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TRITERPENOID SAPONINS FROM *ASTER BELLIDIASTRUM*

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Key Word Index—*Aster bellidiastrum*; Asteraceae; triterpenoid saponins; bellidiastroside C_2; bellissaponins; besysaponin C_{12}; polygalacic acid; chemotaxonomy.

Abstract—Seven triterpenoid saponins were isolated from both the aerial and the underground parts of *Aster bellidiastrum*. Five of the compounds were common to both parts. The structures were elucidated mainly from their NMR and mass spectral data, and showed significant similarity to the corresponding data for the saponins from plants of the *Bellis* genus. Two of the saponins are novel compounds with the structures 3-*O*-*β*-D-xylopyranosyl-2*β*,3*β*,23-trihydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl(1 → 6)-*β*-D-glucopyranoside and 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,16*α*,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-*α*-L-arabinofuranosyl(1 → 3)-[*α*-L-rhamnopyranosyl(1 → 2)]-*β*-D-fucopyranoside.

INTRODUCTION

Aster bellidiastrum (L.) Scop. is a small perennial herb possessing an outward appearance which is more similar to species of the genus *Bellis* than other asters. In a previous paper [1], we reported the isolation and structural elucidation of the major deacylsaponins obtained from the aerial parts. As a continuation of these studies, we have obtained three further glycosides from the aerial parts and seven compounds from the underground parts of the plants.

Since the structures of saponins of *A. bellidiastrum* show significant similarities to those of the genus *Bellis* [2–8], a strategy of comparison of chromatographic and spectroscopic data, as well as application of mass spectral and two-dimensional (2D) NMR methods, was used for the identification of the compounds. The strategy can be summarized as follows:

1. TLC and HPLC comparison with compounds obtained from plants of the *Bellis* genus and from aerial parts of *A. bellidiastrum*;
2. GC analysis of the pertrimethylsilyl derivatives of the methylglycosides obtained by methanolysis of the saponin to identify the sugar components;
3. electrospray ionization-mass spectrometry (ESI-MS) of underivatized material to establish the molecular mass and MS-MS to determine sugar chains from the fragmentation pattern;
4. one-dimensional (1D) ^1H and ^{13}C NMR spectroscopy and comparison of the data obtained with those obtained previously for saponins
5. 2D NMR experiments (COSY, HMQC, HMBC) to confirm the molecular fragments and to finally establish their sequence.

Absolute configurations of the sugars were either determined by GC of the pertrimethylsilylated L-cysteine methyl ester derivatives, which were prepared according to Hara *et al.* [9], or the common D configuration was assumed for glucose, and xylose and the L configuration assumed for rhamnose.

RESULTS AND DISCUSSION

Column chromatographic and HPLC fractionation of the mild alkaline hydrolysate of the saponin mixture obtained from the aerial and underground parts of *A. bellidiastrum* afforded seven compounds from each part of the plants. TLC and HPLC comparison indicated that five of the compounds were present in both plant parts (compounds **1**–**5**), while two of them were obtained from the aerial parts (compounds **6** and **7**) and two from the underground parts (compounds **8** and **9**) only. Comparison of the ^{13}C NMR spectra confirmed the identity of **1**–**4** as the major deacylsaponins described previously [1].

