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Effective Synthesis of a Novel Tetrahydrofuran Containing Triterpenoid: 5′(Z)-Benzylidene-tetrahydrofurano[3,2-*b*]lup-20(29)-en-28-oate

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Abstract: The title compound 5′(Z)-benzylidene-tetrahydrofurano[3,2-*b*]lup-20(29)-en-28-oate was synthesized with high chemo-, regio-, and stereoselectivity by 5-exo-dig cycloisomerization of methyl 2*α*-phenylpropynyl-3-oxolup-20(29)-en-28-oate with use of $KN(SiMe_3)_2$-DME. The novel betulinic acid derivative was fully characterized by conventional analytical methods and all proton and carbon signals have been completely assigned by 2D-NMR experiments.

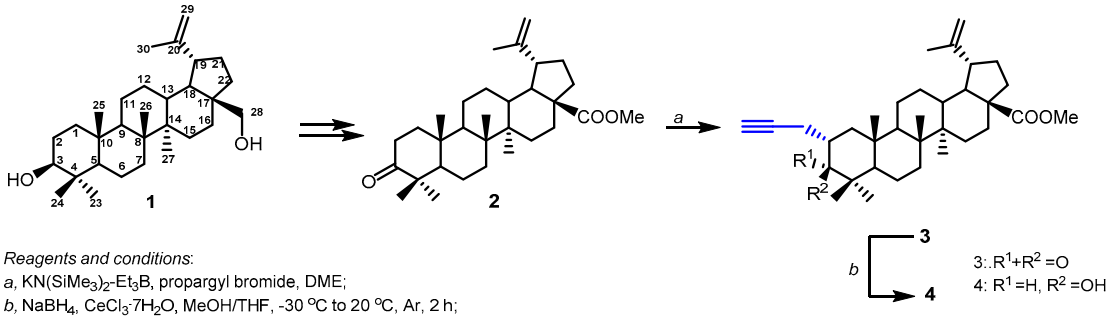
Keywords: betulinic acid; alkynes; tetrahydrofuranes; 5-exo-dig heterocyclization

1. Introduction

The available plant metabolite, that is betulinic acid and its semi-synthetic derivatives, represent an important class of biologically active substances, which are in high demand in medicinal chemistry and pharmacological studies [1–4]. In the synthesis of numerous derivatives of betulinic acid, directed at enhancing its biological potential, particular emphasis is focused on the approaches aimed at constructing of various types of heterocyclic fragments at triterpenoid core [5]. The ketone carbonyl at C-3 of betulinic acid was utilized in syntheses of various fused heterocycles at the 2,3-position of the lupane skeleton including isoxazole, pyrazine, benzopyrazine, pyridine, indole, and pyrazole rings [5–9]. These triterpenoid derivatives modified with heterocyclic rings attached to the A-ring of the triterpene have shown antitumor, anti-inflammatory and leishmanicidal activities. In this group of heterocyclic ring-substituted triterpenoids, betulinic acid analogues containing furan or tetrahydrofuran rings are little-known compounds. At the same time, polysubstituted furans, tetrahydrofurans and their precursors, 2-alkylidenetetrahydrofurans, are present in numerous natural products or used as important synthetic building blocks in the synthesis of promising biologically active substances [10–13]. Recently, we developed an efficient method for the synthesis of 2-propargyl 3-oxo-triterpene acid derivatives [14]. The resulting triterpene compounds containing a 4-pentyn-1-one structural unit in ring A have been successfully used in the anionic 5-exo-dig cycloisomerization induced by a strong base, $KN(SiMe_3)_2$-DME [15]. The heterocyclization of these compounds also was performed in the presence of $Au(I)^+$ phosphine complexes [16]. In the continuation of our studies, this article describes the preparation of new [3,2-*b*] tetrahydrofuran-fused lupane triterpenoid **6**—That is, 5′(Z)-benzylidene-tetrahydrofurano [3,2-*b*]lup-20(29)-en-28-oate, by employing the 5-exo dig heterocyclization of accessible 2-phenylpropynyl derivative of betulinic acid.

