FULL PAPER

Highly Polar Triterpenoid Saponins from the Roots of *Saponaria officinalis* L.

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Five new triterpenoid saponins, including 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**1**), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-(6-*O*-acetyl)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**2**), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**3**), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**4**), 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-(6-*O*-acetyl)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (**5**) together with two known congeners, saponariosides A (**6**) and B (**7**) were isolated from the roots of *Saponaria officinalis* L. Their structures were elucidated by extensive spectroscopic methods, including 1D- (^{1}H, ^{13}C) and 2D-NMR (DQF-COSY, TOCSY, HSQC, and HMBC) experiments, HR-ESI-MS, and acid hydrolysis.

Keywords: *Saponaria officinalis*, Triterpenoid saponins, Quillaic acid.

Introduction

Saponaria officinalis L., commonly named soapwort or bouncing-bet, is a perennial plant belonging to the family Caryophyllaceae. Its native range extends throughout Europe, northern Africa, and west to central Asia, but currently it is also cultivated in many countries across the world [1 – 3]. Roots of *S. officinalis* are a rich source of saponins [4] that consist of a hydrophobic triterpenoidal C_{30} backbone and two hydrophilic glycoside moieties attached to the backbone. Due to this structural composition, these saponins are amphiphilic glycoconjugates which give soap-like foams in water [5] [6]. Therapeutically, *S. officinalis* has been used as an expectorant in bronchitis, and it is still used for skin complaints and in rheumatic disorders [7]. Saponins from *S. officinalis* exhibit a wide variety of biological activities like spermicidal, hemolytic, cytotoxic, immunogenic, anti-inflammatory, anticholesterolemic, anticancer, molluscicidal, and antifungal. Saponins similar in structure to these found in *S. officinalis* are used as adjuvants in vaccines [8 – 13].

Saponins from *S. officinalis* can be divided into a polar and nonpolar fraction. The known saponins are mainly nonpolar [1]. There is some confusion about type of aglycone present in *S. officinalis.* So far it was thought, that one of the aglycones, besides gipsogenic and quillaic acid, is gipsogenin, like in the whole Caryophyllaceae family [14]. But in recent studies it has not been found [1 – 3][15]. *Bötger* and *Melzig* in their investigation about triterpenoid saponins in Caryophyllaceae and Illecebraceae family demonstrated that the occurrence of gypsogenin is 1.5-fold higher in Caryophyllaceae family than that of gypsogenic or quillaic acid, which occur with the same frequency. In contrast, all examined species of the Illecebraceae family were found not to contain gypsogenin [14]. Therefore, *S. officinalis* could belong to the family Illecebraceae.

Thus, this work has been carried out with the aim to deeply investigate saponins occurring in the roots of *S. officinalis* and confirm the absence of gypsogenin as an aglycone.

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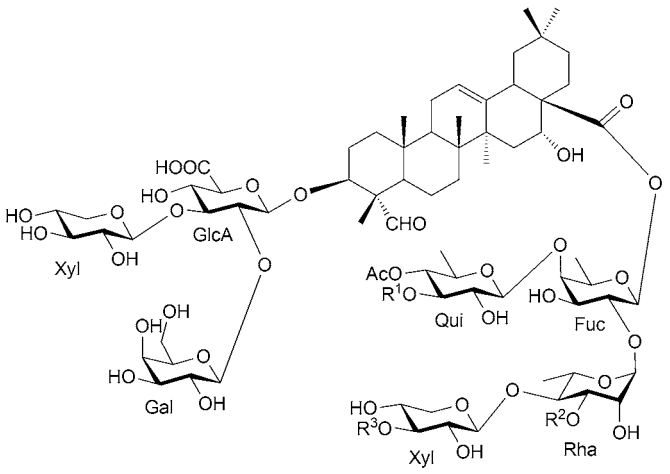
Results and Discussion

A 70% MeOH extract of the dry roots of *S. officinalis* was subjected to successive chromatographic steps like vacuum–liquid chromatography (VLC), liquid–liquid extraction with AcOEt and BuOH, and high-pressure liquid chromatography (HPLC) on hydrophilic interaction liquid chromatography (HILIC) column to provide seven saponins **1** – **7** (*Fig.*). Their structures were elucidated by extensive spectroscopic methods including 1D-(^{1}H and ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC, and TOCSY) experiments as well as ESI-MS analysis.

Compound **1**, obtained as an amorphous solid, exhibited in the HR-ESI-MS, the $[M - H]^-$ peak at *m/z* 1861.7736 consistent with the molecular formula $C_{83}H_{129}O_{46}$. Its negative-ion-mode ESI-MS displayed a *quasi*molecular ion peak at *m/z* 1861 $[M - H]^-$. A fragment ion peak was observed at *m/z* 955 $[M-H-162-146-146-146-132-132-42]^-$, corresponding to the loss of a hexosyl moiety, three deoxyhexosyl moieties, two pentosyl moieties, and an acetyl moiety, respectively.

The ^{13}C-NMR spectrum showed 83 C-atom signals, of which 30 were assigned to the aglycone moiety, 51 to the oligosaccharide moieties, and the remaining two to an acetyl group (*Tables 1* and *2*). The six sp^3 hybrid C-atom signals at δ(C) 11.0, 15.6, 16.9, 24.8, 26.8, 33.1, and the two sp^2 hybrid C-atom signals at δ(C) 122.1 and 144.7 together with the information from ^{1}H-NMR analysis (six Me H-atom *singlets* at δ(H) 0.77, 0.90, 0.94, 1.00, 1.40, 1.77, and a broad *triplet* CH_2=CH H-atom at δ(H) 5.53 (*t*, *J* = 3.2)) indicated that the aglycone possesses an olean-12-ene skeleton [16 – 18]. Moreover, the ^{1}H-NMR spectrum displayed signals at δ 9.85 (*s*) ascribable to the CHO function, at δ 4.05 (*dd*, *J* = 11.5, 4.2, H–C(3)) and at 5.12 (br. *s*, H–C(16)), corresponding to two O-bearing CH H-atoms. The CHO function was assigned to C(23) on the basis of the HMBC correlations between the proton value at δ 9.85 and the carbon resonances at δ 84.3 C(3), 55.3 C(4), 48.5 C(5), and 11.0 C(24). The HMBC correlations between the H-atom resonances at δ 3.34 H–C(18) and 5.12 H–C(16) and the carbon resonance at δ 176.3 allowed us to assign the COOH function to C(28). The multiplicity of H–C(16) which resonated as a broad *singlet*, indicated that OH–C(16) was α-oriented [19]. Detailed NMR analysis identified the aglycone as quillaic acid [1]. The high-frequency shifts of the carbinol function at δ(C) 84.3 C(3) and of the COOH function at δ(C) 176.3 C(28) showed the bisdesmosidic structure of **1**. The observation in the ^{1}H-NMR spectrum of anomeric signals at δ(H) 4.83 (*d*, *J* = 7.5), 4.96 (*d*, *J* = 7.5), 4.98 (*d*, *J* = 7.0), 5.08 (*d*, *J* = 7.5), 5.15 (*d*, *J* = 7.5), 5.31 (*d*, *J* = 7.5), 5.49 (*d*, *J* = 7.5), 5.91 (*d*, *J* = 7.6), and 6.25 (*d*, *J* = 1.2) correlating with signals at δ(C) 103.8, 107.0, 105.6, 106.3, 105.6, 105.0, 104.0, 94.6, and 101.0 in the HSQC spectrum, respectively, suggested that this compound possesses nine sugar moieties.