TLC comparison of **5** with saponins obtained from

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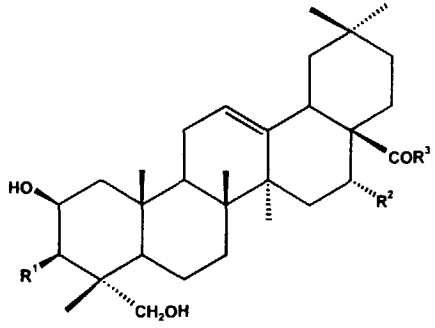
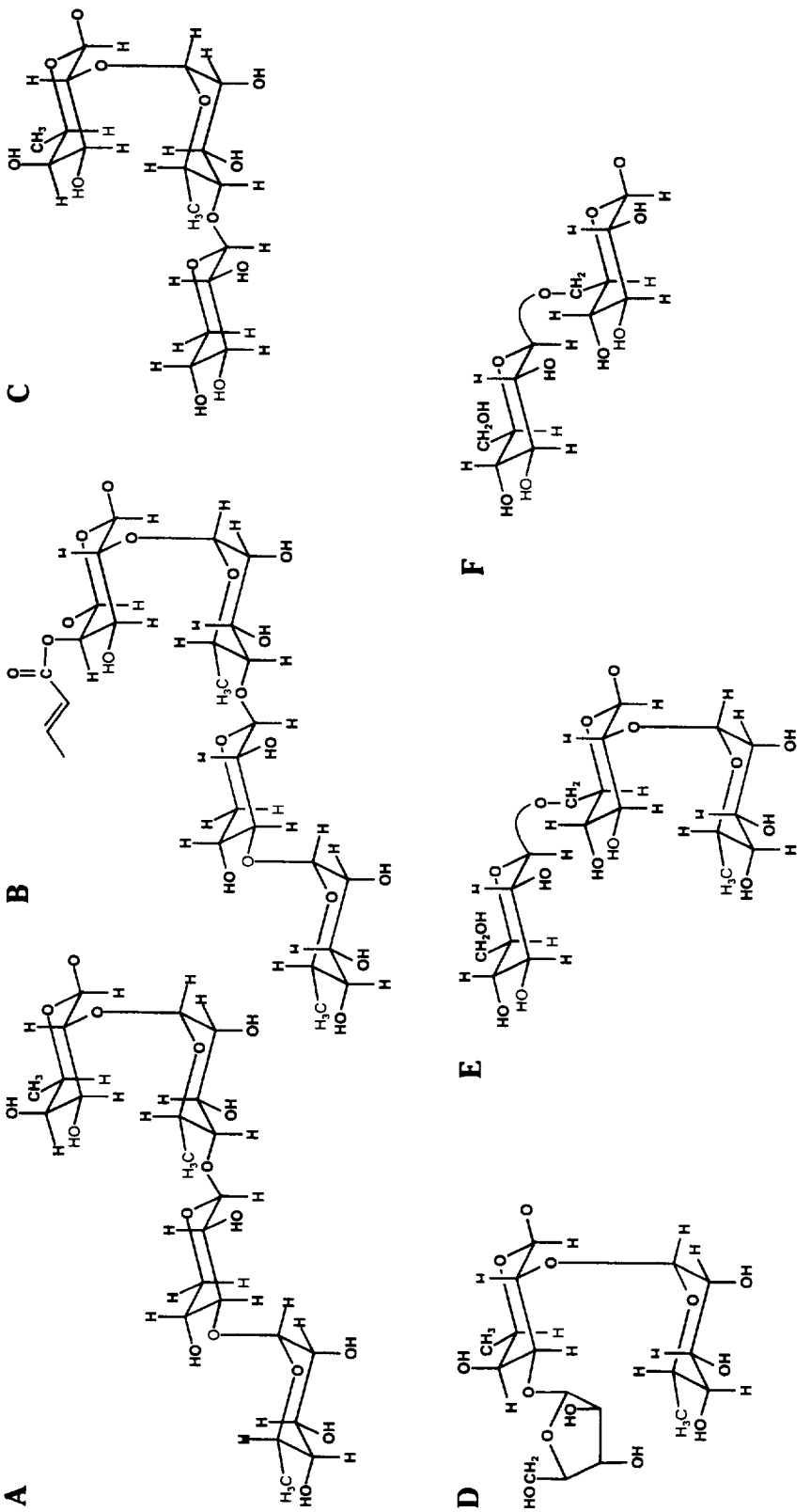


Compound	R^1	R^2	R^3	Name
1	α-L-Rha	OH	C	**Besysaponin C_{12}**
2	α-L-Rha	OH	A	**Bellissaponin BS1**
3	β-D-Glc	OH	C	**Bellidiastroside C_2**
4	β-D-Glc	OH	A	**Bellissaponin BS2**
5	α-L-Rha	OH	B	**Bellissaponin BA_1**
6	β-D-Glc	H	D	**Bellidiastroside B_3**
7	β-D-Glc	H	E	**Bellissaponin BS6**
8	α-L-Rha	OH	OH	**Polygalacic acid 3-O-rhamnoside**
9	β-D-Xyl	H	F	**Bellidiastroside U_{D2}**


A
B
C
D
E
F

Bellis spp. indicated similarity with bellissaponin BA_1 [3]. This was further supported by the ^1H NMR spectrum, which showed signals of a crotonic acid moiety (δ 7.10, *dd*; 6.05, *dd*; 1.97, *dd*; J(H-2, H-3) = 15.5 Hz, J(H-2, H-4) = 1.7 Hz, J(H-3, H-4) = 6.9 Hz) and ESI-MS which gave a molecular ion at *m/z* 1311 $[M + Na]^+$. MS-MS investigation of this ion afforded an intense signal of a daughter ion at *m/z* 661, corresponding to the acylglycosidically-bound carbohydrate moiety, [dhex + dhex + dhex + pent + crotonic acid $- H_2O + Na]^+$ [7, 8]. Full agreement of the ^{13}C NMR data with those of saponins obtained from *Bellis* spp. confirmed that **5** is bellissaponin BA_1 [3]. A similar comprehensive analysis of **7** indicated that this compound corresponds to BS6 from *Bellis perennis* [5].

