SYNTHESIS OF TRITERPENE DERIVATIVES OF D-GLUCOSAMINE - MODIFIED ANALOGS OF GLYCYRRHIZIC ACID

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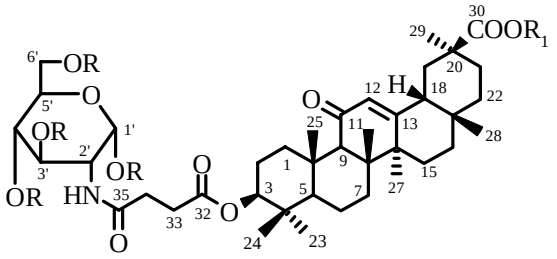
Triterpene D-glucosamine amides containing 18α- and 18β-glycyrrhetic acid succinates were synthesized.

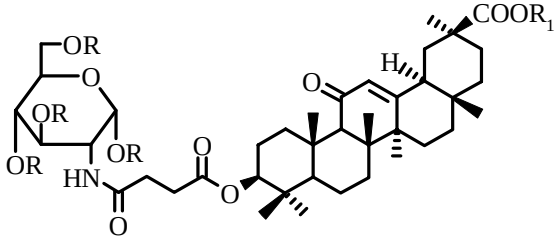
Key words: D-glucosamine, glycyrrhetic acid, glycyrrhizic acid.

Glycyrrhizic acid (GA) and its aglycon 18β-glycyrrhetic acid (GLA) are the principal triterpene components of smooth (*Glycyrrhiza glabra* L.) and Siberian (*G. uralensis* Fisher) licorice root. Their derivatives include anti-inflammatory, antiulcer, antiallergic, antitumor, and antiviral agents [1-3].

In continuation of studies on the synthesis of new bioactive derivatives and modified analogs of GA [4-6], we synthesized for the first time triterpene amides of α-D-glucosamine (α-D-GlcN) (**1-4**). Methyl esters of 3β-O-carboxypropionyl-18β- and -18α-glycyrrhetic acids were used as the starting triterpenes [7].



1: R = CH_3CO, R_1 = CH_3
2: R = R_1 = H



3: R = CH_3CO, R_1 = CH_3
4: R = R_1 = H

2-Amino-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose (amino component) were acylated by the corresponding 3-O-acyl acid chlorides in dry CH_2Cl_2 in the presence of Et_3N at 20-22°C. The yields of the target amides **1** and **3** were 42-44% after purification by column chromatography (CC) over silica gel. The yields of **1** and **3** were higher (52-53%) using for the condensation N,N′-dicyclohexylcarbodiimide (DCC) in DMF:pyridine (5:1). However, N-acylurea was formed as an impurity during the reaction [6]. Rechromatography of the reaction products was required to remove it from the target amides.

The structures of the compounds were confirmed by IR, UV, PMR, and ^{13}C NMR spectral methods. Signals in the NMR spectra of the new compounds **1-4** were assigned using literature data for the starting triterpenes [7-10] and D-glucosamine derivatives [11-13]. Thus, the signal for H-1′ in the PMR spectrum of **1** is observed at 6.15 ppm as a doublet with J = 2.5 Hz. This confirms the α-configuration for the OAc group on the glycoside. In addition to known signals of the triterpene skeleton [7, 8], additional signals of carbohydrate C atoms in 2-amino-2-deoxy-α-D-glucosamine appear in the ^{13}C NMR spectra of **2** and **4**. The signal for C-12 in the ^{13}C NMR spectrum of **3**, which is the 18α-stereoisomer of **1**, is shifted to strong field by about 4 ppm. This was observed previously in ^{13}C NMR spectra of this type of triterpenes [7, 8]. Signals of

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C-8 (45 ppm) and C-14 (43 ppm) in the ^{13}C NMR spectra of **1** and **3** were assigned based on literature data for 18β-GLA derivatives (high-resolution two-dimensional NMR spectra) [10].

Protected conjugates **1** and **3** were deprotected using KOH (1%) in MeOH with subsequent work up with cation exchanger KU-2-8 (H^+ form). The free triterpene derivatives of D-glucosamine **2** and **4** were obtained in yields of 75-80%. The anomeric C-1′ of α-D-GlcN was observed in the spectra of **2** and **4** at weak field (90-92 ppm, α-anomers) [4].

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded on a Bruker AM 300 instrument at working frequency 300 and 75.5 MHz, respectively, in $CDCl_3$ and DMF-d_7 with TMS internal standard. IR spectra were recorded on a Specord M80 spectrophotometer as mineral-oil mulls. Elecronic absorption spectra were recorded on a Specord UF-400 instrument. Specific rotations were determined in a Perkin—Elmer 241 MC polarimeter tube 1 dm in length. Melting points were measured on a Boetius microstage.

TLC was performed on Silufol (Czech Rep.) plates using CH_2Cl_2:CH_3OH (25:1, A) and C_6H_6:CH_3OH (10:1, B; 5:1, C). Spots were detected using phosphotungstic acid (20%) in EtOH with subsequent heating at 110-120°C for 2-3 min. CC was carried out over silica-gel L (40/100 μm, Czech Rep.) or Al_2O_3 (Brockmann neutral).

