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SYNTHESIS OF NEW DERIVATIVES OF 3β-HYDROXY-18βH-OLEAN-9,12-DIEN-30-OIC ACID

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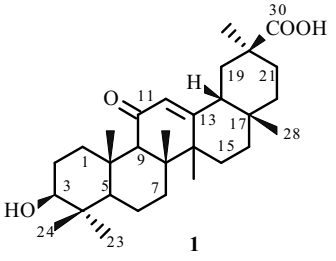
*Ring A was transformed and new A-homo-4-aza- and 3-cyano-3,4-seco-olean-4-ene derivatives of 3*β*-hydroxy-18*β*H-olean-9,12-dien-30-oic acid were synthesized.*

Key words: triterpenoids, 3β-hydroxy-18βH-olean-9,12-dien-30-oic acid, derivatives, Beckmann rearrangement.

Synthetic transformations of biologically active natural compounds represent a critical area of modern organic and bioorganic chemistry related to the synthesis of new biologically active compounds and compounds of new structural types [1-3]. Plant triterpenoids are a widely distributed class of natural compounds that have become especially interesting in the last decade due to the observation of several highly active antitumor, antiviral, anti-inflammatory, and anti-ulcer agents among derivatives of oleanolic, betulinic, ursolic, glycyrrhizic, and glycyrrhetic acids, among others [4-12]. The chemistry and pharmacology of glycyrrhetic acid (GLA) (**1**), the principal oleanane triterpenoid of licorice roots (*Glycyrrhiza glabra* L. and *G. uralensis* Fisher), have been well studied [13]. GLA and its derivatives are effective for treating allergic conditions and are promising for therapy of inflammatory skin diseases, eczema of various etiologies, psoriasis, and allergic dermatitises [14]. GLA amides inhibit reverse transcriptase of HIV-1, a key enzyme in the life cycle of HIV that is necessary in early stages of cell infection [15]. GLA exhibited high antitumor activity in skin tumor models caused by cancerogens [16].

Herein we report the synthesis of new derivatives of 3β-hydroxy-18βH-olean-9,12-dien-30-oic acid (**2**), a GLA analog modified in ring C.

Reduction of GLA (**1**) by an excess of $NaBH_4$ in THF:H_2O (1:1) in the presence of NaOH produced an epimeric mixture (α/β) of 11-hydroxy derivative **3**, which was dehydrated upon refluxing with conc. HCl in THF to 3β-hydroxy-9(11),12-diene **2** [17], the yield of which after purification by column chromatography (CC) over Al_2O_3 was 67%. Methylation by diazomethane formed 30-methyl ester **2a**. The structures of the triterpenoids were confirmed by PMR and ^{13}C NMR spectra. Thus, The ^{13}C NMR spectra of dienes **2** and **2a** contained additional resonances for olefinic C atoms C-9 at δ 116.36 and 115.71 ppm; C-11, 155.23 and 154.53. Resonances of C-5 shifted to strong field by 3.5-4.0 ppm; of C-14, to weak field by 3 ppm (Table 1).



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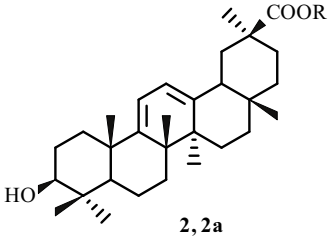
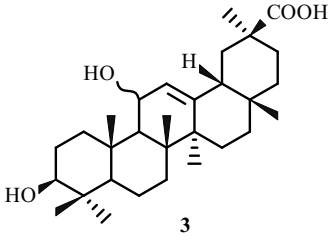