The structures of **6**, **8** and **9** have not been found by us previously and were established as follows. Carbohydrate component analysis identified one unit of L-arabinose, L-rhamnose, D-fucose and D-glucose in **6**, L-rhamnose in **8**, and D-glucose and D-xylose in **9**. ESI-MS of **6** gave a molecular ion at *m/z* 1097 $[M + Na]^+$, which on MS-MS afforded an intense daughter ion at 447. These data indicate that **6** is a bisdesmoside possessing a trisaccharide (dhex + dhex + pent) bound acylglycosidically and has a hexosyltrihydroxyolean-12-enoic acid as the prosapogenin moiety. Assignment of the ^1H and ^{13}C NMR data (see Tables 1 and 2) on the basis of the COSY-45, HMQC and HMBC spectra showed that bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid) is the aglycone and that a furanoid arabinose is present. Cross-peaks between H-1 of glucose (δ 4.46) and C-3 of the aglycone (δ 83.9) and between C-1 of glucose (δ 105.5) and H-3 of the aglycone (δ 3.66) confirmed that glucose is bound at C-3 to the aglycone. The sequence of the acylglycosidic sugar chain was also derived from the HMBC spectrum. Thus, cross-peaks were observed between H-1 of arabinose (δ 5.16) and C-3 of fucose (δ 83.4), and H-1 of rhamnose (δ 5.13) and C-2 of fucose (δ 74.5), and the reverse cross-peaks between C-1 of arabinose (δ 111.6) and C-3 of fucose (δ 3.81), and C-1 of rhamnose (δ 102.6) and H-2 of fucose (δ 3.89). A cross-peak between H-1 of fucose (δ 5.47) and C-28 of the aglycone (δ 178.0) confirmed that the trisaccharide is bound acylglycosidically. The H_1,H_2 coupling constants of 7.9 and 7.8 Hz indicate that glucose and fucose occur as the β-anomers in $^4C_1$ configurations. The appearance of H-1 of arabinose as a broad singlet showed that arabinose occurs as an α-anomer. A J(C-1, H-1) value of 170 Hz and a J(H-4, H-5) value of about 9 Hz clearly established the presence of rhamnose as the α-anomer in the $^1C_4$ configuration [10]. Hence, **6** is 3-*O*-β-D-glucopyranosyl-2β,3β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-α-L-arabinofuranosyl(1 → 3)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-fucopyranoside.

ESI-MS of **8** showed a molecular ion at *m/z* 673 $[M + Na]^+$. Hence only one molecule of rhamnose is present and the aglycone corresponds to a molecular weight of 504, from which the appearance of H-19A at δ 2.30 (*t*, J = 13.5 Hz) in the ^1H NMR spectrum must be polygalacic acid. Comparison of the ^{13}C NMR data for rings A–C showed full agreement with those of the bisdesmosides of polygalacic acid possessing 3-*O*-rhamnosylation. Differences regarding C-16, C-18 and C-22 are in agreement with the data for 28-unsubstituted polygalacic acid [11], although the carboxyl signal could not be detected in the ^{13}C spectrum. On the assumption that rhamnose is present as the α-L-enantiomer, then **8** is 3-*O*-α-L-rhamnopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid.

ESI MS-MS of the molecular ion of **9** at *m/z* 967 yielded an intense daughter ion at *m/z* 347 [hex + hex $- H_2O + Na]^+$, which is indicative of a bisdesmosidic saponin consisting of a diglucoside bound acylglycosidically and an olean-12-enoic acid xyloside as prosapogenin moiety. Comparison of the ^{13}C NMR data showed agreement of the aglycone signals with those of the glycosides of bayogenin obtained from *Bellis perennis* [4]. A downfield shift of C-6 of one glucose moiety to δ 69.5 clearly indicated a 1,6-linkage of this glucose, and all sugars are bound in their β-configurations from the vicinal H-1,H-2 coupling constants of about 8 Hz. Hence, **9** is 3-*O*-β-D-xylopyranosyl-2β,3β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside.

In our previous paper [1] we showed that, from a chemical point of view, *Aster bellidiastrum* is more closely related to *Bellis* than to *Aster*. This conclusion was based on the detection of identical major deacylsaponins and their relative concentrations in the two species. Here we have identified the partly deacylated bellissaponin BA_1 (**5**), which provides evidence that these similarities may be extended to include the genuine acylated saponins.

Thus, to generalize, the composition of the deacylsaponins in all species of the *Bellis* genus is characterized by the presence of bellissaponins BS1 (**2**), BS2 (**4**), besysaponin C_{12} (**1**) and bellidiastroside C_2 (**3**) in typical concentrations [2, 6, 8, 12, 13]. These saponins are 3-*O*-rhamnosides or 3-*O*-glucosides of polygalacic acid. The sugar chain bound acylglycosidically at C-28 consists of an α-L-rhamnopyranosyl(1 → 3)-β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranoside, which in some compounds may be shortened to β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranoside. Besides the glycosides of polygalacic acid, *Bellis* spp. often contain glycosides of bayogenin as minor components [5, 8, 12, 13]. These possess other carbohydrate units and, in contrast to glycosides of polygalacic acid, glucose is the prevalent monosaccharide constituent, usually bound acylglycosidically as a β-D-glucopyranosyl(1→6)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-glucopyranoside [2, 13] or a β-D-glucopyranosyl(1 → 3)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-glucopyranoside [12]. Thus, the isolation and structural elucidation of bellissaponin BS6 (**7**) and of **9** from *A. bellidiastrum* are of

