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The assignments of ^{1}H and ^{13}C NMR spectra of two new aminoacyl triterpene saponins from roots of *Gypsophila trichotoma* Wend. are reported. In addition to 1D NMR methods, 2D NMR techniques (COSY, TOCSY, ROESY, HSQC, HMBC, and HSQC-TOCSY) were used for the assignments. The structures were completed by analysis of HR-ESI-MS and ESI-MS^{n}.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; saponins; *Gypsophila trichotoma*; *Caryophyllaceae*

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INTRODUCTION

Species belonging to the genus *Gypsophila* are highly valued and important medicinal herbs. Triterpenoid saponins from the genus *Gypsophila* are exploited commercially for a variety of purposes including as medicines, detergents, adjuvants, cosmetics, in the composition of photosensitive surfaces in photography.[1,2] In addition, a number of sterols[3-5], flavonoids[6-8], phenolic acids[9] and oligosaccharides[10] have been reported from the species of *Gypsophila*.

Some of the saponins are considered as the major bioactive components of the drugs, mainly used for their anti-inflammatory, spermicidal[11,12], hypocholesterolaemic[13], and antiviral[14,15] activities. They enhance the immune response in the preparation of vaccines (Freund's adjuvant). The commercial Merck Saponin (E. Merck No. 7695), also known as Saponin Pure White, is used as reference substance for the determination of haemolytic indices.[1] It is also employed in certain expectorants.[1]

Bidesmosides from different *Gypsophila* species are based upon the aglycons gypsogenin, quillaic acid and gypsogenic acid.[16-23] Substituted on the C-3 hydroxyl group and on the C-28 carboxyl group by two oligosaccharide chains, the saponins obtained from white saponin are among the highest glycosilated.[24,25]

In the 1980s the supply source of *Gypsophila* was contaminated after the Chernobil disaster. In order to found reliable source of haemolytic saponins, an investigation of other *Gypsophila* species would be desirable.

G. trichotoma is distributed in the southeast region of Europe, southwest and central Asia.[26] This perennial herb is native to Black Sea region in Bulgaria.[27]

With regard to the chemical constituents of *G. trichotoma*, there are few reports.[28-32] Luchanskaya et al. have isolated and identified four triterpenoid saponins (Trichosides

A-D) from the roots of *G. trichotoma* by column chromatography and gas-liquid chromatography of silylated methylsaponins. Trichosides A-C have a common disaccharide moiety attached to gypsogenin at the C-3 carbon and their structures have been established as 3-*O*-β-D-glucosyl-(1→3)-β-D-glucuronosyl gypsogenin 28-*O*-β-D-galactoside,[29] 3-*O*-β-D-glucosyl-(1→3)-β-D-glucuronosyl gypsogenin 28-*O*-β-D-glucosyl-(1→4)-β-D-galactoside[30] and 3-*O*-β-D-glucosyl-(1→3)-β-D-glucuronosyl gypsogenin 28-*O*-β-D-galactosyl-(1→4)-α-L-rhamnosyl-(1→4)-β-D-fucoside,[31] respectively. Trichoside D contains nine sugar units. His structure was partially characterised by hydrolysis. The sugar chain attached at C-3 of gypsogenin was elucidated as β-D-galactosyl-(1→4)-β-D-xylosyl-(1→3)-[α-L-arabinosyl-(1→4)]-β-D-glucuronoside. The sugar chain linked to C-28 carboxyl is not elucidated fully. It's composed of five sugars. The first sugar unit was a β-D-fucose di-substituted by α-L-rhamnose at position 2 and β-D-galactose at position 4. These two sugars were although substituted by glucose or xylose.[32]

There are no studies on the saponins by analytical techniques such as HPLC and NMR spectral analysis.

The medicinal and commercial importance attached to *Gypsophila* prompted us to reinvestigate the saponins in *G. trichotoma.* In this article we report the isolation and structural study of two new aminoacyl triterpenoid saponins from the roots of the plant. The complete assignment of the ^{1}H and ^{13}C NMR spectra of these two isomeric saponins is reported in this paper.