2, 2a



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2: R = H; **2a:** R = Me

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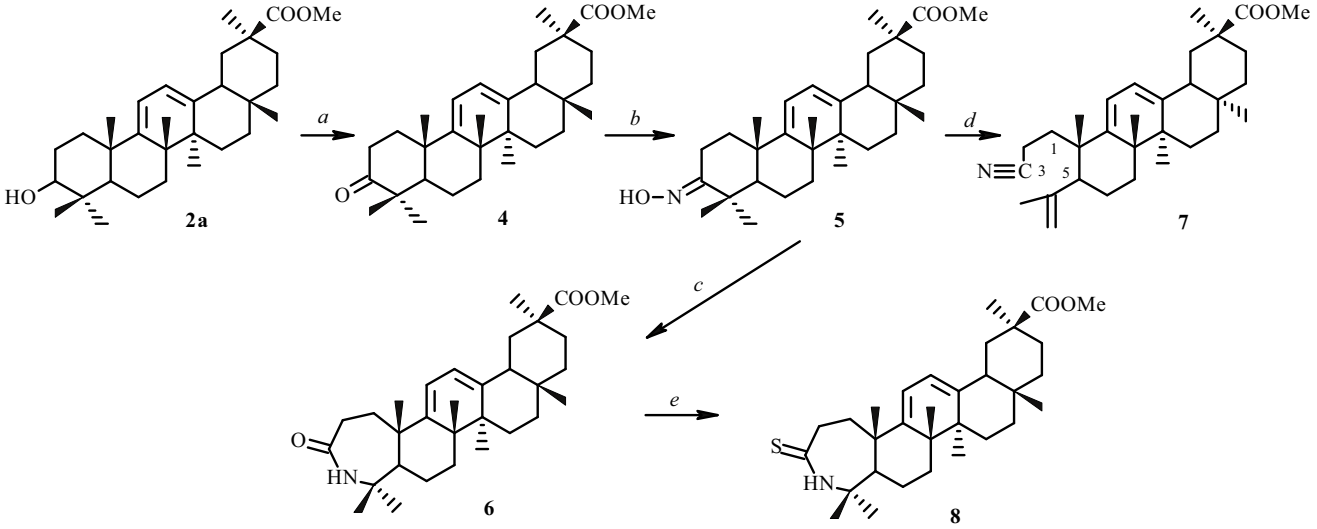
TABLE 1. ^{13}C NMR Spectra of 3β-Hydroxy-18βH-olean-9(11),12(13)-dien-30-oic Acid and Its Derivatives (75.5 MHz, $CDCl_3$, δ, ppm)

C atom	**2** (CD_3OD)	**2a**	**4**	**5**	**6**	**7** ($CDCl_3$+DMSO-d_6)	**8**
1	39.3	39.1	38.0	38.5	40.6	37.7	38.2
2	27.6	27.2	27.0	27.5	27.5	26.2	27.2
3	78.9	78.6	216.7	166.6	175.3	120.3	162.9
4	39.5	39.4	46.1	46.5	46.6	163.7	44.2
5	51.8	51.6	51.5	51.8	56.3	50.7	55.3
6	20.5	20.0	20.3	20.8	20.4	18.1	20.6
7	32.7	32.1	31.3	32.9	30.4	30.6	31.6
8	41.0	40.5	40.1	40.2	41.4	39.6	39.2
9	155.2	154.5	152.2	153.5	154.5	152.8	146.3
10	43.3	42.8	42.5	42.7	42.8	41.8	40.4
11	116.4	115.7	117.0	118.8	118.9	115.6	114.1
12	121.9	121.4	121.0	121.4	121.7	120.4	124.5
13	146.8	146.0	146.3	146.4	146.3	145.3	141.4
14	43.5	43.0	42.6	43.0	43.0	41.9	40.6
15	25.6	25.2	25.4	24.9	25.8	24.6	25.6
16	26.2	25.6	26.6	25.8	25.8	24.0	25.6
17	32.2	31.6	30.9	31.6	31.7	30.6	31.0
18	47.0	46.4	47.0	49.4	49.4	45.5	46.5
19	38.9	38.7	37.5	38.3	38.6	37.3	38.2
20	44.5	44.2	43.8	44.2	44.2	43.2	42.6
21	31.6	31.2	31.0	31.2	31.2	30.2	29.6
22	37.9	37.1	34.1	37.0	38.3	36.1	37.0
23	27.9	27.9	26.6	27.2	27.3	116.5	28.4
24	16.1	15.6	19.3	19.0	20.1	16.5	16.0
25	16.1	15.6	19.7	19.9	20.4	19.0	16.0
26	18.9	18.3	24.9	23.2	22.3	19.9	17.4
27	21.4	21.0	21.2	22.3	22.6	22.6	22.8
28	28.6	28.2	28.0	28.3	28.4	27.0	28.4
29	28.9	28.6	28.2	28.5	28.5	27.6	29.6
30	180.8	177.5	176.9	177.7	177.5	176.5	176.0
31		511	51.2	51.6	51.6	50.9	51.9