Table 1. ^{1}H NMR data of **6** in CD_3OD

Aglycone	δ	Sugars	δ
H-1A	2.10	Arabinose	H-1 5.16 (*bs*)
H-1B	1.22		H-2 4.16 (*dd*, J = 1.2/3.1 Hz)
H-2	4.36 (*dd*, J = 3.5/4.5 Hz)		H-3 3.89
H-3	3.66 (*d*, J = 3.5 Hz)		H-4 4.12 (*ddd*, J = 3.5/5.0/9.0 Hz)
H-5	1.34		H-5A 3.78
H-6A	*ca.* 1.3		H-5B 3.67
H-6B	*ca.* 1.3	Rhamnose	H-1 5.13 (*d*, J = 1.6 Hz)
H-7A	1.43		H-2 3.98 (*dd*, J = 1.6/3.2 Hz)
H-7B	1.43		H-3 3.71
H-9	1.61		H-4 3.42
H-11A	2.05		H-5 3.76 (*dd*, J = *ca.* 6/9 Hz)
H-11B	1.95		H_3-6 1.26 (*d*, J = 6.4 Hz)
H-12	5.31 (*t*, J = 3.5 Hz)	Fucose	H-1 5.47 (*d*, J = 7.9 Hz)
H-15A	1.76		H-2 3.89
H-15B	1.18		H-3 3.81
H-16A	2.10		H-4 3.90
H-16B	1.70		H-5 3.78
H-18	2.88 (*dd*, J = 4.1/13.7 Hz)		H_3-6 1.26 (*d*, J = 6.1 Hz)
H-19A	1.76	Glucose	H-1 5.46 (*d*, J = 7.8 Hz)
H-19B	1.18		H-2 3.31
H-21A	1.45		H-3 3.40
H-21B	1.27		H-4 3.41
H-22A	1.87		H-5 3.34
H-22B	1.60		H-6A 3.84
H-23A	3.65 (*d*, J = 11.3 Hz)		H-6B 3.75
H-23B	3.26 (*d*, J = 11.3 Hz)		
H_3-24	0.98		
H_3-25	1.33		
H_3-26	0.86		
H_3-27	1.21		
H_3-29	0.95		
H_3-30	0.98		

special interest, showing that the similarities between *Bellis* and *A. bellidiastrum* also extend to include the minor glycosides of bayogenin.

Although **9** has a structure similar to that of bellissaponins BS5, BS6 and BS7 [5], it is a new triterpenoid saponin and the trivial name bellidiastroside U_{D2} has been given. In addition, a second new triterpenoid saponin (**6**) has been identified, and named bellidiastroside B_3. The structure of **6** is unique and differs from those of the other compounds obtained from *Bellis* and *A. bellidiastrum*, in that the aglycone is bayogenin and the carbohydrate moiety is similar to the glycosides of polygalacic acid.

EXPERIMENTAL

General. 1D and 2D NMR spectra were recorded in CD_3OD at 300K on Bruker DMX-600 NMR (^{1}H, 600.14 MHz; ^{13}C, 150.91 MHz; **5–7**) or ARX-400 NMR spectrometers (^{1}H, 400.13 MHz; ^{13}C, 100.62 MHz; **1–4**, **8**, **9**), as described previously [2]. MS were obtained on a Finnigan TSQ 700 equipped with a Finnigan electrospray source (ESI-MS and MS-MS). $[\alpha]_D$ values were measured using a Perkin-Elmer 241 C polarimeter. TLC was carried out on silica gel 60 plates or foils (Merck), column chromatography on Diaion HP-20, Sephadex LH-20 (Pharmacia), silica gel 60, 0.063–0.2 mm (Merck) and silica gel 100, 0.063–0.2 mm (Merck), HPLC on a Hitachi/Merck D-6000 equipped with a L-4000 UV detector (column: LiChrosorb RP-18 (7 μm, 250 × 10 mm ID) for preparative and LiChrosorb RP-18 (7 μm, 250 × 4 mm ID) for analytical separations).

Plant material. Plants were collected on 26/27 May 1993 in the Swiss Alps (Kanton Graubünden) at an altitude of about 2000 m. The material was dried at 50–60 . A voucher specimen is deposited at the Herbarium of the Institute of Pharmacy, Humboldt-University, herbal number Scho-21.

Extraction and isolation of saponins from the aerial parts. Compounds **1–4** were isolated from the saponin mixture as described previously [1]. For the isolation of **5–7**, a second portion of the mixture (10.1 g) was subjected to CC on silica gel 60 ($CHCl_3$–MeOH–H_2O 10 : 3 : 1, lower layer) to give 29 fractions (8.4 g). Fractions 2–7 and 9–29 were combined and then hydrolysed with 500 ml 1% KOH for 2 hr at room temp. After neutralization with HCl, the deacylated saponins were extracted three times with n-BuOH (3 × 150 ml) to give 4.8 g of deacylated saponins.