RESULTS AND DISCUSSION

The mixture of compounds **1** and **2** were obtained as a white amorphous powder. The HR-ESI-MS (positive ion mode) exhibited pseudomolecular ion peaks at *m/z* 1705.7628 $[M+Na]^+$, 1689.7999 $[M+Li]^+$ and 1683.7925 $[M+H]^+$ indicating a molecular weight of 1682, compatible with the molecular formula $C_{76}H_{122}N_4O_{37}$. These three pseudomolecular ion were observed in ESI-MS^+ at *m/z* 1705, 1689 and 1683, respectively, with other fragments ions peaks at *m/z* 1213 $[M+Na-470]^+$ and $[M+Na-743]^+$, due to the loss of two glycosidic chains. The chain with 470 *uma* was composed with hexose, pentose and hexosuronic acid. Another fragment ion peaks at *m/z* 743, 727 and 711 corresponded to the ion fragments of the ester chain (743 *uma)* and to the successively loss of 16 *uma* (NH_2). These ions suggest that the ester glycosidic chain was constituted by four sugars, two desoxyhexose, hexose and pentose, and one aminoacyl group with at least two NH_2 functionnal groups, identified as arginin.

The proton and carbon resonances of the aglycon moiety of saponins **1** and **2** were unambiguously assigned from the analysis of COSY, HSQC J-modulate and HMBC NMR spectra (Table 1). The aglycon was identified as gypsogenin by comparison with literature data.[33]

Analysis of COSY, TOCSY, HSQC J-modulate and HSQC-TOCSY experiments and spin-spin couplings in ^1H NMR allowed the identification of a β-D-glucuronic acid (GlcA), a β-D-galactose (Gal), a β-D-glucose (Glc), a β-D-fucose (Fuc), a α-L-rhamnose (Rha), an α-L-arabinose (Ara), and two β-D-xylose units (Xyl) (Table 2). The common D configuration for Glc, Gal, GlcA, Xyl and Fuc and the L configuration for Rha and Ara were assumed according to those most often encountered among the plant glycosides, and corresponded to the D and L configurations previously characterized in the structural elucidation of trichoside D.[28]

The two β-D-xylose units were identified starting from anomeric protons at δ_H 4.65 (Xyl H-1) and 4.71 (Xyl'-H1) and the ^{13}C NMR chemical shifts indicated a terminal position for these sugars.[26] The β-D-glucose unit was identified starting from anomeric signals at δ_H 4.71 and δ_C 104.8, characterized by its large coupling constants up to 8 Hz. The deshielding of both proton H-3 (δ_H 4.26, *t*, *J* = 9.0 Hz) and carbon C-3 (δ_C 85.5) of this glucose suggested a substitution at this position, like esterification of the hydroxyl. The α-L-arabinose (δ_H 4.63 and δ_C 104.4) was identified to be in terminal position as observed by the ^{13}C NMR chemical shifts.[26] The identification of a β-D-galactopyranosyl unit was readily supported by the characteristic equatorial proton H-4 at δ_H 3.85 (*dm*, *J* = 3.1 Hz) (Table 2). The ^{13}C NMR chemical shifts of this galactose indicated a terminal position in the sugar chain and suggested most ramifications in the compound. The methyl doublet at δ_H 1.25 (*J* = 6.2 Hz) and the typical small doublet of anomeric proton at δ_H 5.18 (*J* = 1.3 Hz) were characteristic of a rhamnopyranosyl unit. It was identified as α anomeric configuration by the ^{13}C NMR chemical shift[34] and ROE effects observed between Rha H-1 and Rha H-2 and between Rha H-3 and Rha H-5. The deshielding of both Rha C-3 (δ_C 82.5) and Rha C-4 (δ_C 79.1) indicated a disubstitution of this rhamnose. The β-D-fucopyranosyl was identified starting from the deshielded anomeric proton at δ_H 5.37 (*J* = 7.6 Hz) and characterized by its methyl doublet at δ_H 1.23 (*J* = 6.4 Hz) and its equatorial proton H-4 at δ_H 3.70 (*dd*, *J* = 8.0, 2.6 Hz). The deshielding of anomeric proton and the chemical shift of anomeric carbon at 95.3 suggested that this fucose was attached by an ester linkage to the C-28 carboxylic group of the aglycon. This was confirmed by the long-range correlation observed in the HMBC experiment between signals at δ_H 5.37 (Fuc H-1) and δ_C 178.5 (C-28 of gypsogenin). This observation and the deshielded carbon C-3 (δ_C 86.4) of gypsogenin

