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Two New Saponins from *Solidago gigantea*

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Abstract

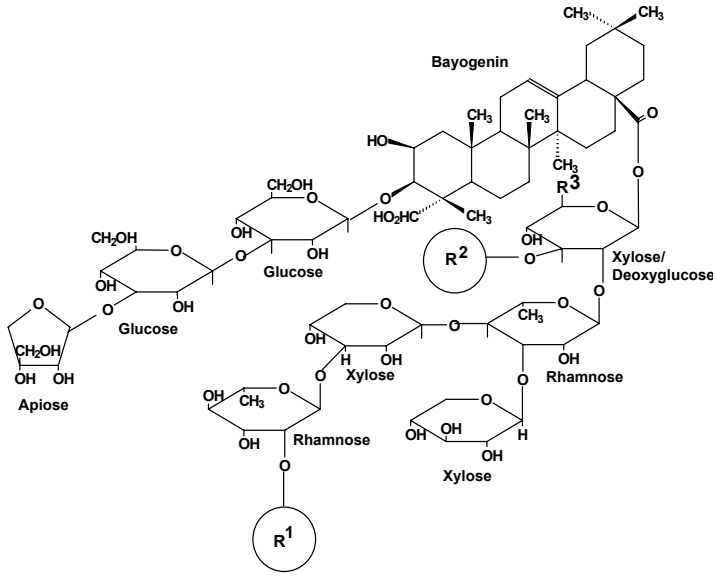
Two new triterpenoid saponins, named giganteasaponin 5 and giganteasaponin 6, were isolated from the aboveground parts of *Solidago gigantea* and were defined to be 3-O-[β-D-Apio-D-furanosyl-(1→3)- β-D-glucopyranosyl-(1→3)- β-D-glucopyranosyl]-28-O {α-L-rhamnopyranosyl-(1→3)- β-D-xylopyranosyl-(1→4)- [β-D-xylopyranosyl-(1→3)]- α-L-rhamnopyranosyl-(1→2) [α-L-rhamnopyranosyl-(1→3)]- β-D-xylopyranosyl}-Bayogenin respectively 3-O- [β-D-Apio-D-furanosyl-(1→3)- β-D-gluco-pyranosyl-(1→3)-β-D-glucopyranosyl]-28-O {β-D-galactopyranosyl-(1→2)- α-L-rhamnopyranosyl-(1→3)- β-D-xylopyranosyl-(1→4)- [β-D-xylopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2) [α-L-rhamnopyranosyl-(1→3)]- β-D-xylopyranosyl}-Bayogenin. The structures were established on the basis of chemical degradation, extensive MS studies and NMR investigations as well.

Keywords

Triterpen, saponin, giganteasaponin, *Solidago gigantea*

Introduction

In previous publications we reported on the isolation and structure elucidation of the main saponins (giganteasaponins 1-4, Figure 1) from *Solidago gigantea* [1-5]. Investigating the saponin content and composition of *Solidago gigantea* from different locations using HPLC [6] we found two additional unknown saponins (giganteasaponin 5 and 6) representing sometimes up to 20% of the total saponin



	R^1	R^2	R^3
Giganteasaponin 1	H	Apiosyl	Methyl
Giganteasaponin 2	H	Rhamnosyl	Methyl
Giganteasaponin 3	Galactosyl	Apiosyl	Methyl
Giganteasaponin 4	Galactosyl	Rhamnosyl	Methyl

Figure 1. Structures of the known giganteasaponins from *Solidago gigantea*

content. The behaviour of giganteasaponin 5 and 6 at the HPLC analysis was similar to that of the other saponins from *S. gigantea* [6, 7]: pairs of saponins (giganteasaponins 1 and 3 respectively giganteasaponins 2 and 4) cannot be separated on reversed phase HPLC, they coelute in one peak and can only be separated using polar stationary phases like silica or polar RP-phases [7]. These pairs of saponins show an identical structure exept an additional terminal galactose in giganteasaponin 3 respectively gigantaesaponin 4. Since giganteasaponin 5 and 6 coelute slightly before the other pairs of saponins on RP-HPLC it could be suggested that these compounds represent an analogous pair of saponins with