Table 2. ^{13}C NMR chemical shifts of compound **6** in CD_3OD

Aglycone		Sugar		
C-1	44.5	Arabinose	C-1	111.6
C-2	71.1		C-2	83.1
C-3	83.9		C-3	78.9
C-4	42.9*		C-4	86.6
C-5	48.1†		C-5	63.3
C-6	18.6‡	Rhamnose	C-1	102.6
C-7	33.5		C-2	71.6
C-8	40.9		C-3	72.1
C-9	49.8		C-4	73.6
C-10	37.5		C-5	70.8
C-11	24.7		C-6	18.4
C-12	123.8	Fucose	C-1	95.4
C-13	144.8		C-2	74.5
C-14	43.1*		C-3	83.4
C-15	29.0		C-4	72.8
C-16	24.1		C-5	72.3
C-17	48.2†		C-6	16.5
C-18	43.2*	Glucose	C-1	105.5
C-19	47.6		C-2	75.4
C-20	31.5		C-3	71.1
C-21	34.9		C-4	78.2
C-22	33.1‡		C-5	77.7
C-23	65.7		C-6	62.3
C-24	14.7			
C-25	17.6			
C-26	17.9			
C-27	26.4			
C-28	178.0			
C-29	33.5			
C-30	24.1			

†,*Assignments may be interchanged.
‡ Assigned by comparison with ref. [5].

These were separated by CC on silica gel 100 ($CHCl_3$–MeOH–H_2O, 10:3:1, lower layer, to give fractions 1–8, and $CHCl_3$–MeOH–H_2O, 6:4:1, to give fractions 9 and 10). Fraction 1 (89 mg) was separated by HPLC with MeOH–H_2O (70:30) to give **5** (60 mg) and **6** (12 mg). HPLC of fractions 9 (43 mg) and 10 (200 mg) with MeOH–H_2O (70:30) afforded compound **7** (11 mg).

Extraction and isolation of saponins from the underground parts. Dried plant material (160 g) was extracted with petrol, CH_3CO_2Et and 80% MeOH. The MeOH extract was dried under reduced pressure. The residue was dissolved in H_2O and extracted five times with n-BuOH. The dried n-BuOH extract was dissolved in MeOH and dropped into an excess of diethyl ether to give 16.9 g of a brown, powdery crude glycoside mixture. This mixture was dissolved in H_2O and chromatographed on a column of Diaion HP-20 using a H_2O, 50%MeOH, MeOH gradient. The MeOH eluate was dried to give 12.6 g of a saponin fraction.

The saponin fraction was separated by CC on silica gel 60 ($CHCl_3$–MeOH–H_2O, 10:3:1, lower layer, to give fractions 1–14, $CHCl_3$–MeOH–H_2O, 6:4:1 to give fractions 15–18). Fractions 8 and 9 (2.6 g) were hydrolysed with 150 ml of 1% KOH for 2 hr at room temp. After neutralization with HCl the deacylated saponins were extracted three times with n-BuOH (3 × 50 ml) to give 2.1 g of deacylated saponins. These were separated by CC on silica gel 100 ($CHCl_3$–MeOH–H_2O, 10:3:1, lower layer) to give six fractions. While fraction 5 consisted of **4** only (407 mg), fraction 1 (23 mg) was separated by HPLC with MeOH–H_2O (73:27) to give **8** (9 mg), fraction 2 (18 mg) by HPLC with MeOH–H_2O (70:30) to give **5** (8 mg), fraction 3 (161 mg) by HPLC with MeOH–H_2O (70:30) to give **1** (25 mg) and **9** (30 mg) and an aliquot (295 mg) of fraction 4 (784 mg) by HPLC with MeOH–H_2O (70:30) to give **2** (88 mg) and **3** (27 mg). Compound **9** was purified further by silica gel 60 CC ($CHCl_3$–MeOH–H_2O, 10:3:1, lower layer) to give 24 mg of the pure compound.

Identification of the component monosaccharides. The determination was performed according to Kusumoto *et al.* [14] using 1 mg of the compound. GLC: column J&W Scientific DB-17 (30 m × 0.25 mm ID, film thickness 0.25 μm), oven temp. 170° for 10 min, then increasing by 2° min^{-1}, 250° injection port and detector temperature, carrier gas He (0.4 ml s^{-1}). Retention times: arabinose 8.07, 8.44, 8.83 and 9.19 min; rhamnose 8.88 and 9.29 min; fucose 8.60, 10.01 and 10.71 min; xylose 11.73 min; glucose 19.13 and 19.49 min.