a. PDC/$CHCl_3$; *b*. NH_2OH·HCl, 115°C; *c*. $SOCl_2$/dioxane, 10°C, 1% KOH; *d*. *p*-TsCl/Py, 115°C; *e*. Lawesson's reagent/toluene, 110°C

Scheme 1

Oxidation of **2a** by pyridinium dichromate (PDC) in CH_2Cl_2 at room temperature with TLC monitoring produced 3-oxo derivative **4** in 65% yield (Scheme 1). Refluxing **4** with $NH_2OH \cdot HCl$ in anhydrous pyridine for 1 h formed 3-hydroxyimine **5**. The ^{13}C NMR spectrum of **5** exhibited a resonance for C-3 at δ 166.6 ppm (C=N–).

We used the Beckmann rearrangement of ketooximes, which has been well studied for 18α- and 18β-GLA, in order to prepare compounds with a seven-membered ring. Depending on the conditions, the reactions could follow two pathways to form lactams (aza derivatives) and *seco*-nitriles [18]. Reaction of **5** with $SOCl_2$ in anhydrous dioxane at 10°C formed a single product, A-homo-4-aza derivative **6**, in 74% yield according to TLC through a first-order Beckmann rearrangement.

The structure of **6** was elucidated using spectral methods. The IR spectrum of **6** contained absorption bands for CONH at 1664 cm^{-1}. The PMR spectrum had resonances at weak field (δ 5.58, 5.62, 5.65 ppm) belonging to NH and olefinic protons (H-11, H-12). The presence of a C=O group in the 3-position of **6** was confirmed by a strong-field shift of the chemical shift (CS) for C-3 (from 166.6 to 175.3 ppm). The resonances of neighboring C atoms C-2 and C-4 shifted to weak field by 2-5 ppm at the same time (Table 1).

Heating **5** with *p*-TsCl in anhydrous Py for 5 h caused a second-order Beckmann rearrangement to form 3-cyano-3,4-*seco*-olean-4-ene (**7**) in 66% yield. The ^{13}C NMR spectrum showed a resonance for the CN group at δ 120.3 ppm; of the additional olefinic bond, at 163.7 and 116.5 (Table 1).

Refluxing A-homo-4-aza-3-oxo derivative **6** with Lawesson's reagent in toluene for 5 h produced 3-thio analog **8** in 65% yield. Its IR spectrum contained absorption bands at 1680 (C=S) and 1572 (NHC=S) cm^{-1}. The ^{13}C NMR spectrum showed a shift of the C-3 resonance from 175.3 ppm to 162.9 as a result of the formation of the C=S bond.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded on Bruker AM-300 spectrometers (operating frequency 300 MHz for ^1H and 75.5 MHz for ^{13}C) with TMS internal standard. Resonances in NMR spectra were assigned in normal mode using the program set ACD LABS and literature data for GLA and its derivatives [19, 20].

