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SYNTHESIS OF METHYL ESTERS OF BETULINIC ACID 2-DEOXY-α-GLYCOSIDES AND 28-OXO-19,28-EPOXYOLEANANE

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New 2-deoxy- and 2,6-dideoxy-α-L-arabinohexopyranosides of betulinic acid and 28-oxoallobetulin methyl esters were synthesized by the glycal method.

Key words: triterpenoids, betulinic acid, glycosylation, glycals, glycosides.

The synthesis of glycosides of triterpene alcohols is interesting because of their valuable biological activity. Addition of a sugar unit to the triterpene increases its water solubility, which is especially important in developing medicinal preparations. Natural and synthetic glycosides of the lupane triterpene betulin (mainly β-glycosides have been reported) exhibited various types of pharmacological activity. Thus, fruticesaponin B, which was isolated from *Bupleurum fruticescens* extract, exhibited high activity toward inflammations caused by carrageenin, tetradecanoylphorbol acetate, arachidonic acid, and ethylphenylpropiolate [1].

Glycosides of betulin administered in lecithin liposomes can increase the effect of liposomal preparations for decreasing the blood level of cholesterol in experimental hypercholesterolemia [2]. The *O*-, *S*-, and *N*-glycosides of betulinic acid have been patented for inhibition of lipoxygenase and intercellular adhesion [3]. 3-*O*-Glucopyranosyl betulinic acid possess high growth-regulating activity for the main root of cucumber (*Cucumis sativus* L., variety KIT) sprouts [4]. Glycosides of betulin and its monoacetates and 18,19-isobetulin were synthesized under Koenigs—Knorr and Helferich reaction conditions and had low stereoselectivity and yields [5-7].

It seemed interesting to synthesize new α-glycosides, in particular, of betulinic acid and 28-oxoallobetulin, during a study of their structure—activity relationships and ways to increase their solubility. We note that 28-oxoallobetulin exhibited distinct anti-flu activity [8]. However, this compound is practically insoluble in water, which has a detrimental effect on the ability to study it further. Furthermore, glycosides of 28-oxoallobetulin have not been reported.

Triterpene glycosides were synthesized by the glycal method [9]. The glycosyl donors were acetates of L-glucal (**I**) and L-rhamnal (**II**); the activators, cation exchanger KU-2-8 (H^+-form) and LiBr. This glycosylation method was successfully used previously for preparation of glycyrrhetic acid and betulin 2-deoxy-α-glycosides [10, 11]. The glycosylation of the methyl ester of betulinic acid (**1**) and 28-oxoallobetulin (**2**) by **I** and **II** proceeded stereoselectively to form 2-deoxy- (**3** and **5**) and 2,6-dideoxy-α-glycosides (**4** and **6**) in 83-88% yields (Scheme 1). The β-anomers were detected by TLC and NMR. Deacetylation of **3**-**6** by KOH (5%) in CH_3OH gave the target 2-deoxy-α-L-arabino- and 2,6-dideoxy-α-L-arabinohexopyranosides **7**-**10** in 80-87% yields.

The structures of the synthesized compounds (**3**-**10**) were established using NMR spectra and were compared with literature data for the aglycon [12] and the carbohydrate part [11]. ^{13}C NMR spectra of the aglycons of the glycosides were analogous to the spectrum of the starting material with the exception of a weak-field shift for C3 by 9.0-9.7 ppm. Signals of anomeric C1′ atoms of **3**-**6** appeared at δ 99.4-99.8 ppm. Spectra of **7**-**10** lacked signals for acetates but retained signals for methoxyls of the aglycon in **3** and **4** (δ 176.6 ppm). Anomeric protons of **3**-**6** were observed in PMR spectra at δ 4.94 ppm as a doublet with $J_{1,2}$ = 2.0-2.6 Hz, which indicated that they were equatorial and, therefore, that the C1′–O bond to the aglycon