Determination of the absolute configuration of the sugars. The determination was performed according to [9] using about 1 mg of the compound. GLC: column J&W Scientific DB-17 (30 m × 0.25 mm ID, film thickness 0.25 μm), 250° oven temp., 280° injection port and detector temperature, carrier gas He (22.3 l h^{-1}). Retention times: D-xylose 8.95 min (L-xylose 9.44 min), L-arabinose 8.95 min (D-arabinose 9.49 min), L-rhamnose 9.72 min, D-fucose 10.26 min (L-fucose 10.93 min). The distinction between D- and L-arabinose in **6** was made as described in [15].

Compound **1**. Crystals (from MeOH); mp 231–233°; $[\alpha]_D^{22}$ −22.9 (*c* = 0.24). TLC: R_f 0.52 ($CHCl_3$–MeOH–H_2O 7:4:1). HPLC: R_t 6.4 min (LiChrosorb RP-18, 7μm, 250 × 4 mm ID, MeOH–H_2O 70:30). ^1H and ^{13}C NMR: see [1].

Compound **2**. Crystals (from MeOH); mp 225–227°; $[\alpha]_D^{22}$ −36.5 (*c* = 1.00). TLC: R_f 0.48 ($CHCl_3$–MeOH–H_2O 7:4:1). HPLC: R_t 6.4 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm ID, MeOH–H_2O 70:30). ^1H and ^{13}C NMR: see [1].

Compound **3**. Crystals (from MeOH); mp 214–217°; $[\alpha]_D^{22}$ −15.3 (*c* = 0.17). TLC: R_f 0.48 ($CHCl_3$–MeOH–H_2O 7:4:1). HPLC: R_t 4.7 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm ID, MeOH–H_2O 70:30). HR-FAB-MS: *m/z* $[M + Na]^+$ 1113.5477 (calc. 1113.5458 for $C_{53}H_{86}O_{23}Na$). ^1H and ^{13}C NMR: see [1].

Compound **4**. Crystals (from MeOH); mp 219–222°; $[\alpha]_D^{22}$ 19.5 (*c* = 0.64). TLC: R_f 0.43 ($CHCl_3$–MeOH–H_2O 7:4:1). HPLC: R_t 4.7 min (LiChrosorb RP-18,

7 μm, 250 × 4 mm ID, MeOH–H_2O 70 : 30). ^1H and ^{13}C NMR: see [1].

Compound **5**. Crystals (from MeOH); mp 231–233 °; $[\alpha]_D^{22}$ −22.8° (c = 0.24). TLC: R_f 0.61 ($CHCl_3$–MeOH–H_2O 7 : 4 : 1). HPLC: R_t 13.6 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm ID, MeOH–H_2O 70 : 30). ESI-MS: m/z 1311 $[M + Na]^+$. ^1H NMR: aglycone δ 0.85, 0.93, 0.96, 1.01, 1.38, 1.43 (6 × CH_3), 2.35 (*t*, J = 13.5 Hz, H-19$_A$), 3.00 (*dd*, J = 3.7 and 14.1 Hz, H-18), 5.39 (*t*, $J \simeq$ 3 Hz, H-12), sugar methyl protons δ 1.28 (*d*, J = 6.2 Hz), 1.29 (*d*, J = 6.1 Hz), 1.37 (*d*, J = 6.8 Hz), sugar anomeric protons δ 4.53 (*d*, J = 7.9 Hz), 4.90 (*bs*), 5.18 (*d*, J = 1.1 Hz), 5.34 (*d*, J = 1.4 Hz), 5.42 (*d*, J = 8.0 Hz). ^{13}C NMR: aglycone δ 45.2 (C-1), 72.0 (C-2), 82.5 (C-3), 43.5 (C-4), 48.0 (C-5), 18.6 (C-6), 33.7 (C-7), 41.0 (C-8), 48.4 (C-9), 37.8 (C-10), 24.7 (C-11), 123.5 (C-12), 144.7 (C-13), 43.1 (C-14), 36.4 (C-15), 74.7 (C-16), 50.3 (C-17), 42.4 (C-18), 47.8 (C-19), 31.3 (C-20), 36.6 (C-21), 32.0 (C-22), 65.5 (C-23), 14.9 (C-24), 18.4 (C-25), 17.9 (C-26), 27.2 (C-27), 177.4 (C-28), 33.4 (C-29), 25.0 (C-30), acid δ 168.0 (C-1), 123.4 (C-2), 147.0 (C-3), 18.2 (C-4), Rha-1 δ 102.5 (C-1), 72.3 (C-2), 72.3 (C-3), 74.0 (C-4), 70.0 (C-5), 18.1 (C-6), Xyl δ 107.1 (C-1), 76.4 (C-2), 84.3 (C-3), 69.9 (C-4), 67.2 (C-5), Rha-1,4 δ 101.7 (C-1), 72.3 (C-2), 72.3 (C-3), 84.5 (C-4), 69.0 (C-5), 18.0 (C-6), Fuc δ 95.0 (C-1), 75.6 (C-2), 74.9 (C-3), 74.7 (C-4), 71.2 (C-5), 16.6 (C-6), Rha-1 (bound to the aglycone) δ 104.2 (C-1), 72.4 (C-2), 72.3 (C-3), 74.1 (C-4), 70.4 (C-5), 18.0 (C-6).