IR spectra in mineral oil mulls were recorded on an IR Prestige-21 spectrometer (Shimadzu). UV spectra were recorded on a UF-400 spectrophotometer. Molecular ions were determined by LC/MS on a Shimadzu LCMS-2010 instrument using atmospheric pressure chemical ionization and solutions in MeOH or CH_3CN.

Optical activity was measured on a Perkin—Elmer 341 polarimeter in a 1-dm tube at 20-22°C (λ_{Na} 546 nm). Melting points were determined on a Boetius microstage.

Column chromatography (CC) was carried out over silica gel (KSK, 50-150 fraction, ZAO Sorbpolimer) (SG) or Al_2O_3 (Brockmann neutral). TLC used Sorbfil (ZAO Sorbpolimer) plates. Spots of compounds were detected using phosphotungstic acid solution (20%) or H_2SO_4 in EtOH (5%) with subsequent heating at 110-120°C for 2-3 min.

Solvents were purified as usual [21] and were evaporated in vacuo at <50°C.

We used PDC (Aldrich) and GLA prepared by the literature method [22] that was recrystallized twice from aqueous EtOH, mp 292-294°C, $[\alpha]_D^{20}$ +168° (*c* 0.03, $CHCl_3$), lit. [22] mp 289°C, $[\alpha]_D^{20}$ +163° (*c* 1.0, $CHCl_3$).

3β-Hydroxy-18βH-olean-9(11),12(13)-dien-30-oic Acid (2). A solution of GLA (2.5 g, 5.3 mmol) in THF (100 mL) and H_2O (100 mL) was treated with NaOH (1.24 g, 31 mmol) and $NaBH_4$ (12.5 g, 330 mmol), refluxed for 4 h, treated with aqueous NaH_2PO_4 (500 mL, 5%), and extracted with EtOAc (300 mL × 3). The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The crude product (2.0 g) was dissolved in THF (100 mL), treated with several drops of conc. HCl, refluxed for 6 h, and diluted with cold H_2O (300 mL). The precipitate was filtered off, washed with water, and chromatographed over a column of Al_2O_3 with elution by $CHCl_3$:CH_3OH (300:1, 200:1, 100:1, v/v) to afford **2** (2.2 g), which was recrystallized from EtOH. Yield 1.6 g (66.7%) (transparent needles), mp >300°C, $[\alpha]_D^{20}$ +343° (*c* 0.06, $CHCl_3$), UV spectrum (λ_{max}, MeOH, nm): 280 (log ε 3.98), lit. [17] $[\alpha]_D^{20}$ +374° (*c* 1.0, THF).

PMR spectrum (CD_3OD, δ, ppm, J/Hz): 0.74 (3H, s, CH_3-28), 0.80 (3H, s, CH_3-27), 0.94 (3H, s, CH_3-26), 0.98 (3H, s, CH_3-25), 1.08, 1.10 (3H, both s, CH_3-23, CH_3-24), 1.14 (3H, s, CH_3-29), 1.30-2.00 (m, CH, CH_2), 3.14 (H_α-3, t, J_1 = 7.3, J_2 = 8.1), 5.52, 5.54 (2H, H-11, H-12).

Table 1 lists the ^{13}C NMR spectrum. $C_{30}H_{46}O_3$. MW 454.7.

Methyl Ester of 3β-Hydroxy-18βH-olean-9(10),11(12)-dien-30-oic Acid (2a). A solution of **2** (0.90 g, 2 mmol) in EtOAc (100 mL) was treated with diazomethane in Et_2O until a yellow color persisted. The solution was evaporated. The solid was recrystallized from EtOH. Yield 0.83 g (90%), R_f 0.70 ($CHCl_3$:CH_3OH, 10:1), mp 243-245°C. IR spectrum (ν, cm^{-1}): 3350-3270 (OH), 1722 (COOMe), 1217, 1159, 1103, 1086, 1038, 991, 822, 721. UV spectrum (λ_{max}, MeOH, nm): 282 (log ε 5.05).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.80 (3H, s, CH_3-28), 0.84 (3H, s, CH_3-27), 0.98 (3H, s, CH_3-26), 1.04 (3H, s, CH_3-25), 1.14 (6H, s, CH_3-23, CH_3-24), 1.20 (3H, s, CH_3-29), 1.25-2.10 (CH, m, CH_2), 3.24 (H_α-3, dd, J_1 = 4.2, J_2 = 11.0), 3.69 (3H, s, OCH_3), 5.60, 5.62 (2H, H-11, H-12).