The chemical shifts of the individual H-atoms of the sugar units were established from the combination of 1D-TOCSY and COSY experiments, and ^{13}C-chemical shifts of their relative carbons were assigned on the basis of the HSQC data. Thus, one β-glucuronopyranosyl (δ(H) 4.83), three β-xylopyranosyls (δ(H) 4.96, 5.08, and 5.31), one β-quinovopyranosyl (δ(H) 4.98), one β-glucopyranosyl (δ(H) 5.15), one β-galactopyranosyl (δ(H) 5.49), one β-fucopyranosyl (δ(H) 5.91), and one α-rhamnopyranosyl (δ(H) 6.25) units were identified. In the rhamnose unit, the high H–C(1)/C(1) *J* = 169 Hz and three-bond strong HMBC correlations from the anomeric H-atom to C(3) and C(5), indicated that the anomeric H-atom was equatorial, thus possessed an α-configuration [20]. In the quinovose unit, an acetyl group attached to C(4) was evident for the downfield shift of H–C(4) (δ(H) 5.06, *dd*, *J* = 9.0, 9.0) due to the acetylation effect. The determination of the sequence and linkage sites of the sugar units at C(3) was obtained from the HMBC correlations between the



	R^1	R^2	R^3
1	Xyl	H	Glc
2	Xyl	(6-O-Ac)-Glc	H
3	Xyl	H	H
4	H	H	Glc
5	H	(6-O-Ac)-Glc	H
6	Xyl	Xyl	Xyl
7	H	Xyl	Xyl

Figure. Structures of compounds **1** – **5**.

Table 1. ^{1}H- and ^{13}C-NMR data for the aglycone moieties of compound **1** (600 MHz, in (D_5)pyridine[a]), δ in ppm, J in Hz.

Position	**1**	
	δ(H)	δ(C)
1	1.33 – 1.37 (*m*), 0.79 – 0.83 (*m*)	38.0
2	2.11 – 2.16 (*m*), 1.83 – 1.78 (*m*)	24.9
3	4.05 (*dd*, *J* = 11.5, 4.2)	84.3
4	–	55.3
5	1.32 – 1.36 (*m*)	48.5
6	1.33 – 1.37 (*m*), 1.01 – 1.05 (*m*)	20.4
7	1.52 – 1.56 (*m*, 2 H)	32.8
8	–	40.4
9	1.73 – 1.78 (*m*)	46.6
10	–	36.4
11	1.87 – 1.91 (*m*), 0.92 – 0.96 (*m*)	23.5
12	5.53 (br. *t*, *J* = 3.2)	122.1
13	–	144.7
14	–	42.3
15	2.11 – 2.15 (*m*), 1.94 – 1.97 (*m*)	36.2
16	5.12 (br. *s*)	74.0
17	–	49.4
18	3.34 (*dd*, *J* = 3.0, 13.5)	41.3
19	2.67 – 2.72 (*m*), 1.25 – 1.28 (*m*)	47.2
20	–	30.8
21	2.35 – 2.38 (*m*), 1.24 – 1.28 (*m*)	35.7
22	2.28 – 2.33 (*m*), 2.08 – 2.12 (*m*)	31.8
23	9.85 (*s*)	210.6
24	1.40 (*s*)	11.0
25	0.77 (*s*)	15.6
26	1.00 (*s*)	16.9
27	1.71 (*s*)	26.8
28	–	176.3
29	0.90 (*s*)	33.1
30	0.94 (*s*)	24.8

[a]) The chemical shift values of the aglycone moiety of **2** – **5** were superimposable to those of **1**. The assignments were based on 1D-TOCSY, COSY, 2D-TOCSY, HSQC, and HMBC spectra.

H-atom signal at δ(H) 5.49 H–C(1_{gal}) and the carbon resonance at δ(C) 78.5 C(2_{glcA}), the signal at δ(H) 5.31 H–C(1_{xyl}) with the carbon resonance at δ(C) 86.0 C(3_{glcA}), the H-atom signal at δ(H) 4.83 H-C(1_{glcA}) and the carbon resonance at δ(C) 84.3 C(3). In turn, the sugar chain at C(28) was established from the following HMBC correlations: the H-atom signal at δ(H) 5.15 H–C(1_{glc}) with the carbon resonance at δ(C) 88.1 C($3_{xyl'}$), the H-atom signal at δ(H) 5.08 H–C($1_{xyl'}$) with the carbon resonance at δ(C) 84.0 C(4_{rham}), the H-atom signal at δ(H) 6.25 H–C(1_{rham}) with the carbon resonance at δ(C) 74.0 C(2_{fuc}), the H-atom signal at δ(H) 4.96 H–C($1_{xyl''}$) and the carbon resonance at δ(C) 84.7 C(3_{qui}), and the H-atom signal at δ(H) 4.98 H–C(1_{qui}) with the carbon resonance at δ(C) 83.3 C(4_{fuc}).

The D-configuration of glucose, galactose, glucuronic acid, xylose, fucose, and quinovose units and the L-configuration of rhamnose unit were established after hydrolysis of **1** with 3M HCl and conversion into thiazolizine derivatives to arylthiocarbamate using L-cysteine methyl ester and *o*-tolylisothiocyanate and determination of retention time by HPLC using the method of *Tanaka et al.* [21] with slight modifications.

From the above evidences, the structure of compound **1** was elucidated as 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside.

Compound **2**, obtained as an amorphous solid, exhibited in the HR-ESI-MS, the $[M - H]^-$ peak at m/z 1903.7861 consistent with the molecular formula $C_{85}H_{131}O_{47}$. Its negative-ion-mode ESI-MS displayed a quasimolecular ion peak at m/z 1903 $[M - H]^-$. A fragment ion peak was observed at m/z 955 $[M-H-162-146-146-146-132-132-42-42]^-$, corresponding to the loss of a hexosyl moiety, three deoxyhexosyl moieties, two pentosyl moieties, and two acetyl moieties, respectively.