indicated a bidesmosidic saponin. The fucose was substitued at position 2 as observed by its deshielded Fuc C-2. Starting from the anomeric proton at δ 4.42 (*d*, *J* = 7.5 Hz), two β-D-glucuronic acid units were identified by the dedoubling signals of proton H-3 at δ 3.74 and 3.76 and carbon C-3 at δ 86.0 and 85.0, respectively and of carbonyl C-6 at δ 176.2 and 175.0. The deshielding of carbons C-2 and C-3 indicated a disubstitution of these glucuronic acids (Table 1). Observation of cross-peaks in the HMBC spectrum between anomeric signal at δ_H 4.42 of glucuronic acid units and carbon C-3 of gypsogenin, indicated the presence of two saccharidic chains attached at C-3 of gypsogenin. The long-range proton-carbon coupling between Gal H-1 (δ_H 4.81) and GlcA C-2 (δ 78.0), Xyl H-1 (δ_H 4.65) and GlcA C-3 (δ 86.0) and Ara H-1 (δ_H 4.63) and GlcA' C-3 (δ 85.5), indicated two trisaccharide chains, the β-D-galactopyranosyl-(1–>2)-[β-D-xylopyranosyl-(1–>3)]-β-D-glucuronopyranoside and β-D-galactopyranosyl-(1–>2)-[α-L-arabinopyranosyl-(1–>3)]-β-D-glucuronopyranoside.

Integration of anomeric proton signals showed that the ratio xylose/arabinose was 2:1.

The sequencing of the ester chain was obtained by analysis of HMBC experiment which showed cross-peaks between Fuc H-1 (δ_H 5.37) and carbonyl C-28 of gypsogenin (δ 178.5), Rha H-1 (δ_H 5.18) and Fuc C-2 (δ 75.8), Rha H-3 (δ_H 4.03) and Glc C-1 (δ 104.8), and between Rha H-4 (δ_H 4.03) and Xyl' C-1 (δ 105.0). This sequence was confirmed by the observation of ROE connectivities between Rha H-1 and Fuc H-2, Rha H-3 and Glc H-1 and Rha H-4 and Xyl' H-1. Thus the ester chain was at least tetrasaccharidic.

In the ^{1}H and ^{13}C NMR spectra, supplementary signals were observed attributed to an amino acid unit, identified as arginin by comparison with literature data[35] (Table 1). This arginin substituted the position 3 of the glucose unit as observed by the deshielded

glucose H-3. The presence of arginin in the ester chain was confirmed by the fragmentation of the [ester chain]$^+$ ion fragment at *m/z* 743 which gave in ESI-MS^3 another ions fragments at *m/z* 597 [ester chain-Fuc]$^+$, 581 [ester chain-Fuc-NH_2]$^+$, 449 [ester chain-Fuc-NH_2-Xyl]$^+$, and 174 [$C_6H_{14}N_4O_2$]$^+$. Alkaline hydrolysis of the mixture of saponins afforded arginin, identified by co-TLC with authentic sample, and by analysis in ^{1}H NMR, COSY and HR-ESI-MS (*m/z* 175.1197 for [$C_6H_{15}N_4O_2$]$^+$).

Thus the mixture of saponins was identified as 3-*O*-[β-D-galactopyranosyl-(1–>2)-(β-D-xylopyranosyl-(1–>3))-β-D-glucuronopyranosyl]-28-*O*-[3-*O*-arginin-β-D-glucopyranosyl-(1–>3)-(β-D-xylopyranosyl-(1–>4))-α-L-rhamnopyranosyl-(1–>2)-β-D-fucopyranosyl]-gypsogenin (**1**) and 3-*O*-[β-D-galactopyranosyl-(1–>2)-(α-L-arabinopyranosyl-(1–>3))-β-D-glucuronopyranosyl]-28-*O*-[3-*O*-arginin-β-D-glucopyranosyl-(1–>3)-(β-D-xylopyranosyl-(1–>4))-α-L-rhamnopyranosyl-(1–>2)-β-D-fucopyranosyl]-gypsogenin (**2**).