Compound **6**. Crystals (from MeOH); mp 223–225 °; $[\alpha]_D^{22}$ −22.8 (c = 0.42). TLC: R_f 0.53 ($CHCl_3$–MeOH–H_2O 7 : 4 : 1). HPLC: R_t 6.7 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm ID, MeOH–H_2O 70 : 30). ESI-MS: m/z 1097 $[M + Na]^+$. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2.

Compound **7**. Crystals (from MeOH); mp 219–222 °; $[\alpha]_D^{22}$ 19.5° (c = 0.17). TLC: R_f 0.29 ($CHCl_3$–MeOH–H_2O 7 : 4 : 1). HPLC: R_t 5.1 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm ID, MeOH–H_2O 70 : 30). ESI-MS: m/z 1097 $[M + Na]^+$. ^1H NMR: aglycone δ 2.12 (H-1$_A$), 1.20 (H-1$_B$), 4.36 (H-2), 3.66 (H-3), 1.36 (H-5), 1.55 (H-6$_{A,B}$), 1.65 (H-7$_A$), 1.43 (H-7$_B$), 1.61 (H-9), 2.04 (H-11$_A$), 1.98 (H-11$_B$), 5.33 (*t*, J = 3.5 Hz, H-12), 1.66 (H-15$_A$), 1.24 (H-15$_B$), 2.12 (H-16$_A$), 1.71 (H-16$_B$), 2.86 (*dd*, J = 4.3 and 13.6 Hz, H-18), 1.76 (H-19$_A$), 1.18 (H-19$_B$), 1.44 (H-21$_A$), 1.28 (H-21$_B$), 1.84 (H-22$_A$), 1.63 (H-22$_B$), 3.65 (H-23$_A$), 3.26 (H-23$_B$), 0.98 (H_3-24), 1.34 (H_3-25), 0.87 (H_3-26), 1.21 (H_3-27), 0.94 (H_3-29), 0.99 (H_3-30), Rha δ 5.39 (*d*, J = 1.5 Hz, H-1), 3.97 (*dd*, J = 1.5 and 3.3 Hz, H-2), 3.71 (H-3), 3.42 (H-4), 3.78 (*dd*, J = 6.2 and 9.8 Hz, H-5), 1.29 (*d*, J = 6.2 Hz, H_3-6), Glc^A δ 5.46 (*d*, J = 7.6 Hz, H-1), 3.63 (H-2), 3.60 (H-3), 3.50 (H-4), 3.54 (H-5), 4.14 (*dd*, J = 1.9 and 11.4 Hz, H-6$_A$), 3.79 (*dd*, J = 6.4 and 11.4 Hz, H-6$_B$), terminal glucoses δ 4.47 (*d*, J = 7.8 Hz, H-1 Glc^C), 4.38 (*d*, J = 7.8 Hz, H-1 Glc^B), 3.30 (H-2 Glc^C), 3.24 (*dd*, J = 7.8 and 9.1 Hz, H-2 Glc^B), 3.39 (H-3 Glc^B), 3.31 and 3.28 (H-5), 3.89 and 3.84 (H-6$_A$), 3.73 and 3.71 (H-6$_B$). ^{13}C NMR: aglycone δ 44.4 (C-1), 71.1 (C-2), 83.9 (C-3), 43.1 (C-4), 48.1 (C-5), 18.6 (C-6), 33.3 (C-7), 40.8 (C-8), 49.1 (C-9), 37.5 (C-10), 24.7 (C-11), 123.7 (C-12), 144.9 (C-13), 43.3 (C-14), 29.1 (C-15), 24.4 (C-16), 48.1 (C-17), 42.8 (C-18), 47.3 (C-19), 31.5 (C-20), 34.9 (C-21), 33.1 (C-22), 65.7 (C-23), 14.7 (C-24), 17.7 (C-25), 17.9 (C-26), 26.2 (C-27), 178.1 (C-28), 33.5 (C-29), 24.3 (C-30), Rha δ 101.7 (C-1), 71.9 (C-2), 72.2 (C-3), 73.8 (C-4), 70.3 (C-5), 18.2 (C-6), Glc^A δ 95.2 (C-1), 77.4 (C-2), 78.9 (C-3), 71.3 (C-4), 77.6 (C-5), 69.7 (C-6), terminal glucoses δ 105.5 (C-1 Glc^C), 104.7 (C-1 Glc^B), 75.4 (C-2 Glc^C), 75.2 (C-2 Glc^B), 78.2 (C-3 Glc^B), 78.0 (C-3 Glc^C), 71.6 and 71.1 (C-4), 78.0 and 77.7 (C-5), 62.7 and 62.3 (C-6).