The ^{13}C-NMR spectrum of **2** showed 85 C-atom signals, of which 30 were due to the aglycone moiety and 51 to a saccharide portion made up of nine sugar units and the remaining to two acetyl groups (*Tables 1* and *2*). The comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1** showed that they possess the same aglycone. Differences were observable in the saccharide moieties. For the sugar portion, compound **2** showed signals corresponding to nine anomeric H-atoms at δ(H) 4.81 (*d*, *J* = 7.5), 4.98 (*d*, *J* = 7.5), 5.01 (*d*, *J* = 7.0), 5.23 (*d*, *J* = 7.5), 5.33 (*d*, *J* = 7.5), 5.44 (*d*, *J* = 7.5), 5.49 (*d*, *J* = 7.5), 5.87 (*d*, *J* = 7.6), and 6.21 (*d*, *J* = 1.2) correlating with signals at δ(C) 103.7, 106.6, 105.5, 105.2, 104.9, 104.7, 104.1, 94.6, and 101.6 in HSQC spectrum. The structure elucidation of the sugar units was achieved by extensive 1D- (^{1}H, ^{13}C, TOCSY)- and 2D- (HSQC, HMBC)-NMR analyses. These data showed the presence of nine sugar moieties: *β*-glucuronopyranosyl (δ(H) 4.81), three *β*-xylopyranosyls (δ(H) 4.98, 5.33, 5.44), one *β*-quinovopyranosyl (δ(H) 5.01), one *β*-glucopyranosyl (δ(H) 5.23), one *β*-galactopyranosyl (δ(H) 5.49), one *β*-fucopyranosyl (δ(H) 5.87), and one *α*-rhamnopyranosyl (δ(H) 6.21). The sequence of the oligosaccharidic chain at C(3) of **2** was determined by the HMBC spectrum, which showed the same correlations observable for compound **1** between the H-atom values at δ(H) 5.49 H–C(1_{gal}) and δ(C) 78.4 C(2_{glcA}), δ(H) 5.33 H–C(1_{xyl}) and δ(C) 86.2 C(3_{glcA}), the H-atom signal at δ(H) 4.81 H–C(1_{glcA}) and the carbon resonance at δ(C) 84.3 C(3). The sugar chain at C(28) was established from the following HMBC correlation: δ(H) 5.23 H–C(1_{glc}) and δ(C) 83.0 C(3_{rham}), δ(H) 5.44 H–C($1_{xyl'}$) and δ(C) 78.0 C(4_{rham}), δ(H) 6.21 H–C(1_{rham}) and δ(C) 73.5 C(2_{fuc}), δ(H) 4.98 H–C($1_{xyl''}$) and δ(C) 84.6 C(3_{qui}), and δ(H) 5.01 H–C(1_{qui}) and δ(C) 83.0 C(4_{fuc}). Attachment of the two acetyl groups to C(6) of glucose and C(4) of quinovose was confirmed from HMBC correlations between both H–C(6) (δ(H) 4.92 and 4.69) of glucose and H–C(4) (δ(H) 5.09) of quinovose with the C=O carbons of the two acetyl groups (δ(C) 171.3 and 170.5), respectively. Consequently, the structure

Table 2. ^{1}H- and ^{13}C-NMR data for the sugar moieties of compounds **1** – **5** (600 MHz, in (D_5)pyridine[a]), δ in ppm, J in Hz