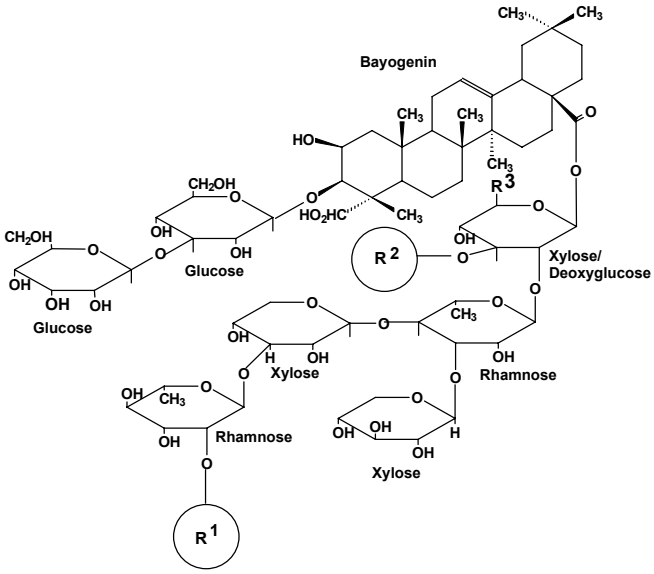
similar polarity and identical structure except an additional galactose. In order to verify this assumption the aim of this study was the isolation and structure elucidation of giganteasaponin 5 and 6.

Results

For the isolation of the saponins from *Solidago gigantea* a methanol-water extract (80%) of dried aboveground parts was prepared. This extract was further separated by column chromatography over Sephadex LH-20 and the fractions were analysed by TLC to detect the saponins. The saponin containing fractions were further purified by SPE with C18 cartridges followed by a separation step using praparative HPLC on RP-8 with methanol-water as mobile phase. The coeluting giganteasaponins 5 and 6 could be separated by preparative TLC on silica and a final purification by preparative HPLC on RP-8 resulted in the single giganteasaponin 5 respectively 6 with high purity.

The spectral features, physicochemical properties, chromatographic behaviour and the hemolytic activity confirmed giganteasaponin 5 and 6 to be triterpenoid saponins. After enzymatic hydrolysis of the single saponins bayogenin could be detected as aglycon on TLC with an authentic reference sample. Hydrolysis of giganteasaponin 5 yielded apiose, glucose, rhamnose and xylose that could be separated and identified by HPIC with authentic reference samples. After trimethylsilylation the sugars were separated and identified by GLC [8] resp. GCMS and the ratio was determined to be approximatively 1:2:3:3. Hydrolysis of giganteasaponin 6 yielded similar results, above that an additional galactose could be identified. These results confirmed the previous suggestions giganteasaponins 5 and 6 to be a pair of saponins with identical sapogenins and identical sugar moieties exept an additional galactose in giganteasaponin 6. To obtain the absolute configurations of the single carbohydrates we followed well established procedures [9] and found D-apiose, D-glucose, L-rhamose as well as D-xylose to be present in gigantaesaponin 5, as expected, giganteasaponin 6 showed the same sugars and an additional D-galactose.

The ESI-mass spectrum of giganteasaponin 5 showed a quasimolecular ion of m/z 1777 $[M-H]^-$ indicating a molecular weight of 1778 like giganteasaponin 1 (Figure 1). This result was confirmed by LSI-MS giving the same quasimolecular ion. The sapogenin bayogenin was found at m/z 487 and the typical series m/z 487-m/z 649-m/z 811-m/z 943, well known from the other giganteasaponins indicating the sugar moiety attached to C-3 of bayogenin consisting of glucose, glucose and a terminal apiose (Figure 1).



	R1	R2	R3
Canadensissaponin 1	H	Apiosyl	Methyl
Canadensissaponin 2	H	Apiosyl	H
Canadensissaponin 3	H	Rhamnosyl	Methyl
Canadensissaponin 4	H	Rhamnosyl	H
Canadensissaponin 5	Galactosyl	Apiosyl	Methyl
Canadensissaponin 6	Galactosyl	Apiosyl	H
Canadensissaponin 7	Galactosyl	Rhamnosyl	Methyl
Canadensissaponin 8	Galactosyl	Rhamnosyl	H

Figure 2. Structures of the known canadensissaponins from *Solidago canadensis*

This sequence could also be observed starting from the quasimolecular ion (m/z 1777- m/z 1645- m/z 1483- m/z 1321). Concerning the sugar moiety at C-28 we found sequences like m/z 1777-m/z 1631-m/z 1499-m/z 1367m that could be explained to be $[M-H]^-$ -desoxyhexose (146)-pentose (132)-pentose (132) as well as m/z 833-m/z 701-m/z 555, suggested to be a hexasaccharide-pentose (132)-desoxyhexose (146). In fact the fragmentation of the acylglycosidic linked sugar moiety was similar but not the same to that of giganteasaponin 1, indicating that there are differences and we found a perfect match with the fragmentation of the respective sugar moiety of canadensissaponin 4 from *Solidago canadensis* [10-12] (Figure 2), where a xylose is directly linked to C-28 instead of a desoxyglucose at giganteasaponin 1. These results confirmed our sugar analyses, where no desoxyglucose could be found. These results could be confirmed by NMR studies, the spectrum was very close to that of giganteasaponin 2 [2], but again no desoxyglucose could be found and instead of this sugar a xylose could be identified. Using various NMR techniques the structure of giganteasaponin 5 could clearly be identified (Figure 3).