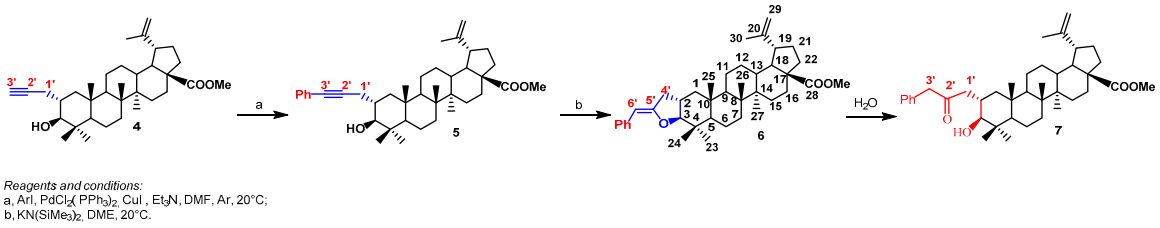
2. Results

In the synthesis of the target triterpenoid **6**, C-2 propargyl derivative of betulinic acid **5** was used as the starting compound, which was obtained in several stages from betulin by the method previously developed by our research group [14] (Scheme 1). The key stage of the scheme was α alkylation with propargyl bromide of potassium enoxytriethylborate generated from methylbetulonate **2** under the action of $KN(SiMe_3)_2$-Et_3B. Stereoselective reduction of the keto group in the propynyl derivative of betulonic acid **3** using $NaBH_4$ modified with $CeCl_3$, produced methylbetulinate **4** in good yield [14] (Scheme 1).



Scheme 1. Preparation of C-2 propargylbetulinic acid **4**.

The starting compound **4** was transformed into triterpenoid **5** by Sonogashira cross-coupling in the presence of $PdCl_2(PPh_3)_2$, CuI and Et_3N (Scheme 2). Triterpenoid **5** heterocyclization was carried out under the action of $KN(SiMe_3)_2$ in DME. The reaction proceeded at room temperature and in a short period of time yielded a single product, that is target triterpenoid **6**, with a yield of 82% (^{1}H- and ^{13}C-NMR spectra). It is interesting to note that only 5-exo dig cyclization occurred and stereoisomerically pure compound (Z-5) was found. We did not detect pyran derivatives derived from 6-endo cyclization and stereoisomer (E-5) even in the trace amounts. Exocyclic enol ethers are known to easily undergo hydrolysis [16,17]. The triterpenoid **6** obtained by us showed protolytic stability during long-term storage (12 months) in an inert atmosphere at a temperature of ±5 °C. However, it was easily hydrolyzed to give phenylaceton-3β-hydroxylup-20(29)-en-28-oate **7** in chloroform-d within 6 h, producing a mixture of compounds **6** and **7** in 60:40 ratio (^{1}H- and ^{13}C-NMR). During the purification of triterpenoid **6** by the method of column chromatography on SiO_2, an analytically pure sample of the compound was isolated in 32% yield along with its hydrolysis product, that is triterpenoid **7** in 51% yield.



Scheme 2. Synthesis of [3,2-*b*]tetrahydrofuran-fused betulinic acid.

The structure of the resulting compound was defined using one-dimensional (^{1}H, ^{13}C) and two-dimensional (COSY, NOESY, HSQC, HMBC) NMR spectroscopy.

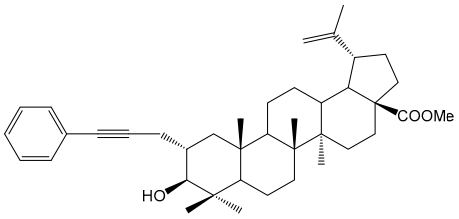
The ^{13}C-NMR spectrum of compound **6**, exhibited no signals for the acetylene group and the 3-OH carbon atom, indicating that these functional groups in the initial compound **5** were involved in the intramolecular cyclization. Along with the characteristic signal of the quaternary carbon atom C-20 (150.6 ppm), a new signal of the quaternary carbon atom (DEPT, HSQC) was registered in the region of 151.3 ppm, which is related to the carbon atom C-5′. The signals of carbon atoms C-6′ and