Saponin **1** was the arginin ester of saponin G4 previously isolated, as a single compound, from *Gypsophilla paniculata* and *G. arostii*.[18] The ^{13}C NMR data of the glycosides chains of saponin **1** were identical to those described for saponin G4 ± 1.5 ppm, except for the signals of the Glc C-3, deshielded in saponin **1** at 85.5 ppm.[18] To our knowledge, saponins **1** and **2** were the first aminoacyl saponins isolated from natural sources.

EXPERIMENTAL

Plant material

G. trichotoma roots were collected in 2003, in Bulgaria, Black Sea region, v. Balgarevo, 50 m above sea level. Voucher specimen of plant material was deposed in the Herbarium of the Faculty of Pharmacy, Université Henri Poincaré Nancy 1, France.

Extraction and isolation

Air-dried powdered roots of the plant (100 g) were homogenized with a Warring blender and extracted with aqueous methanol (25% w/w) containing 1% polyvinylpolypyrolidone and 1 mM gluthation (2 l x 4), agitated for 24 h each time at 25° on a gyratory shaker. The extracts, after filtering, were concentrated *in vacuo* at 35° and lyophilized to yield 40.87 g of the crude extract. An aliquot of the crude extract (5 g) was dissolved in 50 ml water and filtered through a 0.2 μm filter membrane. The aqueous solution was subjected to a low – pressure liquid chromatography. 120 fractions (fraction volume 12 ml) were collected and analysed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). All the fractions containing similar saponin composition were combined, concentrated and lyophilised. The main saponin mixture (352 mg) was subjected to preparative reversed-phase HPLC. An aliquot of the mixture (50 mg) was dissolved in 2 ml water and a preparative HPLC was applied to afford 23 fractions. This procedure was accomplished seven times. Fractions containing saponins (6-19) were pooled and purified further using repeated preparative HPLC to give a mixture (46.8 mg) of saponins **1** and **2**. Analysis of this mixture resulted in a single peak by HPLC having retention time 10.7 min.

Equipment and chromatographic conditions

Low – pressure liquid chromatography

The low – pressure liquid chromatography (pressure 1 - 2 bar, pump Duramat CFG, Prominent, Germany) was accomplished on reversed phase material (Lichroprep RP8, 40-63 μm, 250 g, Merck, Germany). The column was conditioned with water (100 ml) and linear gradient elution from 100% water to 100% ethanol (500 g/ 500 g) was applied.

Analytical HPLC

Analytical chromatographic analyses were performed on a Shimadzu (Japan) chromatographic system equipped with two Model LC-10AT pumps, a Rheodyne injector with 20 μl sample loop, a UV-VIS detector Model SPD-10A set at 210 and 254 nm according to the UV absorption maxima of the compounds determined. The sensitivity was set of 1.00 AUFS. A HPLC method was developed using a reversed phase column Lichrospher RP 18, 5 μm, 250 x 4.6 mm I.D. (Merck, Germany). All data were acquired and processed with Shimadzu CLASS-VP (Version 4.3) chromatography data system.

The mobile phase consisted of acidified water (0.1% concentrated *ortho*- phosphoric acid) (solvent A) and 84% acetonitrile (0.1% concentrated *ortho*- phosphoric acid) (solvent B). The gradient program began at 25% B followed by a linear gradient to 100% B for 15min, an isocratic step (100% B) for 5 min and than changed to the initial condition in 5 min. The flow rate was 1 ml/min. The oven temperature was set at 40°.