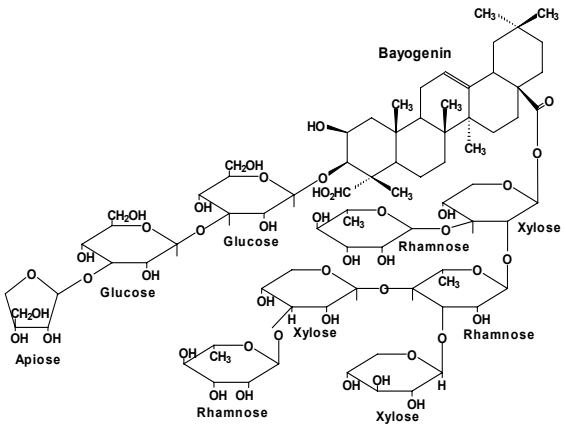


Figure 3. Structure of giganteasaponin 5

The ESI-mass spectrum of giganteasaponin 6 showed a quasimolecular ion of m/z 1939 $[M-H]^-$ indicating a molecular weight of 1940 like giganteasaponin 3

(figure 1). This confirmed our prevoious suggestions that giganteasaponin 6 shows the same structure like giganteasaponin 5 exept an additional galactose. This result was confirmed by LSI-MS giving the same quasimolecular ion. The sapogenin bayogenin was again found at m/z 487 and the typical series m/z 487-m/z 649-m/z 811-m/z 943, as well as m/z 1777-m/z 1645-m/z 1483-m/z 1321 could be observed indicating the sugar moiety attached to C-3 of bayogenin consisting of glucose, glucose and a terminal apiose (Figure 1). Concerning the sugar moiety at C-28 a terminal hexose could be found in contrast to giganteasaponin 5 and the fragmentation pattern was found to be the same as the respective partial structure of canadensissaponin 8 [10-12] (Figure 2). Therefore it could be assumed that the structure of giganteasaponin 6 is identical with that of giganteasaponin 5 exept an additional terminal galactose. These results could be confirmed by NMR studies, the spectrum was very close to that of giganteasaponin 4 [2], but again no desoxyglucose could be found and instead of this sugar a xylose could be identified. Using various NMR techniques the structure of giganteasaponin 6 could be unequivocally identified (Figure 4).

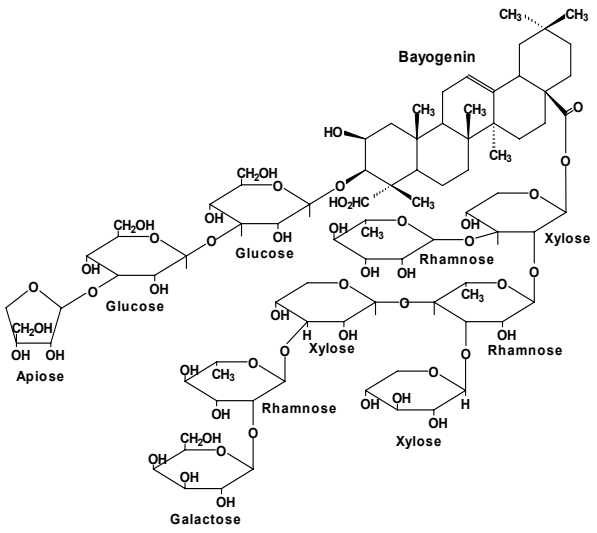


Figure 4. Structure of giganteasaponin 6

Experimental

General Experimental Procedures.