Table 1 lists the ^{13}C NMR spectrum. $[M + H]^+$ 470. $C_{31}H_{48}O_3$. MW 468.7.

Methyl Ester of 3-Oxo-18βH-olean-9(10),11(12)-dien-30-oic Acid (4). A solution of **2a** (1.5 g, 3.2 mmol) in $CHCl_3$ (10 mL) was treated with PDC (1.0 g, 4.8 mmol); stirred at room temperature for 3 h; diluted with $CHCl_3$ (20 mL); and washed with H_2O, Na_2CO_3 solution (5%), saturated NaCl solution, and H_2O again. The organic phase was passed through a small column of Al_2O_3 and evaporated. The dry solid (1.36 g) was chromatographed over a column of SG with elution by toluene and toluene:EtOAc (200:1, 100:1, v/v). Yield 0.80 g (65.0%), R_f 0.38 (benzene), 0.58 (benzene + 1 drop MeOH), mp 233-235°C, $[\alpha]_D^{20}$ +305° (*c* 0.06, MeOH). IR spectrum (ν, cm^{-1}): 1726 (COOMe), 1217, 1180, 1155, 1030. UV spectrum (λ_{max}, MeOH, nm): 282 (log ε 4.66).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.70 (3H, s, CH_3-28), 0.90 (3H, s, CH_3-27), 0.96 (3H, s, CH_3-26), 1.00 (6H, s, CH_3-23, CH_3-25), 1.04 (3H, s, CH_3-24), 1.15 (3H, s, CH_3-29), 1.16-2.50 (CH, m, CH_2), 3.57 (3H, s, OCH_3), 5.52, 5.54 (2H, H-11, H-12).

Table 1 lists the ^{13}C NMR spectrum. $[M + H]^+$ 469. $C_{31}H_{46}O_3$. MW 466.7.

Methyl Ester of 3-Hydroxyimino-18βH-olean-9(11),12(13)-dien-30-oic Acid (5). A solution of **4** (0.7 g, 1.5 mmol) in anhydrous Py (28 mL) was treated with $NH_2OH{\cdot}HCl$ (1.4 g), refluxed for 1 h, and treated with cold H_2O. The precipitate was filtered off, washed with water, dried, and recrystallized from EtOH. Yield 0.59 g (82.4%), R_f 0.74 (toluene:EtOAc, 3:1), 0.57 (benzene:MeOH, 20:1), mp 234-236°C. IR spectrum (ν, cm^{-1}): 3300-3100 (N–OH), 1726 (COOMe), 1217, 1155, 928, 734. UV spectrum (λ_{max}, MeOH, nm, log ε): 282 (4.0), 260 (4.05), 250 (4.1).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.84 (3H, s, CH_3-28), 1.00, 1.02 (6H, both s, CH_3-26, CH_3-27), 1.09 (3H, s, CH_3-25), 1.12, 1.15 (6H, both s, CH_3-23, CH_3-24), 1.19 (3H, s, CH_3-29), 1.30-2.40 (CH, m, CH_2), 3.69 (3H, s, OCH_3), 5.62, 5.65 (2H, H-11, H-12), 8.92 (br.s, 1H, N–OH).

Table 1 lists the ^{13}C NMR spectrum. $C_{31}H_{47}O_3N$. MW 481.7.