C-3 resonated in the range of 97.7 and 96.7 ppm. In the ^{1}H-NMR spectrum, along with the proton signals at C-29, a new singlet signal of the vinylidene proton H-6′ was present in the region of 5.27 ppm. Methylene protons H-4′ resonated in the region of 2.66 and 2.41 ppm. The obtained spectral data allowed us to conclude that the structure of compound 6 contains a trisubstituted double bond and a tetrahydrofuran ring. The stereochemistry of Z-5 triterpenoid was defined applying two-dimensional NMR correlation spectra. In the ^{1}H-NOESY spectrum of compound Z-5, there were cross-peaks between the signals of protons H-4′, H-6′ and H-2.

3. Materials and Methods

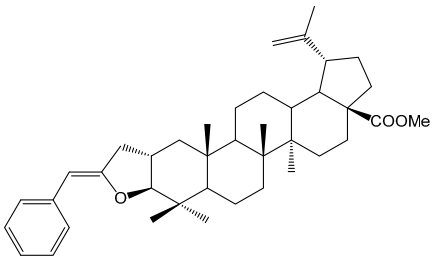
The starting compounds and reagents were purchased from standard commercial suppliers and used without any further purification. Betulonic acid was obtained from betulin according to known procedures [18]. IR spectra were obtained with use of a Vertex 70v spectrometer (Bruker, Karlsruhe, Germany) (solutions in $CHCl_3$). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance-500 instrument (500.13 (^{1}H) and 125.78 MHz (^{13}C)) or on a Bruker Avance-400 instrument (400.13 (^{1}H) and 100.62 MHz (^{13}C)) in $CDCl_3$ with Me_4Si as the internal standard. Mass spectra of new compounds were recorded on an LCMS-2010 EV (Shimadzu, Kyoto, Japan) spectrometer of the UfIC RAS Center for Collective Use "Chemistry". Elemental analysis was carried out on a 1106 analyzer (Carlo Erba, Milan, Italy). TLC was carried out on Sorbfil plates (Sorbpolimer, Krasnodar, Russia) in hexane–EtOAc (from 10:1 to 2:1) or in $CHCl_3$-MeOH (20:1); spots were visualized with anisaldehyde. Silica gel L (KSKG grade, 50–160 μm) was employed for column chromatography. Starting triterpenoid 4 was prepared as previously reported [15]. NMR spectra of all new compounds are in Supplementary Materials.

3.1. 2a-Phenylpropynyl-3β-hydroxylup-20(29)-en-28-oate (**5**)