Preparative HPLC

The preparative HPLC was performed on chromatographic system equipped with two Waters Associates Model 510 pumps, rheodyne injector Model 7010 with 2 ml sample loop, and a Lambda Max Model 481 LC (Waters Associates) spectrophotometer. The UV detection wavelength was set at 210 nm. A semi-preparative reversed phase Lichrospher 100 RP18 (end-capped), 10 μm, 250 x 10 mm I.D., Merck (Germany) column was used. The binary gradient elution system consisted of water (solvent A) and methanol (solvent B) and separation was achieved using the following step-gradient: 0-10 min 30% B; 10-18 min 35% B; 18-28 min 45% B; 28-38 min 50% B; 38-46 min 60% B; 46-54 min 70% B; 54-62 min 80% B; 62-70 min 100B. The flow rate was 10 ml/min. Separation was achieved at ambient temperature of 25±2°C.

Thin layer chromatography

TLC was performed on plate Polygram SilG/UV254 (Macherey-Nagel, Germany) with chloroform-methanol-water at the ratio 6.5:5:1 as the mobile phase. The saponins were visualized as purple spots by spraying 10% sulfuric acid in ethanol and heating at 105°C in an oven.

NMR spectra

The NMR spectra were recorded at 293 K using a BRUKER Avance DRX 500 spectrometer using 5 mm multinuclear inverse probehead with z-shielded gradient operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. 1D and 2D NMR experiments (COSY, TOCSY, ROESY, HSQC-Jmod, HMBC and HSQC-TOCSY) were performed using standard Bruker pulse programs (XWinNMR version 2.6).

Sample was dissolved in CD_3OD. Chemical shifts were referenced to the solvent signal

(δ (CHD_2OD) = 3.33 ppm for ^1H NMR and (δ (CD_3OD) = 49.0 ppm for ^{13}C NMR).

^1H and ^{13}C 1D spectra were acquired with relaxation delay d1=1s, 32K data points and 90° pulses were respectively 10.5 µs at 2 dB and 11.3 µs at -4dB for ^1H and ^{13}C.

2D experiments were recorded with following parameters:

^1H-^1H gradient COSY spectrum: relaxation delay d1 = 1 s; 90° pulse, 10.5 µs for ^1H at 2 dB; number of scans 2; 2K data points in t2; spectral width 10.0 ppm in both dimensions; 256 experiments in t1; zero-filling up to 1K in t1; apodization with pure sine-bell in both dimensions prior to double Fourier transformation.

TOCSY spectrum: relaxation delay d1 = 1 s; 90° pulse, 10.5 µs for ^1H at 2 dB; number of scans 8; spin lock time, 200 ms using 90° pulse of 30 µs at 12 dB; 2K data points in t2; spectral width 10.0 ppm in both dimensions; 512 experiments in t1; apodization with sine-bell (processing parameter SSB=3) in both dimensions; zero-filling with linear prediction up to 1K.

ROESY spectrum: relaxation delay d1 = 1 s; 90° pulse, 10.5 µs for ^1H at 2 dB; number of scans 8; roesy spin lock pulse of 200 ms at 28 dB; 2K data points in t2; spectral width 10.0 ppm in both dimensions; 512 experiments in t1; apodization with squared cosine-bell in both dimensions; zero-filling up to 1K and 4K respectively in t1 and t2.

HSQC-J modulated using Bruker library pulse sequence "inviedgptp": relaxation delay d1 = 1 s; coupling constant $^1J(^1H\text{-}^{13}C)$ = 145 Hz for d4 = 1.7 ms; 90° pulse, 10.5 µs at 2 dB for ^1H, 9 µs at -4.0 dB for ^{13}C and GARP pulse decoupling of 80 µs at 15.5 dB with gradient ratio GPZ1:GPZ2:GPZ3 = 30:80:20.1; 2K data points in t_2; spectral width 10.0 ppm in F2 and 220 ppm in F1; number of scans 16; 512 experiments in t1; apodization with pure cosine-bell in both dimensions; zero-filling with linear prediction up to 1K.

HSQC-TOCSY, pseudo 3D experiment, using Bruker library pulse sequence

"invigpmltp": relaxation delay d1= 1 s; delays and pulses calibration are the same as HSQC and TOCSY; gradient ratio is the same as HSQC; 2K data points in t_2; spectral width 10.0 ppm in F2 and 160 ppm in F1; number of scans 72; 512 experiments in t1; apodization with pure cosine-bell in both dimensions; zero-filling with linear prediction up to 1K.