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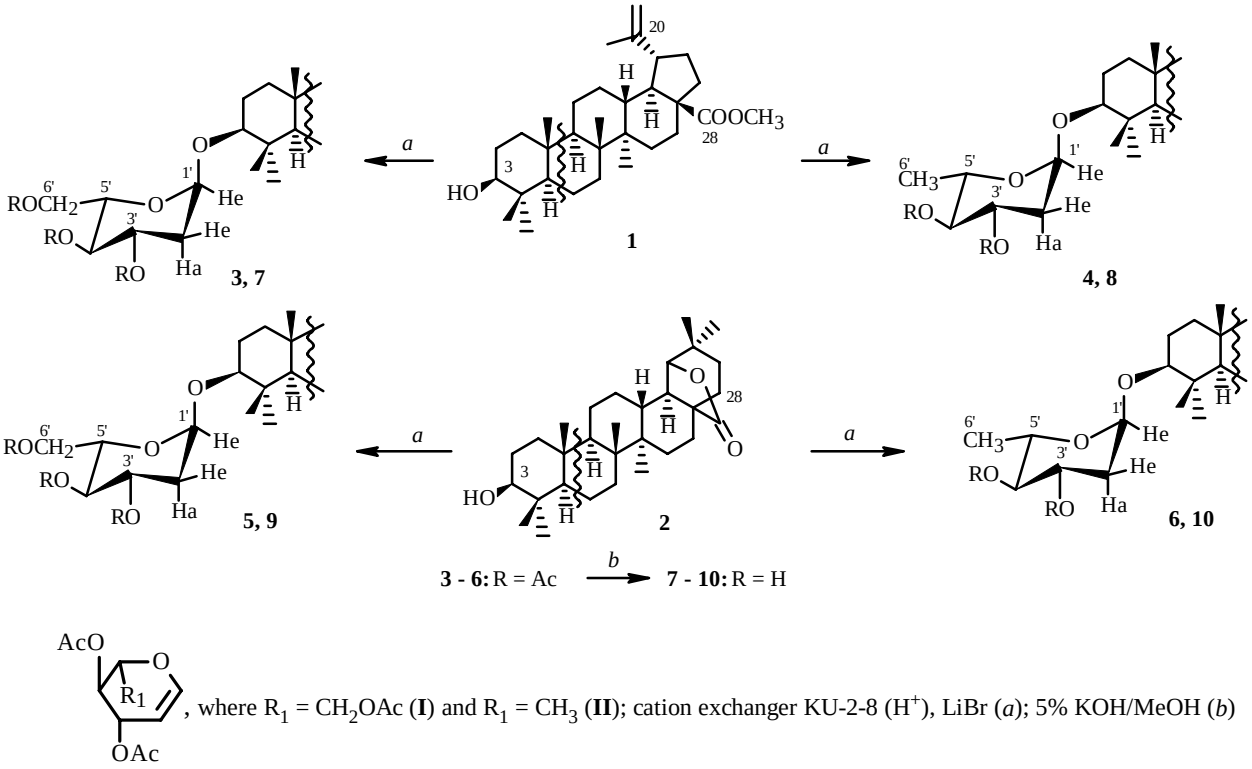
and α-glycoside was axial [11]. Signals of C2′ of **3-6** were found at δ 35.2-35.8 ppm. Sugar atoms C3′, C4′, and C5′ appeared at δ 64.4-75.2 ppm; C6′, at δ 17.3 and 17.6 ppm in spectra of **4** and **6**; at δ 62.5 and 62.7 ppm in spectra of **3** and **5**, respectively.



, where $R_1 = CH_2OAc$ (**I**) and $R_1 = CH_3$ (**II**); cation exchanger KU-2-8 (H^+), LiBr (*a*); 5% KOH/MeOH (*b*)

Scheme 1

Deacetylation produced a weak-field shift of the resonances for C1′ in **7-10** to δ 97.8-100.3 ppm. Signals for sugar atoms in **7-10** were observed at δ 35.3-36.8 ppm (C2′) and 61.8-78.2 ppm (C3′, C4′, C5′).

Thus, new 2-deoxy-α-glycosides of **1** and **2** were synthesized by the glycal method.

EXPERIMENTAL

PMR and ^{13}C NMR spectra in $CDCl_3$ were recorded on a Bruker AM-300 (300 and 75.5 MHz, respectively) with TMS internal standard. Melting points were determined on a Boetius microstage. Optical density was measured on a Perkin—Elmer 241 MC polarimeter in a 1-dm tube. TLC was performed on Silufol plates (Chemapol, Czech Rep.) using $CHCl_3$:CH_3OH (25:1). Compounds were developed by phosphotungstic acid (5%) in ethanol with heating at 100-120°C for 2-3 min. Column chromatography used neutral Al_2O_3. Solvents were dried and reagents were prepared as before [10, 11]. Glycals **I** and **II** were synthesized by the literature method [13].

