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LETTERS TO THE EDITOR

First Conjugate of Glucuronic Acid with Triterpenoid Dihydrobetulin

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Abstract–3β-*O*-Acetyl-28-*O*-succinyl-(2,3,4-tri-*O*-acetyl-5-methoxycarbonyl-β-D-glucopyranosyl)dihydrobetulin was synthesized.

Keywords: triterpenoids, betulin, dihydrobetulin, glucuronic acid

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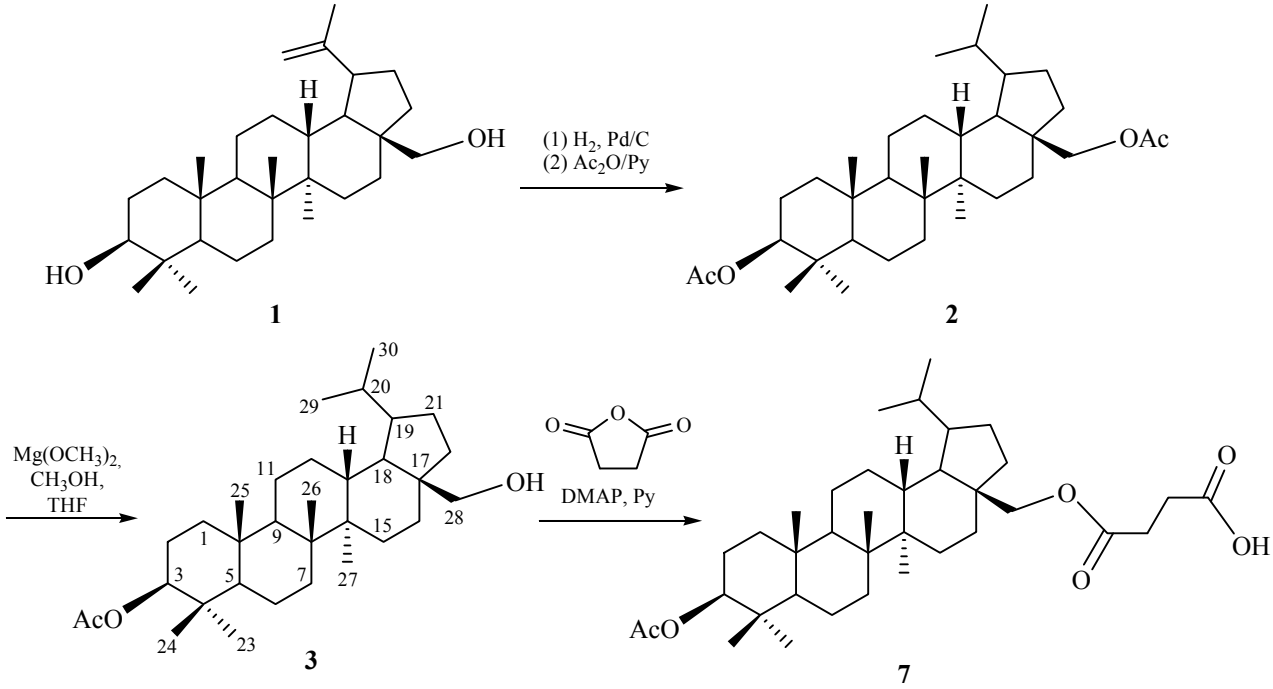
Glucuronic acid is widely used for glycosylation (glucuronidation) of a variety of biologically active compounds [1]. Continuing studies on the synthesis of conjugates of glucuronic acid with natural terpenoids [2–7] we synthesized for the first time a conjugate of glucuronic acid with triterpenoid dihydrobetulin. Using the known procedures [8], the double bond of betulin **1** was subjected to hydrogenation, and the hydroxyl groups to acylation (Scheme 1). Similarly to [9], dihydrobetulin diacetate obtained was converted into 3β-*O*-acetyldihydrobetulin **3** by selective hydrolysis with magnesium methylate in THF. Further glucuronidation of 3β-*O*-acetyldihydrobetulin **3** was performed with the use of methyl 2,3,4-tri-*O*-acetyl-1-bromo-α-D-glucopyranuronate **5** prepared from D-(+)-glucurono-3,4-lactone **4** [10] (Scheme 2). The Koenigs–Knorr glycosylation [11] of 3β-*O*-acetyldihydrobetulin **3** with bromide **5** produced no glucuronide **6** (Scheme 3) probably due to the unfavorable steric interactions between the hydrocarbon skeleton of terpenoid **3** and the acetate groups of monosaccharide **5**. In order to remove the reaction site in the molecule **3** from the bulk triterpenoid skeleton, 3β-acetoxydihydrobetulin **3** was involved into a reaction with an excess of succinic anhydride in pyridine to give 3β-*O*-acetyl-28-*O*-succinyldihydrobetulin **7** in 43% yield (Scheme 1). Then, similarly to [12], a conjugate of glucuronic acid with dihydrobetulin **8** was obtained in 43% yield by reacting triterpenoid **7** with bromide **5** in the presence of potassium and tetrabutylammonium bromide (TBAB) (Scheme 4). The anomeric proton of the glucuronosyl residue in conjugate **8** was registered in the ^{1}H NMR spectrum as a doublet at 5.79 ppm with a vicinal spin-spin coupling constant of 7.6 Hz, which unambiguously indicated the realization of β-glycoside bond.

