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A new acylated and oleanane-type triterpenoid saponin from *Gypsophila arrostii* roots

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ABSTRACT

A new acylated and triterpenoidal saponin, named GS1, was isolated from the roots of *Gypsophila arrostii* Guss. On the basis of acid hydrolysis, comprehensive spectroscopic analyses and comparison with spectral data of known compounds, its structure was established as 3-*O*-*β*-D-xylopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-{21-*O*-[(*E*)-3,4,5trimethoxycinnamoyl]}21-hydroxygypsogenin 28-*O*-*β*-D-glucopyranosyl-(1→2)- [*β*-D-arabinopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→3]-*α*-L-rhamnopyranosyl ester. This article deals with the isolation and structural elucidation of new acylated and oleanane-type saponin.

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Saponin; *Gypsophila arrostii* var. *nebulosa*; HPLC; ESI-TOF-MS; NMR

Introduction

Saponins are secondary metabolites which are widely distributed in the plant kingdom. It acts as a chemical barrier or shield in the plant defense system to counter pathogens and herbivores.[1] The saponin structure, which can easily exceed molecular weights of 1000 Da, is built up of a polar section which is characterized by at least one sugar unit or chain connected to the less polar aglycone (sapogenin) which can be roughly classified as triterpene or steroidal character.[2] Saponins have been ascribed a number of pharmacological actions, the important ones being permeabilizing of the cell membrane, decreasing of serum cholesterol levels, stimulation of luteinizing hormone release leading to abortifacient properties, immunomodulatory potential via cytokine interplay, cytostatic and cytotoxic effects on malignant tumor cells, adjuvant properties for vaccines as immunostimulatory complexes, inhibition of adipogenesis, and synergistic enhancement of the toxicity of immunotoxins.[3–9]

In addition to pharmaceutical applications, saponins have been used in foods as natural surfactant and serve as preservative in controlling microbial spoilage of food. More recently, due to consumer preferance for natural substance, *Quillaja* saponin has been used as a natural small molecule surfactant in beverage emulsions in replacing synthetic surfactant of Tweens.[10] *Gypsophila* species are called as çöven (local name) and widely used in production of tahini halvah in Turkey. Our preliminary studies on *Gypsophila* (Caryophyllaceae) genus led to the isolation of bidesmosidic saponins containing gypsogenin, hederagenin, gypsogenic acid, and quillaic acid as aglycon and a sugar portion made up of four to nine sugar units.[11,12] The aim of this study was the isolation and characterization of the predominant saponin from *Gypsophila arrostii* roots with regard to a comprehensive LC/MS and 2D-NMR analyses including their fragmentation characteristics.

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Material and methods

Plant material

Gypsophila arrostii Guss. plants were harvested from Antalya region in July 2013. The roots were separated from the rest of the plant and used for isolation of saponin fraction. Voucher specimens of *Gypsophila arrostii* were assigned by Prof. Celik and deposited at the Department of Biology (Pamukkale University, Denizli, Turkey; Vouchers ACG-1-3).

Isolation of predominant saponin compound

The cut fresh roots (120 g) were grounded and extracted with 3 L of an MeOH/water (5/3; *v/v*), each step for 2 h at room temperature following filtration through a filter paper placed in a Büchner funnel. The filtrates were collected and MeOH was removed *in vacuo* by rotovaporation (55°C). The remaining syrup was kept at 4°C for 24 h. The suspension was then filtered to remove water-insoluble tannins and other insoluble compounds. Thereafter, the solution was fractionated (5 × 50 mL) and kept in the freezer. In order to hydrolyse crufts like water-soluble tannins each 50 mL fraction was adjusted with 10 mM KOH to pH ~11. The chemical degradation with KOH reduced the matrix complexity of the plant raw extract which facilitated an increased separation efficiency of the *Gypsophila* saponins by high-performance liquid chromatography (HPLC). The solution was swirled 12 h at 40°C and filtered through a Nalgene™ Disposable Filterware (0.45 µm). To remove the low molecular compounds the solution was dialyzed against ultrapure water by using dialysing biomembrane (MWCO 1000) and finally freeze dried. After dissolving in 20% MeOH, the saponins (0.5 mL,~40 mg) were subjected to RP18 (7 µm, 250 × 8 mm) column. Elution was performed with a gradient of MeOH (A) CF_3COOH (TFAA; 0.01%; B) starting with 20% A to 70% A over 60 min. The flow rate was 0.5 mL/min (25°C). The separated saponin, GS1 (14 mg, t_R = 38.5 min) was collected and analyzed by ESI-TOF-MS/MS.