Position	**1**		**2**		**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
3-*O*-Sugars										
GlcA-1	4.76 (*d*, *J* = 7.5)	103.4	4.83 (*d*, *J* = 7.5)	103.5	4.83 (*d*, *J* = 7.5)	103.7	4.81 (*d*, *J* = 7.5)	103.7	4.83 (*d*, *J* = 7.5)	103.8
2	4.29 (*dd*, *J* = 7.5, 9.0)	78.6	4.30 (*dd*, *J* = 7.5, 9.0)	78.4	4.32 (*dd*, *J* = 7.5, 9.0)	78.4	4.30 (*dd*, *J* = 7.5, 9.0)	78.4	4.30 (*dd*, *J* = 7.5, 9.0)	78.5
3	4.29 (*dd*, *J* = 9.0, 9.0)	85.4	4.26 (*dd*, *J* = 9.0, 9.0)	86.0	4.26 (*dd*, *J* = 9.0, 9.0)	86.0	4.25 (*dd*, *J* = 9.0, 9.0)	86.2	4.26 (*dd*, *J* = 9.0, 9.0)	86.0
4	4.48 (*dd*, *J* = 9.0, 9.0)	70.3	4.38 (*dd*, *J* = 9.0, 9.0)	71.5	4.38 (*dd*, *J* = 9.0, 9.0)	71.7	4.45 (*dd*, *J* = 9.0, 9.0)	71.5	4.36 (*dd*, *J* = 9.0, 9.0)	71.8
5	4.28 (*d*, *J* = 9.0)	78.2	4.30 (*d*, *J* = 9.0)	78.2	4.26 (*d*, *J* = 9.0)	78.5	4.36 (*d*, *J* = 9.0)	77.6	4.28 (*d*, *J* = 9.0)	78.5
6		172.0		172.0		172.0		172.0		172.0
Gal-1	5.46 (*d*, *J* = 7.5)	104.2	5.50 (*d*, *J* = 7.5)	103.8	5.51 (*d*, *J* = 7.5)	104.2	5.49 (*d*, *J* = 7.5)	104.1	5.49 (*d*, *J* = 7.5)	104.0
2	4.42 (*dd*, *J* = 7.5, 9.0)	73.4	4.44 (*dd*, *J* = 7.5, 9.0)	73.6	4.46 (*dd*, *J* = 7.5, 9.0)	73.9	4.42 (*dd*, *J* = 7.5, 9.0)	73.7	4.42 (*dd*, *J* = 7.5, 9.0)	73.7
3	4.09 (*dd*, *J* = 4.0, 9.0)	75.3	4.10 (*dd*, *J* = 4.0, 9.0)	75.2	4.14 (*dd*, *J* = 4.0, 9.0)	75.6	4.12 (*dd*, *J* = 4.0, 9.0)	75.2	4.09 (*dd*, *J* = 4.0, 9.0)	75.5
4	4.50 (*dd*, *J* = 2.5, 4.0)	70.1	4.53 (*dd*, *J* = 2.5, 4.0)	70.0	4.53 (*dd*, *J* = 2.5, 4.0)	70.3	4.52 (*dd*, *J* = 2.5, 4.0)	70.1	4.51 (*dd*, *J* = 2.5, 4.0)	70.3
5	3.92 – 3.97 (*m*)	76.5	3.95 – 3.99 (*m*)	76.0	4.00 – 4.04 (*m*)	76.7	4.04 – 4.08 (*m*)	75.2	3.98 – 4.02 (*m*)	76.4
6	4.44 (*dd*, *J* = 4.5, 12.0)	61.7	4.48 (*dd*, *J* = 4.5, 12.0)	61.7	4.48 (*dd*, *J* = 4.5, 12.0)	62.0	4.42 (*dd*, *J* = 4.5, 12.0)	61.8	4.46 (*dd*, *J* = 4.5, 12.0)	61.8
	4.40 (*dd*, *J* = 2.0, 12.0)		4.39 (*dd*, *J* = 2.0, 12.0)		4.42 (*dd*, *J* = 2.0, 12.0)		4.48 (*dd*, *J* = 2.0, 12.0)		4.38 (*dd*, *J* = 2.0, 12.0)	
Xyl-1	5.38 (*d*, *J* = 7.5)	104.6	5.32 (*d*, *J* = 7.5)	104.7	5.32 (*d*, *J* = 7.5)	104.9	5.33 (*d*, *J* = 7.5)	104.9	5.31 (*d*, *J* = 7.5)	105.0
2	3.98 (*dd*, *J* = 9.2, 7.5)	75.2	3.95 (*dd*, *J* = 9.2, 7.5)	75.0	3.97 (*dd*, *J* = 9.2, 7.5)	75.5	4.01 (*dd*, *J* = 9.2, 7.5)	75.8	3.97 (*dd*, *J* = 9.2, 7.5)	75.3
3	4.11 (*t*, *J* = 9.2)	78.2	4.10 (*t*, *J* = 9.2)	78.5	4.11 (*t*, *J* = 9.2)	78.4	4.11 (*t*, *J* = 9.2)	78.3	4.10 (*t*, *J* = 9.2)	78.3
4	4.11 – 4.16 (*m*)	71.0	4.10 – 4.15 (*m*)	71.0	4.09 – 4.13 (*m*)	71.1	4.09 – 4.13 (*m*)	71.0	4.08 – 4.12 (*m*)	71.2
5	4.29 (*dd*, *J* = 11.7, 5.2)	67.2	4.24 (*dd*, *J* = 11.7, 5.2)	66.8	4.27 (*dd*, *J* = 11.7, 5.2)	67.4	4.27 (*dd*, *J* = 11.7, 5.2)	67.3	4.26 (*dd*, *J* = 11.7, 5.2)	67.6
	3.68 (*t*, *J* = 11.7)		3.64 (*t*, *J* = 11.7)		3.65 (*t*, *J* = 11.7)		3.68 (*t*, *J* = 11.7)		3.65 (*t*, *J* = 11.7)	
28-*O*-Sugars										
Fuc-1	5.91 (*d*, *J* = 7.6)	94.6	5.87 (*d*, *J* = 7.6)	94.6	5.89 (*d*, *J* = 7.6)	94.6	5.92 (*d*, *J* = 7.6)	94.2	5.88 (*d*, *J* = 7.6)	94.5
2	4.50 (*dd*, *J* = 7.6, 8.5)	74.0	4.51 (*dd*, *J* = 7.6, 8.5)	73.5	4.52 (*dd*, *J* = 7.6, 8.5)	73.7	4.55 (*dd*, *J* = 7.6, 8.5)	73.9	4.59 (*dd*, *J* = 7.6, 8.5)	73.5
3	4.15 (*dd*, *J* = 8.5, 2.5)	76.6	4.21 (*dd*, *J* = 8.5, 2.5)	76.4	4.17 (*dd*, *J* = 8.5, 2.5)	76.6	4.16 (*dd*, *J* = 8.5, 2.5)	76.7	4.21 (*dd*, *J* = 8.5, 2.5)	76.4
4	3.94 (*d*, *J* = 2.5)	83.3	4.01 (*d*, *J* = 2.5)	83.0	3.95 (*d*, *J* = 2.5)	83.2	3.96 (*d*, *J* = 2.5)	83.9	4.01 (*d*, *J* = 2.5)	84.2
5	3.90 – 3.95 (*m*)	71.5	3.92 – 3.97 (*m*)	71.6	3.91 – 3.95 (*m*)	71.3	3.91 – 3.95 (*m*)	71.5	4.01 – 3.97 (*m*)	71.9
6	1.46 (*d*, *J* = 6.5)	17.5	1.48 (*d*, *J* = 6.5)	17.6	1.47 (*d*, *J* = 6.5)	17.2	1.50 (*d*, *J* = 6.5)	17.9	1.55 (*d*, *J* = 6.5)	17.0
Rham-1	6.25 (*d*, *J* = 1.2)	101.0	6.21 (*d*, *J* = 1.2)	100.6	6.32 (*d*, *J* = 1.2)	100.8	6.32 (*d*, *J* = 1.2)	100.9	6.32 (*d*, *J* = 1.2)	100.6
2	4.68 (*dd*, *J* = 1.2, 3.2)	71.8	4.93 (*dd*, *J* = 1.2, 3.2)	70.9	4.69 (*dd*, *J* = 1.2, 3.2)	71.9	4.73 (*dd*, *J* = 1.2, 3.2)	71.6	4.95 (*dd*, *J* = 1.2, 3.2)	71.3
3	4.58 (*dd*, *J* = 3.2, 9.3)	72.2	4.63 (*dd*, *J* = 3.2, 9.3)	83.0	4.62 (*dd*, *J* = 3.2, 9.3)	72.5	4.62 (*dd*, *J* = 3.2, 9.3)	72.4	4.62 (*dd*, *J* = 3.2, 9.3)	82.9
4	4.28 (*t*, *J* = 9.3)	84.0	4.47 (*t*, *J* = 9.3)	78.0	4.36 (*t*, *J* = 9.3)	82.5	4.34 (*t*, *J* = 9.3)	83.6	4.52 (*t*, *J* = 9.3)	78.2
5	4.33 – 4.37 (*m*)	68.2	4.49 – 4.47 (*m*)	68.4	4.35 – 4.39 (*m*)	68.2	4.39 – 4.43 (*m*)	68.2	4.52 – 4.55 (*m*)	68.2
6	1.53 (*d*, *J* = 6.5)	18.6	1.56 (*d*, *J* = 6.5)	18.3	1.56 (*d*, *J* = 6.5)	18.3	1.57 (*d*, *J* = 6.5)	18.2	1.65 (*d*, *J* = 6.5)	18.9
Xyl′-1	5.08 (*d*, *J* = 7.5)	106.3	5.44 (*d*, *J* = 7.5)	104.7	5.19 (*d*, *J* = 7.5)	106.2	5.11 (*d*, *J* = 7.5)	105.9	5.18 (*d*, *J* = 7.5)	103.6
2	3.97 (*dd*, *J* = 9.2, 7.5)	75.3	3.90 (*dd*, *J* = 9.2, 7.5)	75.8	3.95 (*dd*, *J* = 9.2, 7.5)	75.5	3.98 (*dd*, *J* = 9.2, 7.5)	75.2	3.90 (*dd*, *J* = 9.2, 7.5)	75.7
3	3.97 (*t*, *J* = 9.2)	88.1	4.11 (*t*, *J* = 9.2)	78.3	4.11 (*t*, *J* = 9.2)	78.0	4.00 (*t*, *J* = 9.2)	88.0	4.11 (*t*, *J* = 9.2)	78.2
4	3.96 – 3.99 (*m*)	69.3	4.10 – 4.13 (*m*)	71.0	4.06 – 4.10 (*m*)	71.0	3.99 – 4.03 (*m*)	69.0	4.08 – 4.12 (*m*)	71.2
5	4.09 (*dd*, *J* = 11.7, 5.2)	66.7	4.11 (*dd*, *J* = 11.7, 5.2)	67.0	4.12 (*dd*, *J* = 11.7, 5.2)	67.2	4.12 (*dd*, *J* = 11.7, 5.2)	66.8	4.14 (*dd*, *J* = 11.7, 5.2)	66.9
	3.36 (*t*, *J* = 11.7)		3.36 (*t*, *J* = 11.7)		3.40 (*t*, *J* = 11.7)		3.38 (*t*, *J* = 11.7)		3.37 (*t*, *J* = 11.7)	
Glc-1	5.15 (*d*, *J* = 7.5)	105.6	5.23 (*d*, *J* = 7.5)	105.2			5.17 (*d*, *J* = 7.5)	105.4	5.20 (*d*, *J* = 7.5)	105.3
2	4.08 (*dd*, *J* = 7.5, 9.0)	75.6	3.98 (*dd*, *J* = 7.5, 9.0)	75.2			4.09 (*dd*, *J* = 7.5, 9.0)	75.6	3.98 (*dd*, *J* = 7.5, 9.0)	75.2
3	4.18 (*t*, *J* = 9.0)	78.4	4.11 (*t*, *J* = 9.0)	78.3			4.19 (*t*, *J* = 9.0)	78.1	4.11 (*t*, *J* = 9.0)	78.2
4	4.14 (*t*, *J* = 9.0)	71.3	3.93 (*t*, *J* = 9.0)	71.2			4.11 (*t*, *J* = 9.0)	71.0	3.90 (*t*, *J* = 9.0)	71.5
5	3.95 – 3.91 (*m*)	78.7	4.05 – 4.09 (*m*)	78.3			3.91 – 3.96 (*m*)	78.3	4.06 – 4.10 (*m*)	78.2
6	4.46 (*dd*, *J* = 3.5, 12)	62.5	4.92 (*dd*, *J* = 3.5, 12)	64.3			4.48 (*dd*, *J* = 3.5, 12)	62.4	4.90 (*dd*, *J* = 3.5, 12.0)	64.3
	4.23 (*dd*, *J* = 4.5, 12)		4.69 (*dd*, *J* = 4.5, 12)				4.23 (*dd*, *J* = 4.5, 12)		4.64 (*dd*, *J* = 4.5, 12.0)	