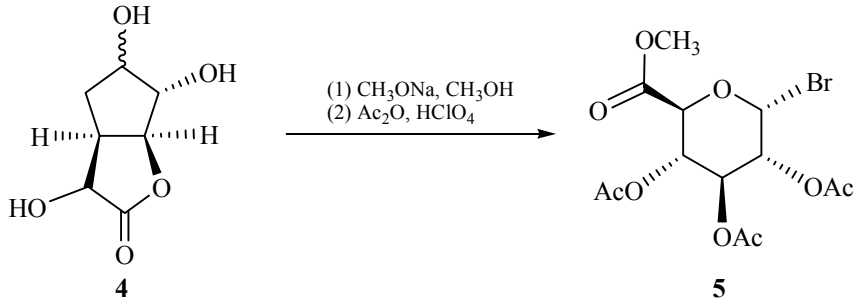
Betulin **1** was kindly provided by N.I. Medvedeva (Ufa Institute of Chemistry of the Russian Academy of Sciences). 3β,28-Di-*O*-acetyldihydrobetulin **2** and 3β-*O*-acetyldihydrobeltulin **3** were prepared by the procedures reported in [8] and [9], respectively; the constants and spectral characteristics corresponded to those described in [8]. D-(+)-Glucurono-3,4-lactone and succinic anhydride were purchased from Acros (Belgium).

3β-*O*-Acetyl-28-*O*-succinyldihydrobetulin (7). A solution of 0.64 g (1.3 mmol) of dihydrobetulin 3β-*O*-acetate **3**, 0.39 g (3.9 mmol) of succinic anhydride, 10 mL of anhydrous pyridine, and 0.48 g (3.9 mmol) of dimethylaminopyridine (DMAP) was refluxed for 16 h. After cooling, the reaction mixture was acidified with 10% HCl solution, poured into ice water, and extracted with $CHCl_3$. The organic layer was washed successively with water, 5% HCl solution, a saturated NaCl solution, and water, then dried with $MgSO_4$, concentrated under reduced pressure, and recrystallized from MeOH. Yield 0.33 g (43%), white amorphous powder, mp 117–119°C, $[\alpha]_D^{20}$ –10.5° (*c* 1.33, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$), δ, ppm: 0.80–1.90 m (27H, triterpenoid skeleton), 0.77 d [3H,