HMBC using Bruker library pulse sequence "inv4gplplrnd": relaxation delay d1 = 2 s; same pulse calibration as HSQC; delay of the low-pass J-filter d2 = 3.44 ms (corresponding to $^{1}J(^{1}H\text{-}^{13}C)$ = 145 Hz); delay for evolution of long-range coupling d6 = 70 ms; gradient ratio GPZ1:GPZ2:GPZ3 = 50:30:40; 2K data points in t2; spectral width 10.0 ppm in F2 and 220 ppm in F1; number of scans 20; 512 experiments in t1; ; apodization with pure sine-bell in both dimensions; zero-filling with linear prediction up to 1K.

MS spectra

HR-ESI-MS, ESI-MS and MS-MS experiments were performed using a Micromass Q-TOF.micro instrument (Manchester, UK) with an electrospray source (eV== 60V, 80°C, flow of injection 5µl/min).

Mass data of 1 and 2

$C_{76}H_{122}N_4O_{37}$; HR-ESI-MS (positive ion mode): *m/z* 1705.7628 (calc. 1705.7686) $[C_{76}H_{122}N_4O_{37} + Na]^+$, 1689.7999 (calc. 1689.7948) $[C_{76}H_{122}N_4O_{37} + Li]^+$, 1683.7925 (calc. 1683.7866) $[C_{76}H_{122}N_4O_{37} + H]^+$; ESI-MS (positive ion mode): *m/z* 1705 $[M+Na]^+$, 1689 $[M+Li]^+$, 1683 $[M+H]^+$, 1213 $[M+H\text{-}470]^+$, 963 $[M+Na\text{-}743]^+$, 1667 $[M+H\text{-}NH_2]^+$, 1651 $[M+H\text{-}2\ NH_2]^+$, 743 [ester chain]$^+$, 727 [ester chain $-NH_2]^+$, 711 [ester chain -2 $NH_2]^+$; ESI-MS-MS : MS^3 (743): *m/z* 597 [ester chain-Fuc]$^+$, 581 [ester chain-Fuc-$NH_2]^+$, 449 [ester chain-Fuc-NH_2-Xyl]$^+$, 174 $[C_6H_{14}N_4O_2]^+$; MS^3 (727): *m/z*

581 [ester chain-Fuc-NH_2]$^+$, 449 [ester chain-Fuc-NH_2-Xyl]$^+$, 158 [$C_6H_{14}N_4O_2$-NH_2]$^+$; MS^3 (711): *m/z* 565 [ester chain-Fuc-2 NH_2]$^+$, 449 [ester chain-Fuc-2 NH_2-Xyl]$^+$, 142 [$C_6H_{14}N_4O_2$-2 NH_2]$^+$.

Alkaline hydrolysis

Alkaline hydrolysis of the mixture of saponins **1** and **2** with 5% NaOH in EtOH (reflux 4h at 100°C) afforded arginin**:** HR-ESI-MS (positive ion mode): *m/z* 175.1197 (calc. 175.1195) [$C_6H_{14}N_4O_2$ + H]$^+$; ^{1}H NMR (D_2O): 2.15 (H-4a, m), 2.21 (H-4b, *m*), 2.35 (H-3, *m*), 3.43 (H-5, *t*, *J* = 6.8 Hz), 4.36 (H-2, *t*, *J*=6.2 Hz).