Melting points were measured using a Reichert Heiztischmikroskop RCH, LSI-MS was carried out on a Finnigan MAT 8500 (matrix glycerol, negative mode, Cs) and ESI-MS were recorded with PE-Sciex API 150 EX mass spectrometer (ESI, negative mode). ^{1}H and ^{13}C NMR were recorded on a 500 MHz Bruker AM 500 spectrometer referring to TMS. TLC was used for saponin fraction control either on silica 60 F_{254} Merck precoated plates, mobile phase $CHCl_3$:CH_3OH:aqueous HCOOH (1%) = 6:5:1, or on HPTLC RP-8 F_{254} MERCK, mobile phase CH_3OH:aqueous HCOOH (1%) = 65:35, detection by spraying with anisaldehyde-sulfuric acid reagent (saponins give green spots). TLC for analysing the sapogenins was carried out on silica 60 F_{254} Merck precoated plates, mobile phase $CHCl_3$:CH_3OH = 95:5, detection by spraying with anisaldehyde-sulfuric acid reagent (sapogenins give blue-grey spots). Sephadex LH-20 was used for column chromatography under normal pressure and solid phase extraction (SPE) was carried out with cartridges Inchrom Clean-UP C18, 5000 mg. Preparative HPLC was carried out using a Shimadzu preparative HPLC-system on a reversed phase column (LiChrosorb RP-8, 7 µm, 25mm i.d. x 250 mm, detection UV 205 nm or split to evaporative light scattering detector Sedex 75). For carbohydrate analyses a Dionex 4500 HPAEC system was used (Carbo Pac PA-100, 4 x 250 mm, precolumn 4 x 40 mm, detection with PAD, mobile phase gradient 100mM NaOH to 1mM NaOAc in 100mM NaOH). Sugars were also analysed after trimethylsilylation using a Shimadzu QP2010 GCMS-system (capillary column Macherey-Nagel SE-54 CB, 50 m x 0,25 mm i.d., film 0,45 µm, injector 270°C, oven 100-250°, rate 6°/min., mobile phase He 5.0, detector EIMS, 250°C, 70eV).

Plant material

Solidago gigantea was collected in the “Konsumwald”, Auhof, Vienna, Austria in August 1996. Voucher specimen are deposited in the herbarium of the Department of Pharmacognosy, University of Vienna.

Extraction and Isolation

700g of the aboveground parts were dried and grinded and extracted exhaustively 3 times with 3 l 80% methanol. The solvent was evaporated (residue 230g) and 20g of the extract were separated by column chromatography on Sephadex LH-20 using methanol as mobile phase. Further purification was achieved by solid phase extraction (SPE) with cartridges Inchrom Clean-UP C18, 5000 mg, using methanol-water (40:60) and chloroform to purifiy the extract and methanol to elute the saponins. The saponins could be separated by repeated preparative HPLC on reversed phase (RP-8) using isocratic methanol-water (60:40) as mobile phase. The separation of giganteasaponin 5 and 6 could be achieved by repeated preparative TLC on silica 60 with chloroform-methanol-water (6:4:1) as mobile phase. A final purification step with preparative HPLC on RP-8 using again methanol-water (60:40) as mobile phase gave giganteasaponin 5 (17,66 mg) as well as giganteasaponin 6 (15,45 mg) with high purity.

Giganteasaponin 5

3-O- [β-D-Apio-D-furanosyl-(1→3)- β-D-glucopyranosyl-(1→3)- β-D-glucopyranosyl]-28-O {α-L-rhamnopyranosyl-(1→3)- β-D-xylopyranosyl-(1→4)- [β-D-xylopyranosyl-(1→3)]- α-L-rhamnopyranosyl-(1→2)[α-L-rhamnopyranosyl-(1→3)]-β-D-xylopyranosyl}-Bayogenin. White amorphous solid, mp 230–234°C (uncorr.). LSI-MS: m/z 1777 $[M-H]^-$, 1645, 1631, 1499, 1483, 1367, 1321, 943, 833, 811, 701, 649, 555, 487.

Giganteasaponin 6

3-O- [β-D-Apio-D-furanosyl-(1→3)- β-D-glucopyranosyl-(1→3)- β-D-glucopyranosyl]-28-O {β-D-galactopyranosyl-(1→2)- α-L-rhamnopyranosyl-(1→3)- β-D-xylopyranosyl-(1→4)- [β-D-xylopyranosyl-(1→3)]- α-L-rhamnopyranosyl-(1→2) [α-L-rhamnopyranosyl-(1→3)]-β-D-xylopyranosyl}-Bayogenin. White amorphous solid, mp 253–258°C (uncorr.), LSI-MS: m/z 1939 $[M-H]^-$, 1807, 1777, 1645, 1631, 1513, 1499, 1483, 1465, 1367, 995, 943, 863, 811, 717, 701, 649, 555, 487.

Discussion

The main saponins from *Solidago gigantea* [1-5] and *Solidago canadensis* [10-12] are very similar, whereas the saponins from *Solidago virgaurea* have the same aglycone bayogenin but show different structures with less carbohydrates [13]. All saponins from *S. canadensis* have attached a disaccharide to C-3, consisting of two glucoses, in contrast to that the saponins from *S. gigantea* show a trisaccharide with two glucoses and an apiose at this position. Concerning the acylglycosidic sugar moiety at C-28, the saponins from *S. gigantea* and *S. canadensis* are quite similar with six ore seven sugars. Taking a closer look to the saponins from the latter two species, pairs of saponins are found (giganteasaponins 1+3, 2+4, 5+6 respectively canadensissaponins 1+5, 2+6, 3+7 as well as 4+8) with the identical structure exept an additional terminal galactose.

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