Analytical procedures and equipment

The optical rotations were determined on a Perkin Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10-cm microcell. $^1H/^{13}C$ NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AMX-500 spectrometer. For measuring the $^1H/^{13}C$ NMR analyses, the samples were prepared by dissolving 5.5 mg of GS1 in 500 mL pyridine-d_5. Chemical shifts were referred to the residual solvent signal (pyridine-d_5: δ_H 7.23, 7.58, 8.75; δ_C 123.8, 135.8, 150.3). The assignments were based on double quantum filter-correlation spectroscopy (DQF-COSY), total correlated spectroscopy (TOCSY), heteronuclear single quantum coherence-total correlated spectroscopy (HSQC), and HMBC experiments at 600/150 MHz. The IR spectra were recorded with a Perkin Elmer 100 FT-IR Spectrometer.

Acid hydrolysis and detection of sugar units by gas chromatography/mass spectrometry (GC/MS)

A solution (2.3 mg) of GS1 in 1 N HCl (0.5 mL) was stirred at 80–85°C for 2.0 h. After cooling, the solution was concentrated by blowing with N_2. The residue was dissolved in 1-(trimethylsilyl)-imidazole and pyridine (0.1 mL), and the solution was stirred at 60°C for 5 min. After drying the solution with a stream of N_2, the residue was partitioned between H_2O and CH_2Cl_2 (1 mL, 1:1 v/v). The CH_2Cl_2 layer was analyzed by GC using an l-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200°C for both. A temperature gradient system was used for the oven, starting at 100 C for 1 min and increasing up to 180 C at a rate of 5°C/min. Retention time, D-glucose (16.20 min), L-rhamnose (12.20 min), D-xylose (11.10 min). From GS1 D-glucose, L-rhamnose, D-xylose, were detected.

Table 1. ^{1}H and ^{13}C NMR spectral data for the aglycone, sugar, and acyl moieties of GS1 in pyridine d_5.

	δ ^{1}H (J in Hz)	δ ^{13}C		δ ^{1}H (*J* in Hz)	δ ^{13}C		δ ^{1}H (*J* in Hz)	δ ^{13}C
Aglycone			3-*O*-sugars			28-*O*-sugars		
1	1.40 m/0.80 m	38.0	Glc			Rha		
2	1.90 m/2.14 m	24.7	1	5.20 d (7.0)	104.6	1	5.92 d (1.0)	102.2
3	4.10 m	86.2	2	4.06	80.3	2	4.50 br s	74.2
4	–	56.2	3	4.24	84.2	3	4.90 d	75.0
5	1.48 m	49.7	4	4.50	71.3	4	3.98	72.6
6	1.28 m/1.58 m	20.0	5	4.53	78.0	5	3.98	64.8
7	0.88 m/1.46 m	34.6	6	4.20/1.40	60.6	6	1.46 d (6.0)	16.2
8	–	44.8	Xyl			Xyl″		
9	1.86 m	48.8	1	5.50 d (7.7)	104.2	1	6.05 d (7.0)	103.8
10	–	36.1	2	4.40	73.6	2	5.05	80.2
11	1.86 m/1.92 m	24.5	3	4.20	75.6	3	4.86	82.2
12	5.55 m	122.8	4	4.44	70.7	4	4.55	78.3
13	–	146.8	5	4.41/4.20	69.0	5	3.20/3.98	18.8
14	–	48.2	Xyl′			Glc′		
15	1.88 m/2.20 d	36.2	1	5.31 d (7.7)	105.6	1	5.60 d (7.0)	105.4
16	3.35 m	35.8	2	3.98	70.6	2	4.20	75.0
17	–	52.2	3	4.20	78.4	3	4.10	78.4
18	3.18 br d	42.5	4	4.12	72.0	4	4.02	71.8
19	2.60 t/1.28 m	47.3	5	3.40/4.23	68.8	5	3.98	78.4
20	–	30.2				6	4.20/4.60	76.0
21	5.18 br s	87.0	TMC–α	–	168.0	Ara		
22	2.28 m	30.6	β	6.66 d 16.1	118.0	1	5.62 d (7.7)	104.5
	2.48 m							
23	9.76 s	210.4	γ	7.96 d 16.1	129.6	2	3.96	79.4
24	1.46 s	14.8	1	–	130.2	3	4.12	76.7
25	0.86 s	15.8	2,6	6.85 s	106.0	4	4.14	71.0
26	1.15 s	17.0	3,5	–		5	4.10	74.0
27	1.76 s	26.8	4	–		6	3.50/4.18	67.2
28	–	176.8	OCH_3 at C3	3.88 *s*	56.0			
29	0.93 s	32.8	OCH_3 at C4	3.90 *s*	60.2			
30	0.96 s	24.4	OCH_3 at C5	3.88 *s*	56.2			

