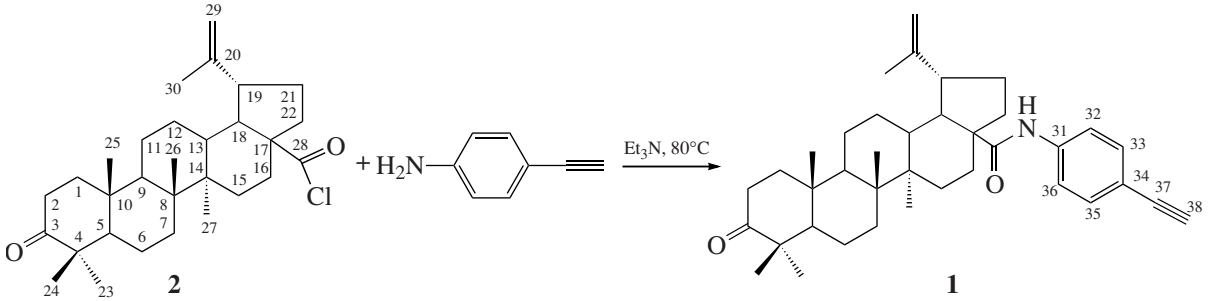
In recent years, there has been growing interest in plant triterpenoids, which provide a basis for the preparation of highly efficient antitumor agents [1]. Lupane triterpenoids, in particular, betulinic acid and its derivatives, are of special interest [1, 2]. Amides and peptides of betulonic acid showing antitumor activity are known [3, 4].

Studies within the last two decades have shown that acetylenic compounds are a rather representative group of natural metabolites produced mainly by high plants, as well as by fungi and microorganisms. In spite of the high anticancer activity of many natural acetylenic metabolites [5, 6], betulonic acid derivatives with triple bonds are still unknown; therefore, the synthesis of such compounds is an independent synthetic task. Moreover, alkyne residue is one of the most convenient fragments in designing new medicinal agents owing to the high reactivity and facile functionalization of the triple bond.

Taking into account that the results of practical importance in the search for antitumor and antiviral agents [2] were obtained by the transformation of the carbon atom at the 28-position of betulonic acid, we have attempted to introduce acetylenic fragments just in the 28-position of betulonic acid.

It should be emphasized that the use of the currently most common method for the preparation of alkynes by the Sonogashira cross-coupling of terminal acetylenes with halogenated derivatives in the $CuI–PdCl_2(PPh_3)_2$ system is not trivial. Indeed, the initial halobetulonic acid contains the terminal vinyl group and a disubstituted olefin can form under the copper–palladium catalysis conditions due to the Heck reaction [7].

Therefore, we used acid chloride function of molecule **2** to introduce acetylene residues into the betulonic acid molecule (Scheme 1). The use of *p*-aminophenylacetylene as the amine component allowed us to prepare, in one step, a highly reactive synthon, a betulonic acid with terminal acetylene group **1**. The synthetic value of compounds with the ethynyl group is determined by the high acidity of the HC≡C fragment, which provides the facile functionalization of a molecule and the formation of new C–C bonds.



Scheme 1.