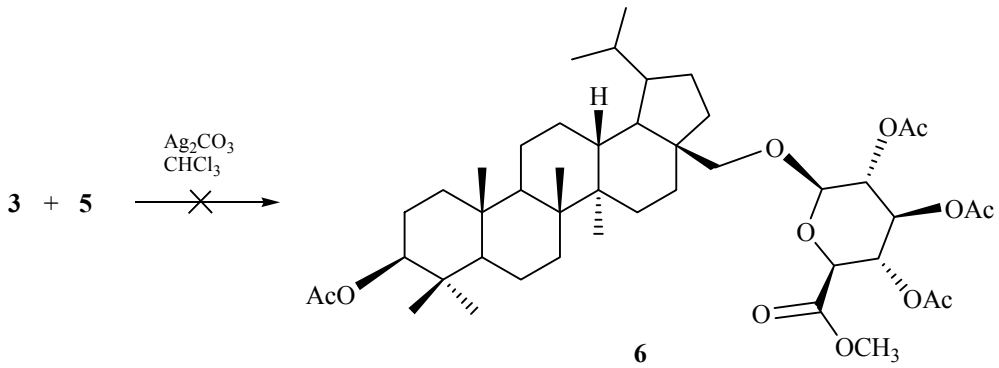
Scheme 1.



Scheme 2.

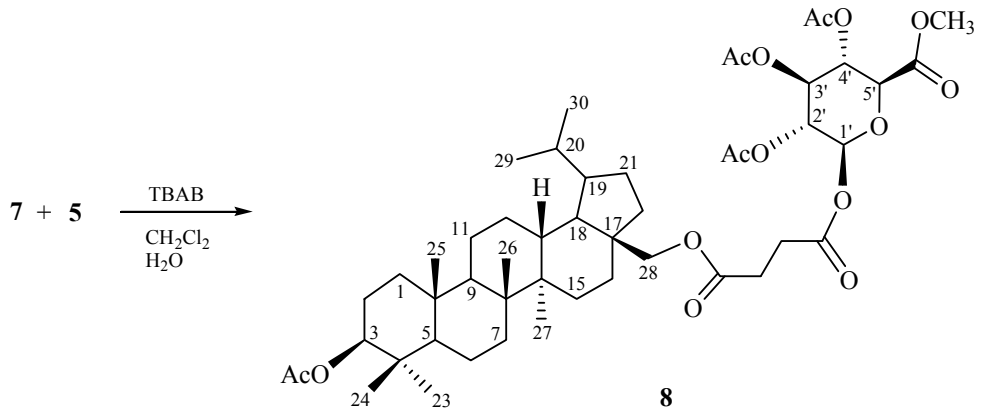


Scheme 3.



$H^{29(30)}$, ^3J = 6.7 Hz], 0.83 d [3H, $H^{30(29)}$, ^3J = 6.7 Hz]; 0.84 s, 0.85 s, 0.86 s, 0.95 s, 1.04 s (5×3H, $H^{23–27}$), 2.04 s [3H, $CH_3C(O)$], 2.60–2.72 m [4H, $(O)CCH_2CH_2C(O)$], 3.86 d (1H, H_A^{28}, ^3J = 10.9 Hz), 4.30 d (1H, H_B^{28}, ^3J = 10.9 Hz), 4.48 d.d (1H, H^3, ^3J = 10.4, 6.1 Hz). ^{13}C NMR spectrum (600 MHz, $CDCl_3$), δ_C, ppm: 14.6, 14.9, 16.0, 16.1, 16.5, 18.2, 20.8, 21.3, 21.6, 22.9, 23.7, 26.8, 26.9, 27.9, 28.9, 29.1, 29.4, 29.8, 31.6, 34.2, 37.0, 37.2, 37.8,

Scheme 4.



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38.4, 40.9, 42.9, 44.5, 46.6, 48.2, 50.0, 55.3, 63.3, 81.0, 171.1, 172.4, 177.4. Mass spectrum (ESI), *m*/*z*: 609.6 $[M + Na]^+$. Found, %: C 73.75; H 9.90. $C_{36}H_{58}O_6$. Calculated, %: C 73.68; H 9.96.