Methyl Ester of A-Homo-4-aza-3-oxo-18βH-olean-9(11),12(13)-dien-30-oic Acid (6). A solution of **5** (0.5 g, 1 mmol) in anhydrous dioxane (50 mL) at 10°C was treated with freshly distilled $SOCl_2$ (1 mL), stirred for 10 min, and poured into cold KOH solution (1%). The precipitate was filtered off, washed with water, and dried. The dry solid was recrystallized from MeOH:$CHCl_3$, yield 0.37 g (74%), R_f 0.54 (benzene + 1 drop MeOH), mp 264-266°C, $[\alpha]_D^{20}$ +442° (*c* 0.04, $CHCl_3$). IR spectrum (ν, cm^{-1}): 3300-3100 (NH), 1726 (COOMe), 1664 (CONH), 1271, 1249, 1215, 1186, 1107, 1085, 1016, 997, 923, 894. UV spectrum (λ_{max}, nm, MeOH): 285 (log ε 4.14).

PMR spectrum ($CDCl_3$, δ, ppm): 0.83 (3H, s, CH_3-28), 0.97, 1.00 (6H, both s, CH_3-26, CH_3-27), 1.10, 1.12 (6H, both s, CH_3-25, CH_3-23), 1.24 (3H, s, CH_3-29), 1.34 (3H, s, CH_3-24), 1.40-2.10 (CH, m, CH_2), 3.68 (3H, s, OCH_3), 5.58, 5.62, 5.65 (3H, NH, H-11, H-12).

Table 1 lists the ^{13}C NMR spectrum. $C_{31}H_{49}O_3N$. MW 483.7.

Methyl Ester of 3-Cyano-3,4-*seco*-18βH-olean-4,9,12-trien-30-oic Acid (7). A solution of **5** (0.1 g, 0.1 mmol) in anhydrous Py (2 mL) was treated with *p*-TsCl (0.3 g), refluxed without admitting moisture for 5 h, and poured into HCl solution (5%, 10 mL). The precipitate was filtered off, washed with water, and dried to afford **7** (0.06 g, 66%), homogeneous according to TLC, R_f 0.23 (benzene:EtOH, 20:1), mp 253-256°C. IR spectrum (ν, cm^{-1}): 2200-2100 (CN), 1728 (COOMe), 1217, 1155, 1088, 926, 735.

PMR spectrum ($CDCl_3$ + DMSO-d_6, δ, ppm): 0.52, 0.66, 0.76, 0.80, 0.83, 0.95 (18H, all s, 6CH_3), 1.00-2.00 (CH, m, CH_2), 3.38 (3H, s, OCH_3), 5.30, 5.32, 5.34 (4H, =CH_2, H-11, H-12).

Table 1 lists the ^{13}C NMR spectrum. $C_{31}H_{46}O_2N$. MW 464.7.

Methyl Ester of A-Homo-4-aza-3-thioxo-18βH-olean-9(11),12(13)-dien-30-oic Acid (8). A solution of **6** (50 mg, 0.1 mmol) in anhydrous toluene (5 mL) was treated with Lawesson's reagent (160 mg, 0.4 mmol), refluxed without admitting

moisture for 5 h, and filtered. The filtrate was washed with Na_2CO_3 solution (5%) and water and evaporated. The solid was chromatographed over a column of SG with elution by $CHCl_3$. Yield 65.2%, R_f 0.77 (toluene:EtOAc, 3:1). IR spectrum (ν, cm^{-1}): 2967 (NH), 1680 (S=C), 1572 (NHC=S).

PMR spectrum ($CDCl_3$, δ, ppm): 0.80, 0.84, 1.00, 1.02, 1.13, 1.26 (21H, all s, $7CH_3$), 1.40-2.7 (CH, m, CH_2), 3.70 (3H, s, OCH_3), 5.62, 5.68 (2H, H-11, H-12), 7.00 (1H, br.s, NH).

Table 1 lists the ^{13}C NMR spectrum. $C_{32}H_{51}NO_2S$.

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