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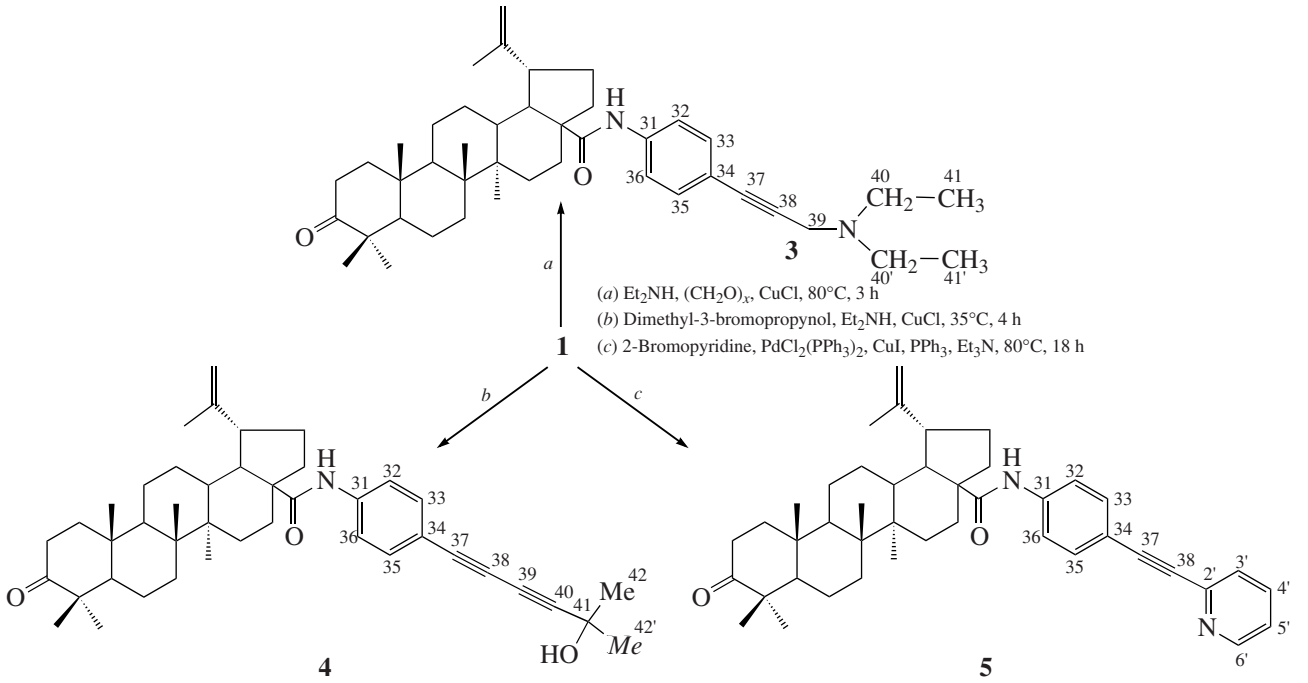
The use of high reactivity of ethynyl derivatives for the modification of betulonic acid was demonstrated by the examples of typical reactions for 1-alkynes.

Aminopropargyl derivatives are of special interest in the search for biologically active compounds in the series of acetylenes. Thus, inhibitors of enzyme, mammalian squalene epoxidase, were found among 3-arylpropyn-2-yldialkylamines of general formula Ar-C≡C–CH_2-$N(Alk)_2$ [8]. The aminoalkylation of acetylene was carried out under typical conditions of the Mannich reaction. The reaction of amidoacetylene **1** with diethylamine and paraformaldehyde in the presence of cuprous chloride on heating in dioxane at 80°C led to the formation of target propargylamine **3** in 68% yield (Scheme 2).



Scheme 2.

The Cadiot–Chodkiewicz cross-coupling is a conventional important method for the synthesis of diacetylene derivatives because high anticancer activity was revealed among diacetylene compounds [5]. Many natural conjugated butanediynylcarbinols were obtained by this method [9]. We synthesized also α-diacetylene tertiary alcohol **4**. The reaction of alkyne **1** with a bromocarbinol in methanol in the presence of CuCl, Et_2NH, $NH_2OH \cdot HCl$ proceeded smoothly at 30–35°C to form butane-1,3-diynylcarbinol **4** in 73% yield.

Taking into consideration the presence of the vinyl group in the molecule of betulonic acid (and, therefore, the possibility of the Heck reaction), it is of fundamental importance to check the selectivity of cross-coupling of terminal acetylene **1** with haloarene under the copper–palladium catalysis conditions. The selective reaction would open a way to the synthesis of a wide variety of betulonic acid derivatives with ethynylaryl and ethynylhetaryl substituents. We used α-bromopyridine as a haloarene component. The reaction of ethynyl derivative **1** with 2-bromopyridine (55°C, 18 h) under standard conditions of the Sonogashira reaction ($PdCl_2(PPh_3)_2$, CuI, Et_3N) gave rise to disubstituted acetylene **5** in 62% yield. No products of the Heck cross-coupling reaction were detected.

Thus, we have obtained first betulonic acid derivatives containing acetylene fragments. The possibility of the selective course of the Sonogashira reaction for the preparation of the acetylene derivatives of betulonic acid (**1**) in spite of the presence of vinyl group in the initial compound has been shown.

The highly reactive derivatives of betulonic acid containing ethynyl fragments (C≡CH) in addition to triterpene derivatives open wide opportunities for preparing a new group of compounds promising for the search for biologically active compounds for medicinal purposes.