Figure 1. Structure of acylated GS1 (MW 1689 da) isolated from *Gypsophila arrostii* roots. compound was ascertained by ^{1}H, ^{13}C and 2D NMR and compared with literature data.[13]

3-*O*-*β*-D-xylopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-{21-*O*-[(*E*)-3,4,5trimethoxycinnamoyl]}21-hydroxygypsogenin 28-*O*-*β*-D-glucopyranosyl-(1→2) – [*β*-D-arabinopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→3]-*α*-L-rhamnopyranosyl ester.

White amorphous powder; $[\alpha]^{20}_{D}$ – 0.21 (*c* 0.0025, distilled water); ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz) data: see Table 1; FT-IR max 3390 cm^{-1} (O–H), 2940cm^{-1} (C–H), 1730cm^{-1} (C–O), 1025cm^{-1} (C–C). ESI-TOF-MS (positive ion mode) $[M+H]^+$ at *m/z* 1690.72.

Results and discussion

A new acylated saponin compound-GS1, an amorphous powder, had a molecular formula of $C_{80}H_{120}O_{38}$ determined by positive ion mode ESI-TOF-MS (at *m/z* 1690.72) $[M+H]^+$ as well as ^{13}C NMR data (Table 1). Its infrared (IR) spectrum featured strong absorbtion at 3390 cm^{-1} relating to the hydroxyl groups. The ESI-TOF-MS spectrum of GS1 showed fragment ion peaks at *m/z* 1557 (M+H-132), 1527 (M+H-162), 1395 (M+H-162-132), which suggested the sequential loss of two pentose and one hexose moiety.

The ^{1}H and ^{13}C NMR spectra of GS1 displayed resonances due to the triterpene part characteristic for gypsogenin, a common aglycone of *Gypsophila* saponins. The *δ* values of C-3 at 86.2 ppm and C-28 at 176.8 ppm, suggested that saponin was a bisdesmosidic glycoside with saccharide units attached to these positions. The sugar portion of separated saponin contained in the ^{1}H NMR spectrum, seven anomeric proton signals at *δ* 5.20 [d, *J* = 7.0 Hz, glucose (Glc)], 5.50 [d, *J* = 7.7 Hz, xylose (Xyl)], 5.31 [d, *J* = 7.7 Hz, xylose (Xyl')], 5.92 [br s, rhamnose (Rha)], 6.05 [d, *J* = 7.0 Hz, xylose (Xyl''], 5.60 [d, *J* = 7.0 Hz, glucose (Glc')], 5.62 [d, *J* = 7.7 Hz, arabinose (Ara)]. Their associated ^{13}C resonances assigned from HSQC and HMBC experiments were at *δ* 104.6, 104.2, 105.6, 102.2, 103.8, 105.4, and 104.5, respectively. On the basis of the assigned protons, the corresponding ^{13}C resonances of the individual monosaccharides were then deduced from the HSQC spectrum. Further supporting information were obtained from the HMBC experiment which also clarified the assignments of some closely related protons and carbons. All the monosaccharides were determined to be in the pyranose form from their ^{13}C NMR data. The anomeric centers of the glucose and xylose units were each determined to have a *β*-configuration based on large $^{3}J_{H\text{-}1,H\text{-}2}$ values.