3β-*O*-Acetyl-28-*O*-succinyl-(2,3,4-tri-*O*-acetyl-5-methoxycarbonyl-β-D-glucopyranosyl)dihydrobetulin (8). To a solution of 0.185 g (0.32 mmol) of terpenoid **7** and 0.15 g (0.38 mmol) of bromide **5** in 10 mL of CH_2Cl_2 was added a solution of 0.11 g (0.80 mmol) of K_2CO_3 and 0.02 g (0.06 mmol) of *t*-butylammonium bromide (TBAB) in 1 mL of H_2O while stirring under argon. The reaction mixture was refluxed for 10 h, then diluted with $CHCl_3$, washed with water, and dried with $MgSO_4$. The solvent was removed at a reduced pressure, and the residue was recrystallized from MeOH. Yield 0.12 g (42.9%), white amorphous powder, mp 173–175°C, $[\alpha]_D^{20}$ –4.8° (*c* 1.53, CH_2Cl_2). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm: 0.80–1.90 m (26H, triterpenoid skeleton), 0.77 d [3H, $H^{29(30)}$, ^3J = 6.8 Hz], 0.83 d [3H, $H^{30(29)}$, ^3J = 6.8 Hz]; 0.84 s, 0.85 s, 0.86 s, 0.94 s, 1.03 s (5×3H, $H^{23–27}$); 2.031 s, 2.035 s, 2.04 s, 2.06 s [4×3H, $CH_3C(O)$], 2.60–2.74 m [4H, $(O)CCH_2CH_2C(O)$], 3.74 s [3H, $CH_3OC(O)$], 3.84 d (1H, H_A^{28}, ^3J = 11.3 Hz), 4.17 d (1H, $H^{5'}$, ^3J = 9.5 Hz), 4.28 d (1H, H_B^{28}, ^3J = 11.3 Hz), 4.48 d.d (1H, $H^{3'}$, ^3J = 10.3, 5.7 Hz), 5.15 t (1H, $H^{2'}$, ^3J = 9.9 Hz), 5.24 t (1H, $H^{4'}$, ^3J = 9.4 Hz), 5.31 t (1H, $H^{3'}$, ^3J = 9.2 Hz), 5.79 d (1H, $H^{1'}$, ^3J = 7.6 Hz). ^{13}C NMR spectrum (400 MHz, $CDCl_3$), δ_C, ppm: 13.7, 14.6, 14.9, 16.0, 16.1, 16.5, 18.2, 20.4, 20.5, 20.8, 21.3, 21.6, 22.9, 23.7, 26.8, 26.9, 27.9, 28.7, 29.0, 29.4, 29.8, 34.2, 34.6, 37.0, 37.2, 37.8, 38.4, 40.9, 42.9, 44.5, 46.6, 48.1, 49.9, 53.0, 55.3, 63.2, 69.0, 70.0, 71.8, 73.1, 80.9, 91.5, 166.7, 169.2, 169.3, 169.8, 170.4, 171.0, 172.1. Mass spectrum (MALDI), *m*/*z:* 925.9 $[M + Na]^+$, 941.9 $[M + K]^+$. Found, %: C 65.25; H 8.17. $C_{36}H_{58}O_6$. Calculated, %: C 65.17; H 8.26.

^{1}H and ^{13}C NMR spectra were recorded on an Avance-400 and Avance-600 spectrometers (Bruker, Germany). Mass spectra (MALDI) were obtained on a time-of-flight mass spectrometer UltraFlex III TOF/TOF (Bruker Daltonik GmbH, Germany) in a linear mode (Nd:YAG laser, λ 355 nm). Data was processed using the FlexAnalysis 3.0 program (Bruker Daltonik GmbH, Germany). The measurements were carried out in the range of *m*/*z* 200–6000. Positively charged ions were registered. 2,5-Dihydroxybenzoic acid and *p*-nitroaniline were used as a matrix. Electrospray ionization mass spectra (ESI) were obtained on an AmazonX mass spectrometer (Bruker Daltonik GmbH, Germany). The measurements were carried out in the *m*/*z* range from 100 to 2800 recording the positively charged ions (the capillary voltage 4500 V). Nitrogen at a temperature of 250°C and a flow rate of 8 L/min was used as the gas drier. A methanol–water solution (70 : 30) was used as the eluent, and the eluent flow rate was 0.2 mL/min. The completeness of the reactions and the purity of the substances were monitored by thin layer chromatography on Sorbfil plates (Imid, Russia), the substances were detected by plate treatment with a 5% solution of sulfuric acid followed by heating to 120°C. Specific rotation was measured on a Model 341 polarimeter (Perkin Elmer Inc., USA) in a temperature-controlled cell at 20°C and at 589 nm. Melting points were measured on a Boetius instrument.

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