Compound **8**. Crystals (from MeOH); mp 229–232 °. TLC: R_f 0.73 ($CHCl_3$–MeOH–H_2O 7 : 4 : 1). HPLC: R_t 8.7 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm ID, MeOH–H_2O 70 : 30). ESI-MS: m/z 673 $[M + Na]^+$. ^1H NMR: aglycone δ 0.87, 0.92, 0.93, 1.02, 1.36, 1.42 (6 × CH_3), 1.06 (*dd*, $J \simeq$ 3.5 and 13.5 Hz, H-19$_B$), 1.21 (H-1$_B$), 1.40 (H-15$_B$), 1.70 (H-9), 1.93 (H-15$_A$), 1.99 (H-11$_B$), 2.02 (H-11$_A$), 2.07 (H-1$_A$), 2.30 (*t*, J = 13.5 Hz, H-19$_A$), 2.94 (*dd*, $J \simeq$ 3.5 and 13.5 Hz, H-18), 3.35 (H-23$_A$), 3.29 (*d*, J = 11.2 Hz, H-23$_B$), 3.71 (*d*, J = 3.7 Hz, H-3), 4.46 (H-2), 4.49 (H-16), 5.36 (*t*, $J \simeq$ 3.5 Hz, H-12), rhamnose δ 4.90 (*bs*, H-1), 3.95 (*dd*, J = 1.6 and 3.2 Hz, H-2), 3.83 (*dd*, J = 3.2/9.5 Hz, H-3), 3.42 (*t*, J = 9.5 Hz, H-4), 3.85 (*dd*, J = 6.3 and 9.5 Hz, H-5), 1.28 (*d*, J = 6.3 Hz, H_3-6). ^{13}C NMR: aglycone δ 45.1 (C-1), 71.9 (C-2), 82.5 (C-3), 43.4 (C-4), 48.0 (C-5), 18.7 (C-6), 33.7 (C-7), 40.8 (C-8), 48.0 (C-9), 37.7 (C-10), 24.6 (C-11), 123.3 (C-12), 145.4 (C-13), 43.0 (C-14), 36.1 (C-15), 75.5 (C-16), 42.3 (C-18), 47.8 (C-19), 31.4 (C-20), 36.6 (C-21), 32.4 (C-22), 65.6 (C-23), 14.7 (C-24), 18.0 (C-25), 17.8 (C-26), 27.5 (C-27), 33.5 (C-29), 25.2 (C-30), Rha δ 104.2 (C-1), 72.4 (C-2), 72.3 (C-3), 74.1 (C-4), 70.4 (C-5), 18.0 (C-6).

Compound **9**. Crystals (from MeOH); mp 208–212 °. TLC: R_f 0.51 ($CHCl_3$–MeOH–H_2O 7 : 4 : 1). HPLC: R_t 5.5 min (LiChrosorb RP-18, 7 μm, 250 × 4 mm ID, MeOH–H_2O 70 : 30). ESI-MS: m/z 967 $[M + Na]^+$. ^1H NMR: aglycone δ 0.86, 0.95, 0.98, 0.98, 1.21, 1.33 (6 × CH_3), 1.14 (H-19$_B$), 1.16 (H-1$_B$), 1.62 (H-9), 1.97 (H-11$_B$), 2.01 (H-11$_A$), 2.12 (H-1$_A$), 2.10 (H-19$_B$), 2.90 (*dd*, $J \simeq$ 3.5 and 13.5 Hz, H-18), 3.26 (H-23$_B$), 3.63 (H-23$_A$), 3.65 (H-3), 4.32 (H-2), 5.31 (*t*, $J \simeq$ 3.5 Hz, H-12), sugar anomeric protons δ 4.37 (*d*, J = 7.7 Hz), 4.39 (*d*, J = 7.8 Hz), 5.39 (*d*, J = 8.1 Hz). ^{13}C NMR: δ 44.4 (C-1), 71.0 (C-2), 83.5 (C-3), 43.5 (C-4), 48.1 (C-5), 18.6 (C-6), 33.4 (C-7), 40.8 (C-8), 48.1 (C-9), 37.5 (C-10), 24.7 (C-11), 123.9 (C-12), 145.0 (C-13), 43.2 (C-14), 28.8 (C-15), 24.7 (C-16), 42.6 (C-18), 48.3 (C-19), 31.5 (C-20), 34.9 (C-21), 33.2 (C-22), 65.5 (C-23), 14.7 (C-24), 17.6 (C-25), 17.6 (C-26), 26.4 (C-27), 178.1 (C-28), 33.5 (C-29), 24.1 (C-30), glc-1 δ 104.7 (C-1), 75.4 (C-2), 77.8 (C-3), 71.6 (C-4), 78.2 (C-5), 62.7 (C-6), glc-1,6 δ 95.8 (C-1), 73.3 (C-2), 78.0 (C-3), 71.6 (C-4), 78.0 (C-5), 69.5 (C-6), xyl δ 106.5 (C-1), 74.5 (C-2), 77.8 (C-3), 70.0 (C-4), 67.3 (C-5).

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