Table 2. (cont.)

Position	**1**		**2**		**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
6-Me*CO*				171.3						170.8
6-*Me*CO			2.10 (*s*)	21.0					2.01 (*s*)	21.0
Qui-1	4.98 (*d*, *J* = 7.0)	105.6	5.01 (*d*, *J* = 7.0)	105.5	4.99 (*d*, *J* = 7.0)	105.5	4.98 (*d*, *J* = 7.0)	106.0	4.99 (*d*, *J* = 7.0)	106.0
2	3.96 (*dd*, *J* = 7.0, 9.0)	75.3	3.99 (*dd*, *J* = 7.0, 9.0)	75.2	3.98 (*dd*, *J* = 7.0, 9.0)	75.2	3.99 (*dd*, *J* = 7.0, 9.0)	75.3	4.02 (*dd*, *J* = 7.0, 9.0)	75.6
3	4.06 (*dd*, *J* = 9.0, 9.0)	84.7	4.05 (*dd*, *J* = 9.0, 9.0)	84.6	4.08 (*dd*, *J* = 9.0, 9.0)	84.2	3.97 (*dd*, *J* = 9.0, 9.0)	75.6	3.97 (*dd*, *J* = 9.0, 9.0)	75.4
4	5.06 (*dd*, *J* = 9.0, 9.0)	74.4	5.09 (*dd*, *J* = 9.0, 9.0)	74.1	5.07 (*dd*, *J* = 9.0, 9.0)	74.2	5.13 (*dd*, *J* = 9.0, 9.0)	76.6	5.13 (*dd*, *J* = 9.0, 9.0)	76.8
5	3.65 – 3.69 (*m*)	70.5	3.64 – 3.68 (*m*)	70.2	3.64 – 3.68 (*m*)	70.5	3.62 – 3.66 (*m*)	70.6	3.63 – 3.66 (*m*)	70.4
6	1.24 (*d*, *J* = 6.5)	18.2	1.24 (*d*, *J* = 6.5)	17.8	1.24 (*d*, *J* = 6.5)	17.8	1.24 (*d*, *J* = 6.5)	18.7	1.26 (*d*, *J* = 6.5)	17.9
4-Me*CO*		170.5		170.5		170.5		170.7		171.3
4-*Me*CO	2.14 (*s*)	21.1	2.14 (*s*)	20.9	2.14 (*s*)	21.3	2.01 (*s*)	20.7	2.12 (*s*)	21.1
Xyl″-1	4.96 (*d*, *J* = 7.5)	107.0	4.98 (*d*, *J* = 7.5)	106.6	4.97 (*d*, *J* = 7.5)	106.8				
2	3.90 (*dd*, *J* = 9.2, 7.5)	75.9	3.90 (*dd*, *J* = 9.2, 7.5)	75.8	3.91 (*dd*, *J* = 9.2, 7.5)	75.8				
3	4.09 (*t*, *J* = 9.2)	78.3	4.11 (*t*, *J* = 9.2)	78.3	4.11 (*t*, *J* = 9.2)	78.4				
4	4.09 – 4.13 (*m*)	71.2	4.09 – 4.13 (*m*)	71.0	4.10 – 4.13 (*m*)	71.0				
5	4.27 (*dd*, *J* = 11.7, 5.2)	67.6	3.68 (*dd*, *J* = 11.7, 5.2)	67.3	4.27 (*dd*, *J* = 11.7, 5.2)	67.4				
	3.65 (*t*, *J* = 11.7)		4.27 (*t*, *J* = 11.7)		3.65 (*t*, *J* = 11.7)					

[a]) The assignments were based on 1D-TOCSY, COSY, 2D-TOCSY, HSQC, and HMBC spectra.

of compound **2** was established as 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-(6-*O*-acetyl)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside.