EXPERIMENTAL

Commercial $PdCl_2(PPh_3)_2$, 2-methyl-3-butyn-2-ol from Aldrich were used in the work. Melting points were determined on a Kofler hot-stage apparatus. IR spectra were recorded on a Vector 22 spectrophotometer as KBr pellets. High-resolution mass spectra were obtained on a Finnigan MAT model 8200 spectrometer (EI, 70 eV). Elemental analysis was performed on a

Carlo Erba model 1106 CHN analyzer (Italy). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-300 spectrometer (operating at 300.13 and 75.47 MHz, respectively) and a Bruker AM-400 spectrometer (operating at 400.13 and 100.61 MHz, respectively). Signal assignment in ^{1}H and ^{13}C NMR spectra was accomplished with the use of different types of proton–proton and carbon–proton shift correlation spectroscopy (COSY, COLOC) (the spectra of compounds **4** and **5** were obtained on a Bruker DRX-500 spectrometer (operating at 500.13 (^{1}H) and 125.76 MHz (^{13}C)); signal assignment for the polycylic cage of compounds **1**–**5** was made taking into account data on the carbon chemical shifts for betulonic acid as a key compound [10]. Chemical shifts were determined relative to the residual signals of the solvent, $CHCl_3$ (δ_H 7.24 ppm and δ_C 76.90 ppm). The multiplicity of signals in ^{13}C NMR spectra was determined in a *J*-modulation mode (JMOD). The data of characteristic signals are presented for the noted compounds in ^{1}H NMR spectra because of the complexity of the assignment of all signals. The main portion of the protons of the triterpenoid skeleton show resonances within the range 2.7–0.8 ppm. The assignment of chemical shifts for the carbon atoms of triple bonds in compounds **1**–**5** is made by comparison with corresponding chemical shifts of acetylenic compounds in ^{13}C NMR spectra described in [11, 12].

***N*-(3-Oxo-20(29)-lupen-28-oyl)-4-ethynylaniline 1.** A mixture of 1.6 g (3.4 mmol) of acid chloride **2**, 0.39 g (3.4 mmol) of *p*-aminophenylacetylene, and 3 mL of triethylamine in 15 mL of dry benzene was heated in an argon flow at 70–75°C for 18 h. After cooling, the precipitate of $Et_3N \cdot HCl$ was filtered off and washed with benzene (3 × 10 mL), and the solvent was removed in a vacuum. The residue was dissolved in 20 mL of benzene, washed with diluted hydrochloric acid (1 : 4), and dried with anhydrous Na_2SO_4; the organic layer was filtered through Al_2O_3 (1.0 × 2.5 cm); the solution was concentrated to 5 mL, loaded onto aluminum oxide column (2.0 × 2.5 cm), and eluted with benzene. The solvent was removed in a vacuum to give 1 g (55%) of compound **1**, mp 159–160°C. High-resolution mass spectrum, found (*m*/*z*): 553.3918 $[M]^+$. For $C_{38}H_{51}NO_2$ calculated: M = 553.3919. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 0.90 (s, 3H, 25-CH_3), 0.95 (s, 3H, 24-CH_3), 0.98 (s, 6H, 26,27-CH_3), 1.03 (s, 3H, 23-CH_3), 1.67 (s, 3H, 30-CH_3), 3.14 (dt, 1H, 19-H, J_1 = 4 Hz, J_2 = 11 Hz), 4.59 (s, 1H, 29-H), 4.73 (s, 1H, 29-H), 3.01 (s, 1H, 38-H), 7.39 (m, 4H, 32,33,35,36-H). IR (KBr, ν, cm^{-1}): 1696 (C=O), 2108 (C≡C), 3387 (C≡C–H).

For $C_{38}H_{51}NO_2$ anal. calcd. (%): C, 82.41; H, 9.28; N, 2.53.

Found (%): C, 82.32; H, 8.72; N, 2.38.

***N*-(3-Oxo-20(29)-lupen-28-oyl)-4-(N,N-diethylaminomethylethynyl)aniline 3.** A mixture of 60 mg (2.0 mmol) of paraformaldehyde and 146 mg (2.00 mmol) of diethylamine in 5 mL of dioxane was kept in an argon flow for 30 min at 45°C. Cuprous chloride (I) (14 mg, 0.14 mmol) was added, and the mixture was stirred until blue color appeared (10 min); compound **1** (550 mg, 0.99 mmol) was added, and the mixture was heated to 80°C for 3 h. After completion of the synthesis, the reaction mixture was washed with aqueous ammonia. The organic layer was dried with Na_2SO_4, filtered through a plug of Al_2O_3 (1 × 1.5 cm). The solvent was removed in a vacuum, the residue was triturated with hexane. The precipitate was filtered off to give 410 mg (68%) of the Mannich base, mp 131–133°C (benzene). High-resolution mass spectrum, found (*m*/*z*): 638.4717 $[M]^+$. For $C_{43}H_{62}N_2O_2$ calculated: M = 638.4811. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 0.94 (s, 3H, 25-CH_3), 0.99 (s, 3H, 24-CH_3), 1.02 (s, 6H, 26,27-CH_3), 1.07 (s, 3H, 23-CH_3), 1.67 (s, 3H, 30-CH_3), 2.61 (m, 4H, N(CH_2)$_2$), 3.13 (dt, 1H, 19-H, J_1 = 4 Hz, J_2 = 11 Hz), 3.61 (s, 2H, 39-CH_2), 4.60 (s, 1H, 29-H), 4.73 (s, 1H, 29-H), 7.40 (m, 4H, 32,33,35,36-H). IR (KBr, ν, cm^{-1}): 1704 (C=O), 2212 (C≡C).