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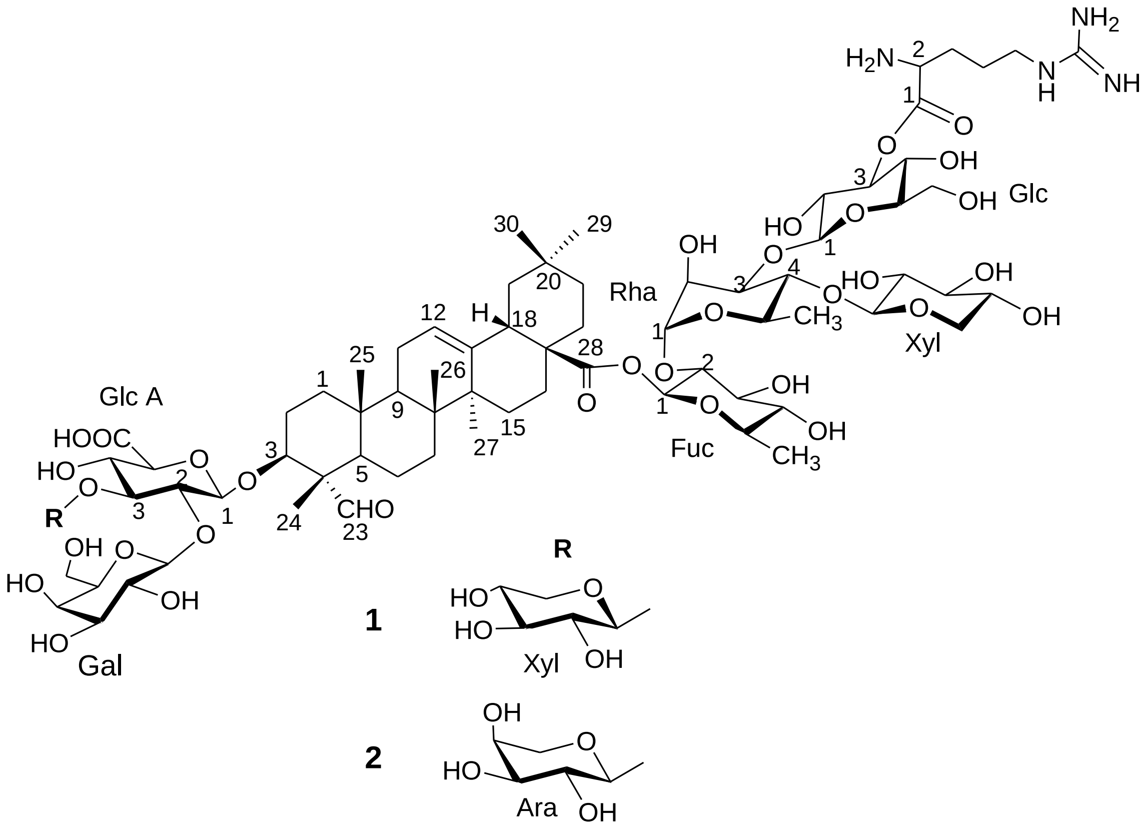
Table 1. ^{1}H and ^{13}C NMR data (δ in ppm) of the aglycon part of **1** and **2** (CD_3OD).

	δ_H, *J*(Hz)	δ_C
1	-	39.1
2	1.82 (*m*) 2.03 (*dm*, 13.7)	25.5
3	3.90 (*m*)	86.4
4	-	56.2
5	1.34 (*m*)	49.2
6		21.3
7	1.30 (*dm*, 8.9) 1.50 (*m*)	33.1
8	-	40.9
9	1.68 (*m*)	48.8
10	-	36.9
11	1.93 (*m*)	24.4
12	5.27 (*tl*, 3.2)	123.5
13	-	144.6
14	-	49.2
15	1.16 (*m*) 1.63 (*m*)	28.8
16	1.64 (*m*) 2.07 (*m*)	23.9
17	-	48.0
18	2.83 (*dd*, 13.2-3.6)	42.7
19	1.14 (*m*) 1.73 (*tm*, 13.5)	47.2
20	-	31.4
21	1.26 (*m*) 1.39 (*m*)	34.7
22	1.57 (*dm*, 13.3) 1.79 (*tm*,13.3)	33.0
23	9.51 (*s*)	212.4
24	1.22 (*s*)	11.0
25	1.00 (*s*)	16.2
26	0.79 (*s*)	17.7
27	1.18 (*s*)	26.2
28	-	178.5
29	0.92 (*s*)	33.5
30	0.94 (*s*)	24.0

Table 2. ^{1}H and ^{13}C NMR data (δ in ppm) of the osidic parts of **1** and **2** (CD_3OD).