Compound **3** in its HR-ESI-MS, gave the $[M - H]^-$ peak at *m*/*z* 1699.7213 consistent with the molecular formula $C_{77}H_{119}O_{41}$. Fragment-ion peak was observed at *m*/*z* 955 $[M-H-146-146-146-132-132-42]^-$. The overall structure assignment was accomplished using the same protocol as in **1** and **2**. Its ^{1}H- and ^{13}C-NMR data revealed that **3** was a bisdesmosidic glycoside with the same aglycone (quillaic acid) as that in **1** and **2** and that it contained eight sugars and one acetyl group. For the sugar portion, compound **3** showed signals corresponding to eight anomeric H-atoms at δ(H) 4.83 (*d*, *J* = 7.5), 4.97 (*d*, *J* = 7.5), 4.99 (*d*, *J* = 7.0), 5.19 (*d*, *J* = 7.5), 5.32 (*d*, *J* = 7.5), 5.51 (*d*, *J* = 7.5), 5.89 (*d*, *J* = 7.6), and 6.32 (*d*, *J* = 1.2) correlating with signals at δ(C) 103.7, 106.8, 105.5, 106.2, 104.9, 104.2, 94.6, and 100.8 in HSQC spectrum. The chemical shifts of the individual H-atoms of the sugar units were established from the combination of 1D-TOCSY and COSY experiments, and ^{13}C-chemical shifts of their relative carbons were assigned on the basis of the HSQC data. Thus, one *β*-glucuronopyranosyl (δ(H) 4.83), three *β*-xylopyranosyls (δ(H) 4.97, 5.19, 5.32), one *β*-quinovopyranosyl (δ(H) 4.97), one *β*-galactopyranosyl (δ(H) 5.51), one *β*-fucopyranosyl (δ(H) 5.89), and one α-rhamnopyranosyl (δ(H) 6.32) units were identified. Compound **3** showed at C(3) the same the trisaccharide chain observed as in **1** and **2**. The remaining five sugars at C (28) were established from the following HMBC correlations: δ(H) 5.19 H–C($1_{xyl'}$) and δ(C) 82.5 C(4_{rham}), δ(H) 6.32 H–C(1_{rham}) and δ(C) 73.7 C(2_{fuc}), δ(H) 4.97 H–C ($1_{xyl''}$) and δ(C) 84.2 C(3_{qui}), δ(H) 4.99 H–C(1_{qui}) and δ (C) 83.2 C(4_{fuc}). Attachment of an acetyl group to C(4) of quinovose was confirmed from the HMBC correlations between H–C(4) (δ(H) 5.07) of quinovose with the C=O carbon of the acetyl group (δ(C) 170.5). On the basis of all the foregoing evidence, compound **3** was elucidated as 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic acid 28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside.

Compound **4**, $C_{78}H_{121}O_{42}$ (HR-ESI-MS $[M - H]^-$ at *m*/*z* 1729.7335), exhibited a fragment-ion peak at *m*/*z* 955 $[M–H–162–146–146–146–132–42]^-$. Of the 78 carbons observed in the ^{13}C-NMR spectrum, 30 were assigned to the aglycone part, 46 to the oligosaccharide moiety, and the remaining two to an acetyl group. ^{1}H- and ^{13}C-NMR data revealed that **4** was a bisdesmosidic glycoside with the same aglycone (quillaic acid) as in **1** – **3** and that it contained eight sugars. For the sugar portion, compound **4** showed signals corresponding to eight anomeric H-atoms at δ(H) 4.83 (*d*, *J* = 7.5), 4.98 (*d*, *J* = 7.5), 5.11

(*d*, *J* = 7.5), 5.17 (*d*, *J* = 7.5), 5.32 (*d*, *J* = 7.5), 5.50 (*d*, *J* = 7.5), 5.92 (*d*, *J* = 7.6), and 6.32 (*d*, *J* = 1.2) correlating with signals at δ(C) 103.5, 106.0, 105.9, 105.4, 104.7, 103.8, 94.2, and 100.9 in HSQC spectrum. The chemical shifts of the individual H-atoms of the sugar units were established from the combination of 1D-TOCSY and COSY experiments, and ^{13}C-chemical shifts of their relative carbons were assigned on the basis of the HSQC data. Thus, one β-glucuronopyranosyl (δ(H) 4.83), two β-xylopyranosyls (δ(H) 5.11 and 5.32), one β-quinovopyranosyl (δ(H) 4.98), one β-glucopyranosyl (δ(H) 5.17), one β-galactopyranosyl (δ(H) 5.50), one β-fucopyranosyl (δ(H) 5.92), and one α-rhamnopyranosyl (δ(H) 6.32) units were identified. Compound **4** showed at C(3) the same trisaccharide chain observed in **1** – **3**. The remaining five sugars at C(28) was established from the following HMBC correlation: δ(H) 5.17 H–C(1_{glc}) and δ(C) 88.0 C($3_{xyl'}$), δ(H) 5.11 H–C($1_{xyl'}$) and δ(C) 83.6 C(4_{rham}), δ(H) 6.32 H–C(1_{rham}) and δ(C) 73.9 C(2_{fuc}), δ(H) 4.98 H–C(1_{qui}) and δ(C) 83.9 C(4_{fuc}). Attachment of an acetyl group to C(4) of quinovose was confirmed from the HMBC correlations between H–C(4) (δ(H) 5.13) of quinovose with the C=O carbons of the acetyl group (δ(C) 170.7). On the basis of all the foregoing evidences, compound **4** was elucidated as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranoside.

Compound **5**, obtained as an amorphous solid, exhibited in its HR-ESI-MS, the $[M - H]^-$ peak at *m*/*z* 1771.7433 consistent with the molecular formula $C_{80}H_{123}O_{43}$. Fragment-ion peaks were observed at *m*/*z* 955 $[M-H-162-146-146-146-132-44-42-42]^-$, corresponding to the loss of the hexosyl moiety, three deoxyhexosyl moieties, one pentosyl moiety, and two acetyl groups, respectively.