Synthesis of Glycosides 3-6. A solution of **1** (0.47 g, 1 mmol) or **2** (0.67 g, 1 mmol) in CH_2Cl_2:CH_3CN (1:1, 30 mL) was treated with **I** (0.27 g, 1 mmol) or **II** (0.22 g, 1 mmol), molecular sieves (4 Å, 0.55 g), anhydrous cation exchanger KU-2-8 (H^+-form, 0.9 g), and anhydrous LiBr (0.7 g). The mixture was stirred at room temperature for 4-5 h (TLC monitoring) and filtered. Solvent was distilled in vacuo. The solid was chromatographed over Al_2O_3 with elution by $CHCl_3$.

Methyl ester of 3β-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-α-L-arabinohexopyranosyl)-20(29)-lupen-28-oic acid (3) was prepared from **1** and **I**, yield 0.40 g (85%), amorphous yellow solid, R_f 0.57, $[\alpha]_D^{20}$ -39° (*c* 1.00, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.78, 0.83, 0.87, 0.90, 0.98 (15H, 5s, $5CH_3$), 1.10-1.90 (30H, m, CH_2, aglycon CH, H2′), 1.67 (3H, s, H30), 2.00, 2.03, 2.07 (9H, 3s, 3OAc), 2.15-2.25 (1H, m, H19), 2.95-3.05 (1H, m, H3), 3.65 (3H, s, OCH_3), 4.03 (1H, dd, J = 1.6, 12.0, H6′a), 4.13 (1H, ddd, J = 1.8, 5.3, 9.7, H5′), 4.24 (1H, dd, J = 5.3, 12.0, H6′b), 4.60, 4.72 (1H each, 2s, H29), 4.88 (1H, t, J = 9.9, H4′), 4.91 (1H, d, J = 2.6, H1′), 5.35 (1H, ddd, J = 5.5, 9.9, 11.8, H3′).

^{13}C NMR spectrum (δ, ppm): 14.8, 15.7, 16.2, 16.3, 18.4, 19.4, 20.8, 20.9, 21.1 (OCO$\underline{C}$H$_3$), 21.3, 25.6, 27.9, 28.1, 29.7, 30.7, 32.2, 34.4, 35.7 (C2′), 36.9, 38.3, 38.6, 38.8, 39.1, 40.8, 42.4, 47.0, 49.5, 50.6, 51.3, 55.6, 56.6, 62.7 (C6′), 67.9 (C4′), 69.3 (C3′), 69.8 (C5′), 89.7 (C3), 99.8 (C1′), 109.6 (C29), 150.7 (C20), 170.0, 170.3, 170.8 (O$\underline{C}$OCH$_3$), 176.7 (C28). $C_{43}H_{66}O_{10}$ (MW 742.984).

Methyl ester of 3*β*-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-*α*-L-arabinohexopyranosyl)-20(29)-lupen-28-oic acid (4) was prepared from **1** and **II**, yield 0.42 g (88%), amorphous yellow solid, R_f 0.65, $[\alpha]_D^{20}$ -35° (*c* 1.00, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.84, 0.98, 1.00, 1.05, 1.18 (15H, 5s, 5CH$_3$), 0.60-1.95 (28H, m, CH$_2$, algycon CH, H2′), 1.15 (2H, d, J = 6.3, H6′), 1.75 (3H, s, H30), 2.00, 2.05 (6H, 2s, 2OAc), 2.15-2.30 (1H, m, H19), 2.95-3.05 (1H, m, H3), 3.65 (3H, s, OCH$_3$), 4.00 (1H, dq, $J_{5',4'}$ = 9.7, $J_{5',6'}$ = 6.5, H5′), 4.60 (1H, br.s, H29), 4.72 (2H, m, J′ = 9.7, H4′), 4.91 (1H, d, J = 2.0, H1′), 5.28 (1H, ddd, J = 5.5, $J_{3',4}$ = 11.7, J = 9.7, H3′).

^{13}C NMR spectrum (δ, ppm): 14.6, 15.8, 15.9, 16.2, 17.3 (C6′), 18.2, 19.3, 19.6, 20.7, 21.6 (OCO$\underline{C}$H$_3$), 25.4, 26.5, 28.0, 29.6, 30.5, 32.1, 35.1, 35.8 (C2′), 36.9, 38.6, 39.0, 40.6, 42.3, 46.9, 49.8, 50.5, 51.2, 54.9, 55.4 (OCO$\underline{C}$H$_3$), 55.8, 56.5, 65.5 (C5′), 69.2 (C3′), 75.1 (C4′), 89.0 (C3), 99.4 (C1′), 109.5 (C29), 150.5 (C20), 170.3, 170.4 (O$\underline{C}$OCH$_3$), 176.6 (C28). $C_{41}H_{64}O_8$ (MW 684.948).