	1		**2**			**1 and 2**	
	Trisaccharide in C-3 of gypsogenin					*Tetrasaccharide in C-28*	
	δ_H, *J*(Hz)	δ_C	δ_H, *J*(Hz)	δ_C		δ_H, *J*(Hz)	δ_C
β-D-GlcA					*β-D-Fuc*		
1	4.42 (*d*, 7.5)	104.2	4.42 (*d*, 7.5)	104.2	1	5.37 (*d*, 7.6)	95.8
2	3.71 (*m*)	78.0	3.71 (*m*)	78.0	2	3.74 (*m*)	75.8
3	3.74 (*m*)	86.0	3.76 (*m*)	85.5	3	3.70 (*dd*, 8.0-2.6)	75.3
4	3.55 (*m*)	71.9	3.55 (*m*)	71.9	4	3.66 (*dm*, 2.6)	73.2
5	3.63 (*d*, 9.8)	78.0	3.63 (*d*, 9.8)	78.0	5	3.73 (*m*)	72.6
6	-	176.2	-	175.0	6	1.23 (*d*, 6.4)	16.5
β-D-Gal					*α-L-Rha*		
1	4.81 (*d*, 7.4)	103.5	4.81 (*d*, 7.4)	103.5	1	5.18 (*d*, 1.3)	101.8
2	3.50 (*m*)	73.2	3.50 (*m*)	73.2	2	4.35 (*dd*, 2.7, 1.3)	71.0
3	3.51 (*m*)	74.9	3.51 (*m*)	74.9	3	4.03 (*dd*, 9.5, 3.1)	82.5
4	3.85 (*dm*, 3.1)	70.7	3.85 (*dm*, 3.1)	70.7	4	3.68 (*t*, 9.5)	79.1
5	3.55 (*m*)	76.8	3.55 (*m*)	76.8	5	3.83 (*m*)	69.2
6	3.72 (*m*) 3.84 (*dd*, 13.1, 6.6)	62.3	3.72 (*m*) 3.84 (*dd*, 13.1, 6.6)	62.3	6	1.25 (*d*, 6.2)	18.6
β-D-Xyl					*β-D-Glc*		
1	4.65 (*d*, 7.8)	104.4			1	4.71 (*d*, 8.0)	104.8
2	3.28 (*dd*, 9.3, 7.8)	75.0			2	3.53 (*dd*, 9.2, 8.0)	73.6
3	3.38 (*t*, 9.0)	77.7			3	4.26 (*t*, 9.0)	85.5
4	3.57 (*m*)	70.8			4	3.60 (*dd*, 9.8, 9.0)	69.9
5	3.28 (*t*, 11.2) 3.95 (*dd*, 11.2, 4.8)	66.7			5	4.42 (*ddd*, 9.8, 5.1, 2.3)	77.0
					6	3.74 (*dd*, 12.1, 5.1) 3.91 (*dd*, 12.1, 2.2)	61.9
α-L-Ara					*β-D-Xyl*		
1			4.63 (*d*, 7.0)	104.4	1	4.71 (*d*, 8.0)	105.0
2			3.59 (*m*)	72.8	2	3.08 (*dd*, 9.2, 8.0)	75.6
3			3.59 (*m*)	74.3	3	3.33 (*m)*	78.2
4			3.87 (*m*)	69.9	4	3.51 (*m)*	70.9
5			3.63 (*dd*, 9.8, 4.0) 3.94 (*brd*, 10.9)	67.6	5	3.21 (*t*, 10.7) 3.86 (*m)*	66.7
					Arginin		
					1	-	174.1
					2	3.65 (*t*, 6.3)	55.5
					3	1.92 (*m*)	29.2
					4	1.72 (*m)* 1.77 (*m*)	25.5
					5	3.25 (*t*, 6.1)	41.8
					6	-	158.3



NH2
H2N
2
1
O
NH
NH
OH
3
OH
Glc
HO
1
OH
30
29
4
HO
OH
20
Rha
3
12
H
18
1
CH3
OH
Xyl
28
25
O
2
OH
26
Glc A
1
9
O
1
OH
HOOC
15
Fuc
3
27
CH3
HO
2
5
R
3
1
24
CHO
23
OH
R
HO
OH
HO
HO
1
HO
Gal
Xyl
OH
OH
2
HO
Ara
OH