Comparison of ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) of **5** with those of **1** – **4** indicated that **5** had the same aglycone. The differences were observable in the saccharide moieties which contained eight sugars and two acetyl groups. Structure elucidation of sugar portions was achieved by 1D-TOCSY, DQF-COSY, HSQC, and HMBC experiments. For the sugar portion, compound **5** showed signals corresponding to eight anomeric protons at δ(H) 4.76 (*d*, *J* = 7.5), 4.99 (*d*, *J* = 7.0), 5.18 (*d*, *J* = 7.5), 5.20 (*d*, *J* = 7.5), 5.38 (*d*, *J* = 7.5), 5.46 (*d*, *J* = 7.5), 5.88 (*d*, *J* = 7.6), and 6.32 (*d*, *J* = 1.2) correlating with signals at δ(C) 103.4, 106.0, 103.6, 105.3, 104.6, 104.2, 94.5, and 100.6 in the HSQC spectrum. These data showed the presence of one β-glucuronopyranosyl (δ(H) 4.76), one β-quinovopyranosyl (δ(H) 4.99), two β-xylopyranosyl (δ(H) 5.18 and 5.38), β-glucopyranosyl (δ(H) 5.23), one β-galactopyranosyl (δ(H) 5.46), one β-fucopyranosyl (δ(H) 5.88), and one α-rhamnopyranosyl (δ(H) 6.32) units. Compound **5** also contained at C(3) the same trisaccharide unit as in **1** – **4**. The remaining five sugars at C(28) were established from the following HMBC correlations: δ(H) 5.20 H–C(1_{glc}) and δ(C) 82.9 C(3_{rham}), δ(H) 5.18 H–C($1_{xyl'}$) and δ(C) 78.2 C(4_{rham}), δ(H) 6.32 H–C(1_{rham}) and δ(C) 73.5 C(2_{fuc}), δ(H) 4.99 H–C(1_{qui}) and δ(C) 84.2 C(4_{fuc}). Attachment of the two acetyl groups to C(6) of glucose and C(4) of quinovose was confirmed from HMBC correlations between both H–C(6) (δ(H) 4.90 and 4.64) of glucose and H–C(4) (δ(H) 5.13) of quinovose with the C=O carbons of the two acetyl groups (δ(C) 170.8 and 171.3), respectively. Thus, the structure of **5** was established as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl quillaic acid 28-*O*-(6-*O*-acetyl)-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranoside.

Furthermore, two known saponins were isolated and identified as saponariosides A (**6**) and B (**7**) by comparing their spectroscopic data to previously published data [1].

This study highlights the presence of new highly polar triterpenoid saponins (**1** – **5**) from the roots of *Saponaria officinalis*, along with saponariosides A (**6**) and B (**7**), previously isolated in this plant. All these compounds show the same aglycone, quillaic acid, confirming the absence of gypsogenin as aglycone, a phytochemical marker for the Caryophyllaceae family. As above discussed on the basis of these results, it could be argued that *S. officinalis* belongs to Illecebraceae family.

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Experimental Part

General

Vacuum liquid chromatography (VLC): *C18* column (100 × 60 mm, *LiChroprep RP-18*, 40 – 63 μm, *Merck KGaA*, Darmstadt, Germany). Semiprep. HPLC: *ZIC®-HILIC* column (10 × 250 mm, 5 μm, *Merck KGaA*) on *Gilson* semipreparative chromatograph equipped with *Evaporative Light Scattering – PrepELS II* (ELSD) detector (*Gilson Inc.*, Middleton, WI, USA). The drift tube temperature was maintained at 65° and the pressure of the nebulizer gas, N_2, was 47 psi (*Gilson Inc.*). The outgoing effluent from HPLC system was diverted through passive splitter to ELSD with a split ratio 1:100. ESI-MS/MS: *Thermo Finnigan LCQ Advantage Max* mass spectrometer (*Thermo Electron Corp.*, San Jose, CA, USA). HR-ESI-MS (pos.): *Maldi SYNAPT G2-S HDMS Qq-TOF* (*Waters Corp*) (Waters, Milford, MA, USA) coupled with *ACQUITY UPLC I-Class System* (*Waters Corp.*) (Waters, Milford, MA, USA). NMR Spectra: the 1D- and 2D-NMR

spectra (^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, TOCSY, HSQC, and HMBC) were performed on a *Bruker DRX-600* spectrometer (*Bruker BioSpin GmBH*, Rheinstetten, Germany) equipped with a *Bruker* 5 mm *TCI CryoProbe* at 300 K in (D_5)pyridine (99.96%, *Sigma-Aldrich*, St. Louis, MI, USA); δ in ppm rel. to Me_4Si as internal standard, J in Hz; UXNMR software.

Plant Material

Dry, ground roots of *Saponaria officinalis* L. were purchased from commercial source (Herbapol in Cracow, Poland). A voucher specimen (IUNG/SOL/2010/1) has been deposited at the Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation, State Research Institute, Pulawy.

Extraction and Isolation

The milled roots of *S. officinalis* (500 g) were defatted with $CHCl_3$/hexane 1:1 in a *Soxhlet* apparatus. Defatted and dried material (497 g) was extracted three times with 70% MeOH (2 l × 3) for 24 h at room temperature. The combined extracts were concentrated under reduced pressure at 40 °C and lyophilized to give a crude extract (233 g). The extract was suspended in dist. H_2O and applied to a VLC RP-18 column preconditioned with water. Sugars and phenolics were removed with H_2O and 30% MeOH, respectively. Saponins were eluted with 90% MeOH to give 49.9 g of crude saponin fraction, which was partitioned between AcOEt and H_2O. Next, the H_2O-soluble part of saponin fraction (45.4 g) was partitioned between H_2O and BuOH (sat. with H_2O). The partition divided saponins according to the polarity obtaining 29.52 g of H_2O-soluble polar saponin fraction. A 50 mg portion of polar saponin fraction was separated by semipreparative HPLC (5 mM $AcONH_4$/MeCN with 0.1% 5 mM $AcONH_4$: 0 – 1.5 min, 15:85; 1.5 – 15.1 min, 15:85 – 18:82; 15.1 – 21 min, 18:82; 21 – 30 min, 15:85, flow rate 5 ml/min) to yield **1** (t_R 21.5 min; 10 mg), **2** (t_R 20.2 min; 4.8 mg), **3** (t_R 15.7 min; 3.1 mg), **4** (t_R 16.8; 8.3 mg), **5** (t_R.12.7 min; 5.2 mg), **6** (t_R 18.3; 7.4 mg), and **7** (t_R 14.5; 6.5 mg).

3-*O*-*β*-D-Galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl quillaic Acid 28-*O*-*β*-D-Glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (=**O**-4-*O*-**Acetyl-3-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-quinovopyranosyl-(1→4)-*O*-[*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-1-*O*-[(3*β*,16α)-3-[[*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranuronosyl]oxy]-16-hydroxy-23,28-dioxoolean-12-en-28-yl]-*β*-D-fucopyranose**; **1**). White amorphous solid; $C_{83}H_{130}O_{46}$; $[\alpha]_D^{25}$ = +18.5 (c = 0.1 MeOH); IR ν^{KBr}_{max} cm^{-1}: 3430 (> OH), 2925 (> CH), 1665 (C=O), 1650 (C=C), 1265 and 1050 (C–O–C); for ^{1}H- and ^{13}C-NMR ((D_5)pyridine, 600 MHz) data of the aglycone moiety and the sugar portion see *Tables 1* and *2*, respectively; HR-ESI-MS $[M - H]^-$ m/z 1861.7736 ($C_{83}H_{129}O_{46}^-$, 1861.7755).

