Triterpenoid Saponins from *Bellis sylvestris*. 2. Structures of Partially Deacylated Saponins

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Four saponins have been isolated from the herbal parts of *Bellis sylvestris* and the same, together with three further compounds, from the underground parts. The structures were elucidated by electrospray ionization mass spectrometry, including tandem mass spectrometry, and by 1D and 2D homonuclear and heteronuclear NMR spectroscopy. Two of the compounds are new saponins with the structures 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,23-trihydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→2)]-*β*-D-glucopyranoside and 3-*O*-*β*-D-glucopyranosyl-2*β*,3*β*,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-*β*-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-[(*E*)-2-butenoic acid(1→3)]-*β*-D-fucopyranoside. Taxonomically these data provide significant evidence of a link between the *Bellis* genus and *Bellium bellidioides*.

Introduction

Previously we reported the isolation and structure elucidation of the major deacylsaponins obtained from the herb of *Bellis sylvestris* Cyr. (Asteraceae).[1] Among these were the common deacylsaponin of the *Bellis* genus, bellissaponin BS1, and a new saponin called besysaponin C_{12}. The present paper describes the isolation and structure elucidation of two new partially deacylated saponins obtained from the underground parts of *B. sylvestris* and of additional known deacylsaponins obtained from both the herb and underground parts of this plant.

Results and Discussion

Four compounds (**1**−**4**) were isolated from the mild alkaline hydrolysate of the saponin mixture obtained from the aerial parts of *B. sylvestris*. Compounds **1**−**4** (Chart 1) were also obtained from the underground parts, together with three additional saponins (**5**−**7**), as described in the Experimental Section.

Co-chromatography of compounds **1**−**4** with bellissaponin BS1 and besysaponin C_{12} showed that **1** and **2** are identical with these saponins obtained previously.[1] TLC comparison of **3** and **4** with deacylsaponins obtained from *Bellis perennis* indicated that **3** is identical with bellissaponin BS2.[2] A molecular ion at *m*/*z* 1259 $[M + Na]^+$ in the electrospray ionization (ESI) mass spectrum and agreement of the ^{13}C-NMR chemical shifts confirmed this assignment. Co-chromatography of compound **4** with the saponins obtained from *B. perennis*, *B. bernardii*, *Bellium bellidioides*, and *Aster bellidiastrum* did not show any correspondence. Methanolysis and GC identification of the methyl monosaccharides afforded glucose and rhamnose as sugar constituents. Hydrolysis and GC identification of the products obtained by reaction with L-cystein methyl ester hydrochloride according to Hara *et al.*[3] established that these were the D-enantiomer and L-enantiomers, respectively, in a ratio of 3:1. ESIMS yielded a molecular ion at *m*/*z* 1143 $[M + Na]^+$ and an intense daughter ion at *m*/*z* 493, comprising the acyl-linked oligosaccharide chain, when subjected to collision-induced dissociation. ^{1}H- and ^{13}C-NMR chemical shifts were assigned (see Experimental Section) from a combination of 2D homonuclear ^{1}H−^{1}H (COSY-45) and heteronuclear ^{13}C−^{1}H (HMQC, HMBC) correlations that allowed unambiguous identification of the aglycon and the various sugar moieties. Hence, bayogenin (2*β*,3*β*,23-trihydroxyolean-12-en-28-oic acid) was unambigously identified as the aglycon of **4**. The observation of cross peaks in the HMBC spectrum arising from through-bond couplings over three bonds between the anomeric protons and carbons in adjacent systems allowed determination of the sugar sequence and aglycon linkage positions. Hence, cross peaks between H-1 (*δ* 4.47) and C-1 (*δ* 105.5) of a glucose (glc^C) and C-3 (*δ* 83.9) and H-3 (*δ* 3.63) of the aglycon respectively, and between H-1 (*δ* 5.63) of a second glucose unit (glc^A) and C-28 (*δ* 178.0) of the aglycon confirmed that glucose moieties are attached at C-3 and C-28 of the aglycon. Cross peaks between C-1 (*δ* 104.2) of the third glucose unit (glc^B) and H-3 (*δ* 3.83) of glc^A, and C-1 (*δ* 101.7) of the rhamnose and H-2 (*δ* 3.86) of glc^A indicated that **4** consists of a 2,3-linked glucose carrying rhamnose at C-2 and a further glucose at C-3. This assignment was confirmed by the appearance of

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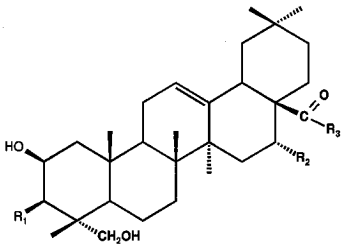
Chart 1