(a)

C(3) aglycone +H+

detected in low abundance

Xyl (132 Da)

m/*z* 1557

(b)

C(28) aglycone +H+

detected in low abundance

Ara (132 Da)

m/*z* 1557

(c)

R_1 = Xyl - Glc - | Xyl

R_2 = Fuc - | Xyl - Glc | Ara

3C and 28C sugar fragmentation

m/*z* 663

(E)-3,4,4-trimethoxycinnamoyl-TMC fragmentation

m/*z* 470

(d) *sugar neutral loss*

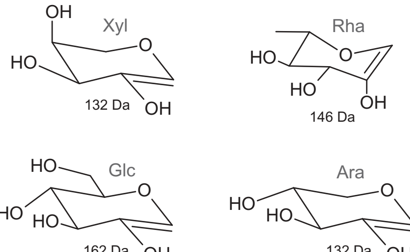

Figure 2. Proposed fragmentation of sugar and acyl groups of GS1 in the positive ion mode; (a) elinination of Xyl moiety (132 Da), (b) fragmentation of Glc moiety (162 Da), (c) 3C and (d) 28C sugar neutral losses (1026 Da) and elimination of trimethoxycinnamoil-TMC group (193 Da).

The large $^{1}J_{H\text{-}1,C\text{-}1}$ values of the Rha (165–168 Hz), confirmed that the anomeric protons were equatorial (α-pyranoid anomeric form). The ^{1}H–^{1}H COSY experiment allowed us to identify the *E*-olefinic protons of a 3,4,5-trimethoxycinnamoyl moiety, which appeared as two doublets at δ_H 7.96 and 6.66 (1H each, J = 16.1 Hz). Further investigations based on the HMBC and HSQC spectra allowed the complete assignments of all protons and carbons of the trisubstituted benzene ring which were in good agreement with those described in literature.[13]

The linkages of the sugar units at gypsogenin C-3 were established from the HMBC correlations between the resonances of Xyl H-1 (*δ* 5.50) and Glc C-3 (*δ* 84.2), Xyl' H-1 (*δ* 5.50) and Xyl C-2 (*δ* 70.6). The HMBC correlation between the resonances of Glc H-1 (*δ* 5.20) and aglycone C-3 (*δ* 86.2) established the attachment of the trisaccharide moiety to C-3 position. Accordingly, the sugar arrangement in the 3-*O*-saccaharide chain was concluded as:

3-*O*-*β*-D-xylopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl (Fig. 1).

GC/MS analysis revealed that GS1 has several sugar units: xylose, glucose, arabinose, and rhamnose. The sugar linkage positions at C-28 were established from the HMBC correlations between the resonances of Ara H-1 (*δ* 5.62) and Xyl''' C-3 (*δ* 82.2). Further correlations between the resonances of Glc H-1 (*δ* 5.60) and Xyl''' C-2 (*δ* 70.2), Xyl''' H-1 (*δ* 6.05) and Rha C-3 (*δ* 70.0) indicated branched sugar arrangement. The correlation between the resonances of the aglycone C-28 (*δ* 176.8) and Rha H-1 (*δ* 5.92) provided definitive evidence for an ester linkage between the tetrasaccharide chain and the aglycone. Therefore, the sugar sequence at C-28 carbonyl group was determined as 28-*O*-*β*-D-glucopyranosyl-(1→2) – [*β*-D-arabinopyranosyl -(1→3)] – *β*-D-xylopyranosyl-(1→3]-*α*-L-rhamnopyranosyl. Similarly, the correlation between the resonances of the aglycone H-21 (*δ* 5.18) and TMCα (*δ* 168.0) provided definitive evidence for an ester linkage between the TMC and aglycone.