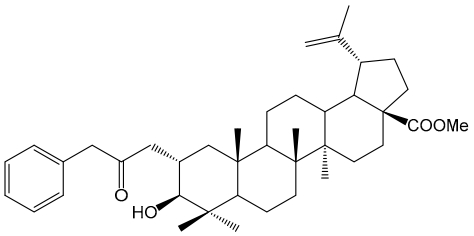
A mixture of triterpenoid **4** (102 mg, 0.2 mmol), iodobenzene (0.019 mL, 0.17 mmol) and Et_3N (0.23 mL, 1.64 mmol) were dissolved in DMF (3.0 mL). Then CuI (3.8 mg, 0.02 mmol) and $PdCl_2(PPh_3)_2$ (5.6 mg, 0.01 mmol) were added to the mixture simultaneously and the resulting mixture was stirred at room temperature for 1.5 h under an argon atmosphere. The completion of reaction was monitored by TLC analysis. The reaction was quenched by addition of water and extracted with $CHCl_3$ (3 × 10 mL). The combined organic extracts were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on SiO_2 with hexane/EtOAc (15:1) as an eluent to afford pure product **5** as a white powder (99 mg, 0.17 mmol, 85%). Rf: 0.15 (10:1 hexan:EtOAc). IR ($CHCl_3$): 3467 (OH), 1726 (C=O) cm^{-1}. m.p. 122–125 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 400 MHz): 7.43 (2H, m, arom), 7.30 (3H, m arom), 4.75, 4.61 (2H, both br s, H-29), 3.69 (3H, s, COOMe), 3.05 (1H, d, *J* = 10.4 Hz, H-3), 3.03 (1H, m, H-19), 2.65 (1H, dd, *J* = 16.8, 4.0 Hz, H^a-1′), 2.50 (1H, dd, *J* = 16.8, 6.4 Hz, H^b-1′), 2.29–0.86 (22H, m, CH, CH_2 in pentacyclic sceleton and 2H, H^a-1, H^b-1), 1.7 (3H, s, H-30), 1.02, 0.99, 0.95, 0.90, 0.82 (3H each, all s, H-23–H-27), 0.77 (1H, d, *J* = 9.2 Hz, H-5).^{13}C-NMR (δ, ppm, $CDCl_3$, 100 MHz): 176.8 (C-28), 150.5 (C-20), 131.7, 128.2, 127.6, 124.0 (arom), 109.6 (C-29), 88.7 (C-2′), 82.2 (C-3′), 81.8 (C-3), 56.6 (C-5), 55.5 (C-17), 51.3 (COOMe), 50.5 (C-9), 49.5 (C-18), 47 (C-19), 45.2 (C-1), 42.5 (C-14), 40.7 (C-8), 39.2 (C-10), 38.3 (C-13), 37.5 (C-4), 36.9 (C-22), 35.5 (C-2), 34.3 (C-7), 32.2 (C-16), 30.6 (C-21), 29.7 (C-15), 28.4 (C-24), 25.5 (C-12), 23.5 (C-1′), 20.9 (C-11), 19.4 (C-30), 18.3 (C-6), 16.9 (C-26), 16.3 (C-23), 15.9 (C-25), 14.8 (C-27). Anal. Calcd for $C_{40}H_{56}O_3$: C, 82.14; H, 9.65. Found: C, 81.99; H, 9.67. MS (APCI): *m*/*z* [M + H]$^+$, calcd for $C_{40}H_{56}O_3$: 585.43; found: 585.5.

3.2. 5′(Z)-Benzylidene-tetrahydrofuran[3,2-b]lup-20(29)-en-28-oate (**6**)



A 1 M solution of $KN(SiMe_3)_2$ (0.26 mL, 0.26 mmol) in THF was added to a solution of triterpenoid **5** (117 mg, 0.2 mmol) in DME (2.8 mL). The reaction mixture was stirred at room temperature under an argon atmosphere. The completion of reaction was monitored by TLC analysis. After 30 min reaction mixture was neutralized with saturated aqueous solution of NH_4Cl (aq). The product was extracted with EtOAc (3 × 10 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was purified by column chromatography on SiO_2 with hexane/EtOAc (30:1) as an eluent to give the appropriate compound **6** as a white powder (37 mg, 0.064 mmol, 32%) and **7** as a white powder (61 mg, 0.10 mmol, 51%). Rf: 0.53 (10:1 hexan:EtOAc). IR ($CHCl_3$): 1725 (C=O), 1674 (C=C) cm^{-1}. m.p. 140–143 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 400 MHz): 7.57 (1H, d, *J* = 7.6 Hz, arom), 7.28 (3H, m, arom), 7.09 (1H, t, *J* = 7.6 Hz, arom), 5.27 (1H, s, H-6′), 4.77, 4.64 (2H, both br s, H-29), 3.7 (3H, s, COOMe), 3.41 (1H, d, *J* = 10.8 Hz, H-3), 3.02 (1H, m, H-19), 2.66 (1H, dd, *J* = 14.8, 6.4 Hz, H^a-4′), 2.41 (1H, t, *J* = 12.8 Hz, H^b-4′), 2.29–0.86 (22H, m, CH, CH_2 in pentacyclic sceleton and 2H, H^a-1, H^b-1), 2.07 (1H, m, H-2), 1.72 (3H, s, H-30), 1.18, 1.01, 0.97, 0.94, 0.93 (3H each, all s, H-23–H-27).^{13}C-NMR (δ, ppm, $CDCl_3$, 100 MHz): 176.6 (C-28), 157.3 (C-5′), 150.5 (C-20), 137.2, 128.1, 127.2, 124.4 (arom), 109.6 (C-29), 97.7 (C-6′), 95.6 (C-3), 56.5 (C-17), 56 (C-5), 51.3 (COOMe), 50.6 (C-9), 49.5 (C-18), 47 (C-19), 42.5 (C-1), 42.5 (C-14), 41.1 (C-8), 39.6 (C-10), 38.2 (C-13), 37.9 (C-4′), 37.5 (C-4), 36.9 (C-22), 34.5 (C-7), 34.1 (C-2), 32.2 (C-16), 30.6 (C-21), 29.7 (C-15), 28.6 (C-23), 25.5 (C-12), 21.0 (C-11), 19.4 (C-30), 17.8 (C-6), 17.3 (C-26), 16.2 (C-25), 15.7 (C-24), 14.7 (C-27). Anal. Calcd for $C_{40}H_{56}O_3$: C, 82.14; H, 9.65. Found: C, 81.94; H, 9.67. MS (APCI): *m*/*z* $[M + H]^+$, calcd for $C_{40}H_{56}O_3$: 585.43; found: 585.5.