3*β*-*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-*α*-L-arabinohexopyranosyl)-28-oxo-19*β*,28-epoxy-18*α*-oleanane (5) was prepared from **2** and **I**, yield 0.39 g (83%), amorphous white solid, R_f 0.69, $[\alpha]_D^{20}$ -198° (*c* 1.00, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.75, 0.80, 0.83, 0.89, 1.01 (21H, 5s, 7CH$_3$), 1.15-1.80 (24H, m, CH$_2$, aglycon CH, H2′), 1.97, 2.00, 2.05 (9H, 3s, 3OAc), 3.05-3.11 (1H, m, H3), 3.90 (1H, s, H19), 4.03 (1H, dd, J = 1.7, 12.0, H6′a), 4.14 (1H, ddd, J = 1.7, 5.5, 9.8, H5′), 4.25 (1H, dd, J = 5.5, 12.0, H6′b), 4.91 (1H, t, J = 9.7, H4′), 4.98 (1H, d, J = 2.4, H1′), 5.35 (1H, ddd, J = 5.5, 9.7, 11.9, H3′).

^{13}C NMR spectrum (δ, ppm): 13.6, 15.4, 16.1, 16.3, 18.1, 19.6, 20.7, 21.9 (COO$\underline{C}$H$_3$), 25.4, 26.4, 27.3, 27.8, 28.7, 30.1, 31.9, 32.3, 33.7, 35.2 (C2′), 35.5, 36.8, 37.1, 38.8, 39.9, 40.5, 46.0, 46.6, 47.9, 48.5, 50.6, 51.2, 55.4, 62.5 (C6′), 67.8 (C4′), 69.1 (C3′), 69.7 (C5′), 85.9 (C19), 89.4 (C3), 99.7 (C1′), 170.2, 170.4, 170.6 (O$\underline{C}$OCH$_3$), 176.7 (C28). $C_{42}H_{64}O_{10}$ (MW 728.958).

3*β*-*O*-(3,4-Di-*O*-acetyl-2,6-dideoxy-*α*-L-arabinohexopyranosyl)-28-oxo-19*β*,28-epoxy-18*α*-oleanane (6) was prepared from **2** and **II**, yield 0.41 g (87%), amorphous white solid, R_f 0.60, $[\alpha]_D^{20}$ -34° (*c* 1.00, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.78, 0.87, 0.92, 0.98, 1.05 (21H, 5s, 7CH$_3$), 1.18 (3H, d, J = 6.2, H6′), 1.20-1.85 (25H, m, CH$_2$, aglycon CH, H2′), 2.07, 2.09 (6H, 2s, 2OAc), 3.06-3.10 (1H, m, H3), 3.93 (1H, s, H19), 4.03 (1H, dq, $J_{5',4'}$ = 9.8, $J_{5',6'}$ = 6.2, H5′), 4.65 (1H, t, J = 9.8, H4′), 4.91 (1H, d, J = 2.4, H1′), 5.30 (1H, ddd, J = 5.4, 11.8, $J_{3',4'}$ = 9.8, H3′).

^{13}C NMR spectrum (δ, ppm): 13.6, 15.4, 16.2, 17.6 (C6′), 17.9, 18.1, 20.9, 21.1 (OCO$\underline{C}$H$_3$), 22.7, 23.9, 25.5, 26.2, 26.5, 26.9, 27.9, 31.9, 32.3, 31.9, 32.3, 33.7, 35.3 (C2′), 36.0, 37.0, 39.2, 39.1, 39.2, 39.9, 40.6, 46.1, 46.7, 51.2, 55.7, 64.4 (C5′), 70.5 (C3′), 75.2 (C4′), 85.9 (C19), 96.7 (C1′), 170.5, 170.5 (O$\underline{C}$OCH$_3$), 176.8 (C28). $C_{40}H_{62}O_8$ (MW 670.922).

Synthesis of Glycosides 7-10. A solution of **3-6** (0.5-0.7 mmol) in MeOH (100-150 mL) was treated with KOH (5%) in MeOH (15-20 mL), stirred for 4 h at room temperature (TLC monitoring), and treated with cation exchanger KU-2-8 (H^+-form). The resin was filtered off. The filtrate was diluted with cold water (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with water (3 × 30 mL), dried over Na_2SO_4, and evaporated in vacuo. The product was recrystallized from alcohol.