3-*O*-*β*-D-Galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl Quillaic Acid 28-*O*-(6-*O*-Acetyl)-*β*-D-Glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (= ***O*-6-*O*-Acetyl-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-xylopyranosyl-(1→3)-4-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)]-1-*O*-[(3*β*,16α)-3-[[*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranuronosyl]oxy]-16-hydroxy-23,28-dioxoolean-12-en-28-yl]-*β*-D-fucopyranose**; **2**). White amorphous solid; $C_{85}H_{132}O_{47}$; $[\alpha]_D^{25}$ = +15.1 (c = 0.1 MeOH); IR ν^{KBr}_{max} cm^{-1}: 3420 (> OH), 2920 (> CH), 1670 (C=O), 1650 (C=C), 1255 and 1055 (C–O–C); ^{1}H-NMR and ^{13}C-NMR ((D_5)pyridine, 600 MHz) data of the aglycone moiety are superimposable with those reported for **1**; for ^{1}H- and ^{13}C-NMR ((D_5)pyridine, 600 MHz) data of the sugar portion see *Table 2*; HR-ESI-MS $[M - H]^-$ 1903.7861 ($C_{85}H_{131}O_{47}^-$, 1903.7861).

3-*O*-*β*-D-Galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl Quillaic Acid 28-*O*-*β*-D-Xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (= ***O*-*β*-D-Xylopyranosyl-(1→3)-*O*-4-*O*-acetyl-*β*-D-quinovopyranosyl-(1→4)-*O*-[*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-1-*O*-[(3*β*,16α)-3-[[*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranuronosyl]oxy]-16-hydroxy-23,28-dioxoolean-12-en-28-yl]-*β*-D-fucopyranose**; **3**). White amorphous solid; $C_{77}H_{120}O_{41}$; $[\alpha]_D^{25}$ = +23.0 (c = 0.1 MeOH); IR ν^{KBr}_{max} cm^{-1}: 3425 (> OH), 2930 (> CH), 1660 (C=C), 1255 and 1030 (C–O–C); ^{1}H-NMR and ^{13}C-NMR ((D_5)pyridine, 600 MHz) data of the aglycone moiety are superimposable with those reported for **1**; for ^{1}H- and ^{13}C-NMR ((D_5)pyridine, 600 MHz) data of the sugar portion see *Table 2*; HR-ESI-MS $[M - H]^-$ 1699.7213 ($C_{77}H_{119}O_{41}^-$, 1699.7227).

3-*O*-*β*-D-Galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl Quillaic Acid 28-*O*-*β*-D-Glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-*β*-D-quinovopyranosyl-(1→4)]-*β*-D-fucopyranoside (= ***O*-4-*O*-Acetyl-*β*-D-quinovopyranosyl-(1→4)-*O*-[*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-1-*O*-[(3*β*,16α)-3-[[*O*-*β*-D-galactopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranuronosyl]oxy]-16-hydroxy-23,28-dioxoolean-12-en-28-yl]-*β*-D-fucopyranose**; **4**). White amorphous solid; $C_{78}H_{122}O_{42}$; $[\alpha]_D^{25}$ = +13.4 (c = 0.1 MeOH); IR ν^{KBr}_{max} cm^{-1}: 3425 (> OH), 2925 (> CH), 1670 (C=O), 1656 (C=C), 1260 and 1050 (C–O–C); ^{1}H-NMR and ^{13}C-NMR ((D_5)pyridine, 600 MHz) data of the aglycone

moiety are superimposable with those reported for **1**; for ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine, 600 MHz) data of the sugar portion see *Table 2*; HR-ESI-MS $[M - H]^-$ 1729.7335 ($C_{78}H_{121}O_{42}^-$, 1729.7332).

3-O-β-D-Galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl Quillaic Acid 28-*O*-(6-*O*-Acetyl)-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranoside (= ***O*-4-*O*-Acetyl-β-D-quinovopyranosyl-(1→4)-*O*-[*O*-6-*O*-acetyl-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)]-1-*O*-[(3β,16α)-3-[[*O*-β-D-galactopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranuronosyl]oxy]-16-hydroxy-23,28-dioxoolean-12-en-28-yl]-β-D-fucopyranose**; **5**). White amorphous solid; $C_{80}H_{124}O_{43}$; $[\alpha]_D^{25}$ = +14.7 (*c* = 0.1 MeOH); IR ν^{KBr}_{max} cm^{-1}: 3420 (> OH), 2930 (> CH), 1660 (C=C), 1615 (C=C), 1260 and 1030 (C–O–C); ^{1}H-NMR and ^{13}C-NMR ((D$_5$) pyridine, 600 MHz) data of the aglycone moiety are superimposable with those reported for **1**; for ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine, 600 MHz) data of the sugar portion see *Table 2*; HR-ESI-MS $[M - H]^-$ 1771.7433 ($C_{80}H_{123}O_{43}^-$, 1771.7438).

Acid Hydrolysis of Compounds and Determination of Absolute Configuration of Monosaccharides

Each compound (2.5 mg) was hydrolyzed in 1.5 ml solution of 3M HCl (dioxane/H$_2$O 1:2) at 90 °C for 5 h. After dioxane was removed from the hydrolysis reaction mixture under a stream of nitrogen, the aglycones were extracted with AcOEt (3 × 1 ml) and dried. The aqueous layer containing sugars was neutralized using an *Amberlite IRA-400* (*Sigma-Aldrich*) ion-exchange resin in its hydroxide form and concentrated under the stream of nitrogen at 60 °C and after this used for sugar determination. To determine the absolute configuration of monosaccharide ingredients of isolated compounds, the method of *Tanaka et al.* [21] with little modifications was used. Sugars of each sample were dissolved in pyridine (0.5 ml) to which 1 mg of L-cysteine methyl ester hydrochloride was added. The mixture was kept at 60 °C for 1 h. 0.2 µl of *o*-tolyl isothiocyanate was added to the mixture, which was heated at 60 °C for 1 h. Each reaction mixture was directly analyzed by reversed-phase HPLC using a *616-Pump, 966-Photodiode Array detector 717-plus Autosampler* (*Waters Corp.*) and an *Eurospher-100 C18* (250 × 4 mm; 5 µm *Knauer GmbH*, Berlin, Germany) column. MeCN/H$_2$O 25:75 containing 50 mM H_3PO_4 was used as mobile phase at a flow rate of 0.8 ml/min and column temperature to 35 °C. Detection was performed in UV at 250 nm. The absolute configuration was determined by comparing the retention times of sugars with derivatives prepared in a similar way from standard monosaccharides (*Sigma-Aldrich*).

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