3.3. 2a-Phenylaceton-3β-hydroxylup-20(29)en-28-oate (**7**)



Rf: 0.11 (10:1 hexan:EtOAc). IR ($CHCl_3$): 3468 (OH), 1725 (C=O) cm^{-1}. m.p. 97–98 °C. ^{1}H-NMR (δ, ppm, $CDCl_3$, 400 MHz): 7.35–7.2 (5H, m, arom), 4.75, 4.62 (2H, both brs, H-29), 3.73 (2H, s, H-3′), 3.68 (3H, s, COOMe), 3.01 (1H, m, H-19), 2.75 (2H, m, H-3, H-1′), 2.27–0.82 (22H, m, CH, CH_2 in pentacyclic skeleton and 3H, H^b-1′, H^a-1), 1.72 (3H, s, H-30), 0.96, 0.95, 0.91, 0.85, 0.77 (3H each, alls, H-23–H-27), 0.68 (1H, d, *J* = 9.2 Hz, H^a-5), 0.58 (1H, m, H^b-1). ^{13}C-NMR (δ, ppm, $CDCl_3$, 100 MHz): 209.9 (C-2′), 176.6 (C-28), 150.5 (C-20), 134.2, 129.5, 128.6, 126.9 (arom), 109.6 (C-29), 83.8 (C-3), 56.5 (C-17), 55.6 (C-5), 51.3 (COOMe), 50.6 (C-9), 50.5 (C-3′), 49.5 (C-18), 47.4 (C-1′), 46.9 (C-19), 46.4 (C-1), 42.4 (C-14), 40.7 (C-8), 39.4 (C-10), 38.3 (C-13), 37.5 (C-4), 36.9 (C-22), 34.3 (C-7), 32.3 (C-16), 32.2 (C-2), 30.6 (C-21), 29.6 (C-15), 28.2 (C-24), 25.5 (C-12), 20.9 (C-11), 19.4 (C-30), 18.5 (C-6), 16.7 (C-26), 15.9 (C-23), 15.9 (C-25), 14.7 (C-27). Anal. Calcd for $C_{40}H_{56}O_3$: C, 79.69; H, 9.70. Found: C, 79.45; H, 9.68. MS (APCI): *m*/*z* $[M - H]^-$, calcd for $C_{40}H_{58}O_4$: 601.43; found: 601.5.

4. Conclusions

Thus, we have presented an economical and chemoselective scheme for production of new 2-alkylidenetetrahydrofuran-fused pentacyclic triterpenoid using a base promoted 5-exo-dig cycloisomerization of 2-alkynyl derivative of betulinic acid.

Supplementary Materials: The supplementary materials are available online.

Author Contributions: R.E. did the synthesis; D.N. analyzed all data; R.G. prepared the manuscript; Y.P. edited and revised the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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