DMF, pyridine, and Et_3N were stored for 1 d over KOH and distilled over $Ba(OH)_2$. DCC was obtained from Aldrich.

Hydrochlorides of 2-amino-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose were prepared as before [14], mp 182-183°C (AcOH), $[\alpha]_D^{20}$ +138° (*c* 0.04, H_2O), lit. [14]: $[\alpha]_D^{20}$ +141° (*c* 1, H_2O).

Elemental analyses of **1-4** for C, H, and N agreed with those calculated.

Synthesis of Amides 1 and 3. Method 1. A solution of the acid chloride of the hemisuccinate of 18β- or 18α-glycyrrhetic acid methyl ester (1.6 g, 2.5 mmol) in dry CH_2Cl_2 (50 mL), which were prepared by treating the corresponding 3-O-acylates [7] in dry benzene with $SOCl_2$, was treated with 2-amino-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose hydrochloride (1.0 g, 2.6 mmol) and Et_3N (0.7 mL, 5.5 mmol). The mixture was held at 20-22°C for 22-24 h; washed with HCl solution (5%), water, and $NaHCO_3$ solution (5%); and dried with $CaCl_2$. The solvent was evaporated in vacuum. The solid was chromatographed over a silica-gel column with elution by CH_2Cl_2:MeOH (300:1, 200:1, 100:1, v/v, stepwise gradient). Fractions that were homogeneous according to TLC with the target products were combined, evaporated, and reprecipitated from aqueous EtOH.

Method 2. A solution of the hemisuccinate of 18β- or 18α-glycyrrhetic acid methyl ester [7] (0.58 g, 1 mmol) in DMF (10 mL) and pyridine (2 mL) at 0-5°C was treated with 2-amino-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose hydrochloride (0.5 g, 1.3 mmol) and DCC (0.24 g, 1.2 mmol), stirred for 1 h at 0-5°C, and held at 20-22°C for 20-22 h. The solid N,N′-dicyclohexylurea was filtered off. The filtrate was diluted with cold water and acidified with HCl (5%) to pH 3-4. The solid was filtered off, washed with water, dried, and chromatographed twice over silica-gel and Al_2O_3 columns with elution by CH_2Cl_2:MeOH (300:1, 200:1, 100:1, silica gel) and C_6H_6:MeOH (200:1, 100:1, 50:1, Al_2O_3) (v/v).

Compound 1. Yield 43.8% (method 1), 53.7% (method 2), R_f 0.43 (A), 0.35 (B). IR spectrum (ν, cm^{-1}): 1750, 1730 (OAc, $COOCH_3$), 1660 (C^{11}=O), 1540 (CONH). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82, 0.88, 0.88, 1.12, 1.16, 1.26 (all s, 21H, $7CH_3$); 2.02, 2.05, 2.10, 2.20 (all s, 12H, 4Ac); 2.35-2.45 (m, 4H, $2CH_2$); 3.68 (s, 3H, OCH_3); 4.04, 4.08 (2H, H-6′a, H-6′b); 4.24 (dd, 1H, $J_{4',5'}$ = 3.8, $J_{5',6'}$ = 3.7, H-5′); 4.48 (dd, 1H, $J_{1',2'}$ = 3.4, $J_{2',3'}$ = 9.6, H-2′); 5.16 (m, 1H, H-3′), 5.68 (s, 1H, H-12), 5.96 (d, 1H, $J_{4',3'}$ = 7.4, H-4′); 6.15 (d, 1H, $J_{1',2'}$ = 2.5, H-1′), 7.20 (s, 1H, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$): 38.8 (C1), 27 (C2), 81.3 (C3), 38.1 (C4), 55.1 (C5), 17.4 (C6), 32.7 (C7), 45.4 (C8), 61.6 (C9), 36.9 (C10), 200.0 (C11), 128.5 (C12), 169.2 (C13), 43.2 (C14), 26.5 (C15), 26.4 (C16), 36.7 (C17), 48.4 (C18), 41.1 (C19), 44.0 (C20), 31.8 (C21), 37.8 (C22), 28.1 (C23), 16.4 (C24), 16.7 (C25), 18.7 (C26), 23.5 (C27), 28.3, 28.5 (C28, C29), 176.9 (C30), 51.7 (C31), 171.7 (C32), 31.0, 29.7 (C33, C34), 172.6 (C35), 90.6 (C1′), 51.0 (C2′), 69.7 (C3′), 67.6 (C4′), 70.6 (C5′), 60.6 (C6′), 170.7, 169.2, 169.1, 168.6 (4C=O OAc), 20.9, 20.7, 20.6, 20.5 (CH_3 Ac).