Cmpd.	R_1	R_2	R_3	Name
1	α-L-Rhap	OH	ß-D-Xylp(1→4)-α-L-Rhap(1→2)-ß-D-Fucp	Besysaponin C_{12}
2	α-L-Rhap	OH	α-L-Rhap(1→3)-ß-D-Xylp(1→4)-α-L-Rhap(1→2)-ß-D-Fucp	Bellissaponin BS1
3	ß-D-Glcp	OH	α-L-Rhap(1→3)-ß-D-Xylp(1→4)-α-L-Rhap(1→2)-ß-D-Fucp	Bellissaponin BS2
4	ß-D-Glcp	H	ß-D-Glcp(1→3)-[α-L-Rhap(1→2)]-ß-D-Glcp	Besysaponin U_{D2}
5	ß-D-Glcp	OH	α-L-Rhap(1→3)-ß-D-Xylp(1→4)-α-L-Rhap(1→2)-[*E*-2-butenoic acid(1→3)]-ß-D-Fucp	Besysaponin U_{B1}
6	α-L-Rhap	OH	α-L-Rhap(1→3)-ß-D-Xylp(1→4)-α-L-Rhap(1→2)-[*E*-2-butenoic acid(1→4)]-ß-D-Fucp	Bellissaponin BA_1
7	ß-D-Glcp	OH	α-L-Rhap(1→3)-ß-D-Xylp(1→4)-α-L-Rhap(1→2)-[*E*-2-butenoic acid(1→4)]-ß-D-Fucp	Bellissaponin BA_2

the reverse cross peaks between H-1 (δ 4.58) of glc^B and C-3 (δ 87.0) of glc^A, and H-1 (δ 5.33) of the rhamnose and C-2 (δ 77.7) of glc^A. All three glucose units had β-configurations from the vicinal H1−H2 coupling constants of 6−8 Hz, whereas rhamnose had an α-configuration from the vicinal H1−H2 coupling constants of 1.7 Hz and H1−C1 coupling constant of ca. 172 Hz.[4] Consequently, compound **4** is 3-*O*-β-D-glucopyranosyl-2β,3β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl(1→3)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside.

Compounds **5**, **6**, and **7** showed signals of an (*E*)-2-butenoic acid system in their ^{1}H-NMR spectra. Comparison of the ^{13}C-NMR spectra with those of bellissaponins BA_1 and BA_2[5] indicated that **6** is identical with bellissaponin BA_1 and that **7** is identical with bellissaponin BA_2. These were further confirmed by the low-field shifts of H-4 of the fucose system obtained from the COSY spectrum and by the appearance of molecular ions at *m/z* 1311 [M + Na^+] and *m/z* 1329 [M + Na^+], respectively, in the ESI MS.

The ^{13}C-NMR data of compound **5** (see Experimental Section) are almost superimposable with those of compound **7**. Differences are found only in the fucose system. These observations and the appearance of a molecular ion at *m/z* 1329 [M + Na^+] in the ESIMS establish that **5** and **1** are isomeric. Cross peaks in the COSY-45 spectrum for the fucose system indicated that the low-field signal at δ 4.96 belonged to H-3 and established the point of attachment of the (*E*)-2-butenoic acid system. The configuration of the carbohydrate components were the same as in **7**; hence, compound **5** is 3-*O*-β-D-glucopyranosyl-2β,3β,16α,23-tetrahydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-[(*E*)-2-butenoic acid(1→3)]-β-D-fucopyranoside.

Although *B. perennis*, the common daisy, has been used in folk medicine over a long period of time,[6] no information is available on the medical use of *B. sylvestris*, the large or autumn daisy. It can be assumed, however, that *B. sylvestris* is used similarly to *B. perennis*. The major saponin components in *B. perennis* are probably responsible for its application as an expectorant in folk medicine.[6] The data presented here demonstrate that both saponin content, as well as saponin composition, of *B. sylvestris* and *B. perennis* are similar. Hence, it can be concluded that the autumn daisy is as useful as the common daisy as a medicinal plant.

Compounds **4** and **5** are new compounds, and the trivial name besysaponins O_{D2} has been given to compound **4** and besysaponin U_{B1} to compound **5**. However, a diacetylated derivative of compound **4** has already been isolated from *Bellium bellidioides* L.[7] The appearance of compound **4** in *B. sylvestris* is of taxonomical significance, as it is the first glycoside in the *Bellis* genus with a 2,3-branched glucose moiety. Consequently, *B. sylvestris* seems to be the link between the *Bellis* genus and *B. bellidioides*. Furthermore, it is evident that there is no significant difference between the saponin composition of *B. bellidioides* and the *Bellis* genus.[8] Hence, from a chemical point of view, *B. bellidioides* should be regarded as a member of the *Bellis* species.