Methyl ester of 3*β*-*O*-(2-deoxy-*α*-L-arabinohexopyranosyl)-20(29)-lupen-28-oic acid (7) was prepared from **3**, yield 0.32 g (80%), white solid, R_f 0.28, mp 153-155°C, $[\alpha]_D^{20}$ -54° (*c* 1.00, $CHCl_3$).

^{13}C NMR spectrum (δ, ppm): 14.7, 15.9, 16.0, 16.5, 18.7, 19.5, 21.2, 25.8, 27.7, 28.3, 29.9, 30.8, 32.5, 34.6, 35.7, 36.8 (C2′), 38.5, 38.7, 38.9, 39.1, 40.9, 42.6, 47.2, 49.7, 50.8, 51.5, 55.8, 56.7, 61.8 (C6′), 68.3 (C4′), 69.3 (C3′), 71.6 (C5′), 89.7 (C3), 100.3 (C1′), 109.6 (C29), 150.8 (C20), 176.8 (C28). $C_{37}H_{60}O_7$ (MW 616.874).

Methyl ester of 3*β*-*O*-(2,6-dideoxy-*α*-L-arabinohexopyranosyl)-20(29)-lupen-28-oic acid (8) was prepared from **4**, yield 0.34 g (82%), white solid, R_f 0.22, mp 132-133°C, $[\alpha]_D^{20}$ -68° (*c* 1.00, $CHCl_3$).

^{13}C NMR spectrum (δ, ppm): 14.6, 15.8, 16.0, 16.1, 17.4 (C6′), 18.2, 19.3, 20.8, 25.4, 25.5, 28.0, 29.0, 30.5, 32.1, 34.2 (C2′), 35.5, 36.8, 36.8, 38.2, 38.3, 38.6, 39.0, 40.6, 42.3, 46.9, 49.4, 50.4, 51.2, 55.5, 56.5, 67.4 (C5′), 69.4 (C3′), 78.3 (C4′), 89.7 (C3), 100.0 (C1′), 109.5 (C29), 150.5 (C20), 176.6 (C28). $C_{38}H_{62}O_6$ (MW 614.902).

3*β*-*O*-(2-Deoxy-*α*-L-arabinohexopyranosyl)-28-oxo-19*β*,28-epoxy-18*α*-oleanane (9) was prepared from **5**, yield 0.31 g (80%), white solid, R_f 0.20, mp 138-140°C, $[\alpha]_D^{20}$ -135° (*c* 1.00, $CHCl_3$).

^{13}C NMR spectrum (δ, ppm): 13.8, 15.5, 16.3, 16.5, 25.7, 26.6, 27.5, 27.7, 27.9, 28.8, 30.3, 31.7, 32.5, 33.9, 35.5, 35.9, 36.4 (C2′), 36.8, 37.0, 38.6, 39.7, 40.4, 46.0, 46.9, 47.5, 50.3, 51.5, 55.3, 61.7 (C6′), 68.3 (C4′), 69.3 (C3′), 71.5 (C5′), 85.9 (C19), 89.4 (C3), 100.2 (C1′), 176.7 (C28). $C_{36}H_{58}O_7$ (MW 602.847).

3β-*O*-(2,6-Dideoxy-α-L-arabinohexopyranosyl)-28-oxo-19β,28-epoxy-18α-oleanane (10) was prepared from **6**, yield 0.36 g (87%), white solid, R_f 0.21, mp 168-170°C, $[\alpha]_D^{20}$ -56° (*c* 1.00, $CHCl_3$).

^{13}C NMR spectrum (δ, ppm): 13.8, 15.3, 16.0, 17.7 (C6′), 17.8, 18.3, 22.9, 23.7, 25.4, 26.0, 26.7, 26.9, 27.8, 31.7, 32.5, 31.7, 32.5, 33.8, 35.3 (C2′), 36.0, 36.6, 37.2, 38.5, 39.9, 40.8, 46.3, 50.6, 51.3, 55.7, 67.5 (C5′), 69.3 (C3′), 78.2 (C4′), 85.9 (C19), 89.6 (C3), 100.2 (C1′), 176.7 (C28). $C_{36}H_{58}O_6$ (MW 586.848).

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