Compound 3. Yield 42.5% (method 1), 52.8% (method 2), R_f 0.35 (B). IR spectrum (ν, cm^{-1}): 3340 (NH), 1750, 1730 (OAc, $COOCH_3$), 1670 (C^{11}=O), 1560 (CONH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.62, 0.78, 0.78, 1.06, 1.14, 1.16, 1.25 (all s, 21H, $7CH_3$); 1.97, 1.99, 2.04, 2.12 (all s, 12H, 4Ac); 2.25-2.35, 2.48-2.58 (m, 4H, $2CH_2$); 3.62 (s, 3H, OCH_3); 3.95, 4.00 (2H, H-6′a, H-6′b); 4.15 (dd, $J_{4',5'}$ = 4.0, $J_{5',6'}$ = 3.7, H-5′); 4.40 (dd, 1H, $J_{1',2'}$ = 3.4, $J_{2',3'}$ = 9.5, H-2′), 5.15 (t, 1H, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 11.5, H-3′), 5.48 (s, 1H, H-12), 5.94 (d, 1H, $J_{4',3'}$ = 10.9, H-4′), 6.05 (d, 1H, $J_{1',2'}$ = 2.5, H-1′).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$): 38.7 (C1), 26.7 (C2), 81.2 (C3), 37.9 (C4), 55.0 (C5), 17.4 (C6), 33.6 (C7), 44.8 (C8), 61.4 (C9), 36.6 (C10), 199.6 (C11), 124.0 (C12), 165.7 (C13), 43.8 (C14), 26.6 (C15, C16), 35.4 (C17), 40.3 (C18), 35.4 (C19), 42.5 (C20), 31.7 (C21), 28.0 (C22, C23), 16.5 (C24), 16.7 (C25), 18.4 (C26), 23.4 (C27), 17.4 (C28), 20.9 (C29), 177.7 (C30), 51.9 (C31), 171.6 (C32), 30.8, 29.4 (C33, C34), 172.5 (C35), 90.5 (C1′), 50.8 (C2′), 69.6 (C3′), 67.4 (C4′), 70.5 (C5′), 60.4 (C6′), 170.6, 169.1, 168.6, 168.5 (C=O OAc), 20.9, 20.7, 20.6, 20.5 (CH_3 Ac).

General Method for Deprotecting Amides 1 and 3. A solution of **1** or **3** (0.5 mmol) in KOH/MeOH (10-15 mL, 1.0%) was stirred at 20-22°C with TLC monitoring (for disappearance of the acetate spot). After the reaction was complete the mixture was treated with MeOH (10-15 mL) and cation exchanger KU-2-8 (H^+ form) until the pH was 4-5. The resin was filtered off, washed with MeOH and filtrate was evaporated. The solid was reprecipitated from aqueous EtOH.

Compound 2. Yield 80.1% (white powder), $[\alpha]_D^{20}$ +79° (*c* 0.04, MeOH), R_f 0.35 (B). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH), 1710 (COOH), 1660 (C^{11}=O), 1550 (CONH). UV spectrum (MeOH, λ_{max}, nm): 249 (log ε 3.95).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$): 39.2 (C1), 27.3 (C2), 81.8 (C3), 39.2 (C4), 55.1 (C5), 17.5 (C6), 32.9 (C7), 45.5 (C8), 61.4 (C9), 37.2 (C10), 200.2 (C11), 128.7 (C12), 169.2 (C13), 43.9 (C14), 26.6 (C15, C16), 36.5 (C17), 48.5 (C18), 41.2 (C19), 44.1 (C20), 31.9 (C21), 37.9 (C22), 27.4 (C23), 16.4 (C24), 16.6 (C25), 18.8 (C26), 23.5 (C27), 28.2, 28.6 (C28, C29), 178.8 (C30), 171.0, 173.0, 31.3, 29.7, 91.6 (C-1′), 51.8 (C-2′), 76.6 (C-3′), 71.2 (C-4′), 77.0 (C-5′), 61.9 (C-6′).

Compound 4. Yield 79.6% (white powder), $[\alpha]_D^{20}$ +45° (*c* 0.02, MeOH), R_f 0.32 (B). IR spectrum (ν, cm^{-1}): 3600-3200 (OH, NH), 1710 (COOH), 1660 (C^{11}=O), 1560 (CONH). UV spectrum (MeOH, λ_{max}, nm): 243 (log ε 4.0).

^{13}C NMR spectrum (75.5 MHz, DMF-d_7): 39.4 (C1), 28.0 (C2), 80.7 (C3), 38.3 (C4), 55.2 (C5), 18.0 (C6), 34.0 (C7), 45.5 (C8), 61.0 (C9), 37.4 (C10), 199.6 (C11), 123.8 (C12), 166.9 (C13), 44.2 (C14), 27.0 (C15, C16), 40.4 (C18), 43.0 (C20), 32.2 (C21), 28.4 (C22), 27.6 (C23), 16.0 (C24), 16.3 (C25), 18.7 (C26), 23.4 (C27), 16.8 (C28), 20.8 (C29), 178.9 (C30), 172.7, 174.7, 91.8 (C-1′), 52.0 (C-2′), 76.0 (C-3′), 71.7 (C-4′), 77.8 (C-5′), 62.4 (C-6′).

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