Experimental Section

General Experimental Procedures. 1D (^{1}H, ^{13}C, DEPT) and 2D [^{1}H COSY, HMBC (^{1}H-detected multiple-bond ^{13}C multiple-quantum coherence spectroscopy), HMQC (^{1}H-detected ^{13}C multiple-quantum coherence spectroscopy)] NMR spectra of compound **4** were recorded in CD_3OD at 300 K on a Bruker AM 600 NMR spectrometer (^{1}H, 600.14 MHz; ^{13}C, 150.91 MHz). ^{1}H, ^{13}C, DEPT, and COSY spectra of compounds **1**, **5**, **6**, and **7** were recorded on a Bruker ARX 400 spectrometer (^{1}H, 400.13 MHz; ^{13}C, 100.63 MHz). ^{1}H, ^{13}C, and DEPT spectra of compound **2** were recorded on a Bruker ARX 400 spectrometer and ^{1}H, ^{13}C, and DEPT spectra of compound **3** on a Bruker AM-300 spectrometer (^{1}H, 300.14 MHz; ^{13}C, 75.47 MHz). MS were recorded on a Finnigan TSQ 700 spectrometer equipped with a Finnigan electrospray source (ESIMS and MS/MS). GC was performed on a Hewlett-Packard HP 5890 Series II; TLC was carried out on Si gel 60 plates or foils (Merck); column chromatography was performed on Diaion HP-20 and Si gel 60, 0.063−0.2 μm (Merck), and HPLC was carried out on a Hitachi/Merck, model D-6000 equipped

with a L-4000 UV detector (detection at 206 nm). $[\alpha]_D$ Values were measured on a Perkin-Elmer 241 C polarimeter and melting points on a Galen III melting point apparatus (Cambridge Instruments).

Extraction and Isolation of Saponins from the Herbal Parts. Plant material (400 g) was extracted as described previously.[1] The dried extract was dissolved in 1% KOH and hydrolyzed at room temperature for 8 h. The hydrolysate was neutralized with 10% HCl, defatted with $CHCl_3$, and then subjected to column chromatography on a Diaion HP-20 instrument using H_2O, 50% MeOH, and MeOH as solvents. The saponins were eluted in the MeOH fraction, giving 23.4 g of a saponin mixture. Of this mixture 21 g were chromatographed on Si gel ($CHCl_3$−MeOH−H_2O 7:3:1, lower layer) affording 1.1 g of compound **1**, 10.4 g of compound **2**, and 2.3 g of another saponin fraction (fraction C). Repeated HPLC of 1 g of fraction C on LiChrospher RP-18 (10 μm, 250 mm × 10 mm i.d.) using MeOH−H_2O (65:35) afforded 87 mg of compound **3** and 30 mg of compound **4**.

Extraction and Isolation of Saponins from the Underground Parts. Plant material (170 g) was extracted as described previously.[1] The dried extract was defatted with $CHCl_3$, dissolved in 1% KOH, and hydrolyzed at room temperature for 2 h. The hydrolysate was neutralized with 10% HCl and then subjected to Diaion HP-20 column chromatography using H_2O, 50% MeOH, and MeOH as solvents. The saponins were eluted in the MeOH fraction, giving 7.2 g of a saponin mixture. This mixture was chromatographed on Si gel ($CHCl_3$−MeOH−H_2O 7:3:1, lower layer), affording saponin fractions A (120 mg), B (230 mg), C (1.05 g), and D (1.2 g). HPLC of fraction A on LiChrosorb RP-18 (7 μm, 250 mm × 10 mm i.d.) using MeOH−H_2O (72:28) afforded 30 mg of compound **6**; HPLC of fraction B using MeOH−H_2O (68:32) afforded 6 mg of compound **5**, 14 mg of compound **1**, and 50 mg of compound **7**; HPLC of 400 mg of fraction C using MeOH−H_2O (74:26) afforded 220 mg of compound **2**, and repeated HPLC of 400 mg of fraction D using MeOH−H_2O (66:34 and 62:38) afforded 130 mg of compound **3** and 8 mg of compound **4**.

Identification of the Component Monosaccharides. The determination was performed according to Kusumoto *et al.*[9] using 1 mg of each compound. GLC conditions: column, J&W Scientific DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature, 170 °C for 10 min, then increasing by 2 °C × min^{-1}; 250° injection port and detector temperature, carrier gas He (0.4 mL/s); retention times, xylose 11.73 min, rhamnose 8.88 and 9.29 min, fucose 10.01 and 10.71 min, and glucose 19.13 and 19.49 min.

Determination of the Absolute Configuration of the Sugars. The determination was performed according to Hara *et al.*[3] using about 1 mg of compounds **2**, **3**, **4**, **6**, and **7**. GLC conditions: column, J&W Scientific DB-17 (30 m × 0.25 mm i.d., film thickness 0.25 μm), 250 °C oven temperature, 280° injection port and detector temperature, carrier gas He (22.3 L/h); retention times, D-xylose 9.11 min (L-xylose 9.72 min), L-rhamnose 9.97 min, and D-fucose 10.57 min (L-fucose 11.31 min).