***N*-(3-Oxo-20(29)-lupen-28-oyl)-4-(2-hydroxy-5-methylhexadiyn-1,3-yl)aniline 4.** A mixture of 188 mg (0.339 mmol) of compound **1**, 0.2 mL of diethylamine, 3.4 mg (0.05 mmol) of hydroxylamine hydrochloride, and 1 mg (0.01 mmol) of cuprous chloride in 2 mL of methanol was stirred in an argon flow. After formation of a bright yellow precipitate of copper acetylenide, 62 mg (0.38 mmol) of 1-bromo-3-methylbutyn-1-ol was added. The mixture was heated at 30–35°C for 4 h. After completion of the reaction, 20 mL of toluene was added, and the reaction mixture was washed with aqueous ammonia and dried with Na_2SO_4. The organic layer was filtered to remove the drying agent, and the solvent was removed in a vacuum. Hexane was added to the residue and the precipitate was filtered off to give 156 mg (73%) of compound **4**, mp 183–184°C (benzene). High-resolution mass spectrum, found (*m*/*z*): 635.4318 $[M]^+$. For $C_{43}H_{57}NO_3$ calculated: M = 635.4333. ^{1}H NMR (300.13 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 0.89 (s, 3H, 25-CH_3), 0.94 (s, 6H, 26,27-CH_3), 0.98 (s, 3H, 24-CH_3), 1.03 (s, 3H, 23-CH_3), 1.66 (s, 3H, 30-CH_3), 1.54 (s, 6H, 42,42'-H), 3.12 (dt, 1H, 19-H, J_1 = 4 Hz, J_2 = 11 Hz), 4.58 (s, 1H, 29-H), 4.72 (s, 1H, 29-H), 7.42 (m, 4H, 32,33,35,36-H). IR (KBr, ν, cm^{-1}): 1692 (C=O), 2145 and 2232 (C≡C).

For $C_{43}H_{57}NO_3$ anal. calcd. (%): C, 81.21; H, 9.03; N, 2.20.

Found (%): C, 81.17; H, 10.10; N, 2.01.

***N*-(3-Oxo-20(29)-lupen-28-oyl)-4-(2-ethynylpyridine)aniline 5.** A mixture of 78 mg (0.49 mmol) of α-bromopyridine, 7 mg (0.04 mmol) of CuI, 7 mg

(0.01 mmol) of $PdCl_2(PPh_3)_2$, 4 mg (0.02 mmol) of PPh_3, 300 mg (0.542 mmol) of acetylene **1**, and 3 mL of triethylamine in 10 mL of toluene was stirred in an argon atmosphere at 55°C for 18 h. After cooling of the reaction mixture, the precipitate of $Et_3N \cdot HBr$ was separated by filtration, washed with toluene (3×10 mL), and the solvent was removed in a vacuum. The residue was triturated with hexane, and the precipitate was filtered off to give 193 mg (62%) of compound **5**, mp 181–182°C. ^1H NMR (300.13 MHz, $CDCl_3$, δ, ppm, *J*, Hz): 0.89 (s, 3H, 25-CH_3), 0.94 (s, 3H, 24-CH_3), 0.97 (s, 6H, 26,27-CH_3), 1.06 (s, 3H, 23-CH_3), 1.66 (s, 3H, 30-CH_3), 3.13 (dt, 1H, 19-H, J_1 = 4 Hz, J_2 = 11 Hz), 4.62 (s, 1H, 29-H), 4.76 (s, 1H, 29-H), 7.50 (m, 4H, 32,33,35,36-H), 7.46 (d, 1H, 3'-H, *J* = 3 Hz), 7.64 (t, 1H, 4'-H, J_1 = 1.7 Hz, J_2 = 7.7 Hz), 7.20 (m, 1H, 5'-H), 8.58 (d, 1H, 6'-H, *J* = 4.9 Hz). IR (KBr, ν, cm^{-1}): 1703 (C=O), 2219 (C≡C).

For $C_{43}H_{54}N_2O_2$ anal. calcd. (%): C, 81.80; H, 8.62; N, 4.44.

Found (%): C, 81.80; H, 8.43; N, 4.38.

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