The structure of saponin was elucidated as 3-*O*-*β*-D-xylopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-{21-*O*-[(*E*)-3,4,5trimethoxycinnamoyl]}21-hydroxygypsogenin 28-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-arabinopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→3]-*α*-L-rhamnopyranosyl ester. The branched sugar chain connected to C-3 and C-28 of the aglycone consist of seven units, of which xylose and arabinose constitute the terminal parts and are expected to cleave first (Fig. 2). It can be concluded that the 132 Da loss presents the "normal" scission of one of these sugars. As it can be clearly seen in Fig. 2,the acyl group fragmentation was observed both 3-C and 28-C neutral losses of sugar units. This findings imply a substantial stability of this acyl group within MS fragmentation in the positive ion mode. The linking points for sugar moieties to the triterpenoid aglycone in the family of Caryophyllaceae usually are at C-3 and C-28. The most of saponins isolated from Caryophyllaceae family have gypsogenin, quillaic acid, gypsogenic acid, and hederagenin as aglyconic backbone[8] and our findings are compatible with this data.

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References

1. Augustin, J.M.; Kuzina, V.; Anderson, S.B.; Bak, S. Molecular Activities, Biosynthesis and Evolution of Triterpenoid Saponins. Phytochemistry **2011**, *72*, 435–457.
2. Vincken, P.L.; Heng, L.; Groot, A.; Gruppen, A. Saponins, Classification and Occurence in The Plant Kingdom. Phytochemistry **2007**, *68*, 275–297.
3. Lee, H.; Kang, R.; Kim, Y.S.; Chung, S.I.; Yoon, Y. Platycodin D İnhibits Adipogenesis of 3T3-L1 Cells by Modulating Kruppel-Like Factor 2 and Peroxisome Proliferator-Activated Receptor Gamma. Phytotherapy Research **2010**, *2*, 161–167.
4. Sun, H.X.; Xie, Y.; Ye, Y.P. Advances in Saponin-Based Adjuvants. Vaccine **2009**, *27*, 1787–1796.
5. Bachran, C.; Bachran, S.; Sutherland, M.; Bachran, D.; Fuchs, H. Saponins in Tumor Therapy. Mini Review Medicinal Chemistry **2008**, *8*, 575–584.
6. Francis, G.; Kerem, Z.; Makkar, H.P.; Becker, K. The Biological Action of Saponins in Animal Systems: A Review. British Journal of Nutrition **2002**, *88*, 587–605.
7. Arslan, I.; Ili, P. Genotoxicological Assessment of Nebuloside a A Triterpenoid Saponin Compound on Whole Blood DNA. International Journal of Food Properties **2015**, *18*(11): 2374–2379.
8. Hostettmann, K.; Marston, A.; *Saponins*, Vol 1. 1st ed.; Cambridge University Press: Cambridge, MA, 1995.
9. Rui, L.; Baojun, Xu. Inhibitory Effects of Phenolics and Saponins From Commonly Consumed Food Legumes in China Against Digestive Enzymes Pancreatic Lipase and alpha-Glycosidase. International Journal of Food Properties **2015**, *18*(10), 2246–2255.
10. Piorkowski, D.T.; McClements, D.J. Beverage Emulsions: Recent Developments in Formulation, Production, and Applications. Food Hydrocolloids **2014**, *42*, 5–41.
11. Arslan, I. Isolation and Structure Elucidation of Hederagenin a Triterpenoid Compound from *Gypsophila Simonii*. Research Journal of Chemistry and Environment **2013**, *17*, 105–107.
12. Arslan, I.; Celik, A.; Melzig, M.F.; Nebulosides A-B, Novel Saponins from Gypsophila Arrostii Var.Nebulosa. Bioorganic & Medicinal Chemistry **2013**, *21*(2), 1279–1283.
13. Elbandy, M.; Miyamoto, T.; Delaude, C.; Lacaille-Dubois, M.A. New Acylated Presenegenin Saponins from Two Species of Muraltia. Helvetica Chimica Acta **2004**, *87*, 340–348.