Compound 1: white, amorphous powder; mp 228−229 °C; TLC R_f 0.52 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 10.7 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 65:35); ESIMS *m/z* 1097 $[M + Na]^+$; MS/MS of 1097 *m/z* 447 [pent + 2dhex − H_2O + $Na]^+$; ^{1}H NMR aglycon δ 0.82, 0.92, 0.94, 0.99, 1.37, 1.43 (6 × CH_3), 2.08 (H-1A), 1.22 (H-1B), 4.25 (H-2), 3.70 (H-3), 1.69 (H-9), 2.00 (H-11A/B), 5.38 (H-12), 1.74 (H-15A), 1.51 (H-15B), 4.53 (H-16), 2.98 (dd, $J \sim$ 3.5/13.5 Hz, H-18), 2.34 (t, J = 13.2 Hz, H-19A), 1.10 (H-19B), sugar anomeric protons δ 4.53 (d, J = 7.4 Hz), 4.90 (bs), 5.34 (d, J = 8.2 Hz), 5.46 (bs), sugar methyl protons δ 1.26 (d, J = 6.6 Hz), 1.28 (d, J = 6.5 Hz), 1.35 (d, J = 5.9 Hz); ^{13}C NMR (δ) aglycon C-1 45.2, C-2 71.9, C-3 82.5, C-4 43.4, C-5 48.1, C-6 18.8, C-7 33.8, C-8 40.9, C-9 48.5, C-10 37.7, C-11 24.7, C-12 123.7, C-13 144.8, C-14 43.0, C-15 36.5, C-16 74.7, C-17 50.1, C-18 42.3, C-19 48.0, C-20 32.3, C-21 36.5, C-22 32.0, C-23 65.5, C-24 14.7, C-25 18.0, C-26 17.8, C-27 27.3, C-28 177.1, C-29 33.4, C-30 24.9, xyl 1,3 C-1 107.2, C-2 76.1, C-3 78.2, C-4 71.1, C-5 67.3, rha 1,4 C-1 101.1, C-2 72.4, C-3 72.3, C-4 84.3, C-5 68.8, C-6 18.3, fuc 1,2 C-1 95.2, C-2 74.1, C-3 76.7, C-4 73.6, C-5 72.7, C-6 16.5, rha 1 (bound to the aglycon) C-1 104.2, C-2 72.4, C-3 72.3, C-4 74.0, C-5 70.4, C-6 17.9.

Compound 2: white, amorphous powder; mp 239−240 °C; $[\alpha]^{22}_D$ −37.2° (*c* 1.09); TLC R_f 0.45 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 10.1 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 65:35); ESIMS *m/z* 1243 $[M + Na]^+$, 1221 $[M + H]^+$; MS/MS of 1243 *m/z* 593 [pent + 3dhex − H_2O + $Na]^+$; ^{1}H NMR aglycon δ 0.83, 0.92, 0.96, 0.99, 1.37, 1.43 (6 × CH_3), 2.34 (t, J = 13.6 Hz, H-19A), 2.98 (dd, $J \sim$ 3.4/13.7 Hz, H-18), 5.39 (t, $J \sim$ 3.5 Hz, H-12), sugar anomeric protons δ 4.53 (d, J = 7.5 Hz), 4.91 (bs), 5.18 (bs), 5.35 (d, J = 8.2 Hz), 5.41 (bs), sugar methyl protons δ 1.26 (d, J = 6.4 Hz), 1.28 (d, J = 6.2 Hz), 1.29 (d, J = 6.0 Hz), 1.36 (d, $J \sim$ 6 Hz); ^{13}C NMR (δ) aglycon C-1 45.1, C-2 71.7, C-3 82.4, C-4 43.4, C-5 48.3, C-6 19.0, C-7 33.6, C-8 40.8, C-9 48.3, C-10 37.7, C-11 24.6, C-12 123.6, C-13 144.6, C-14 42.9, C-15 36.5, C-16 74.8, C-17 50.1, C-18 42.3, C-19 48.0, C-20 31.3, C-21 36.5, C-22 31.9, C-23 65.8, C-24 14.9, C-25 18.0, C-26 17.8, C-27 27.2, C-28 177.3, C-29 33.4, C-30 24.8, rha 1 C-1 102.5, C-2 72.3, C-3 72.2, C-4 74.0, C-5 70.0, C-6 17.9, xyl 1,3 C-1 107.1, C-2 76.4, C-3 84.3, C-4 68.8, C-5 67.1, rha 1,4 C-1 101.3, C-2 72.3, C-3 72.2, C-4 84.5, C-5 69.8, C-6 18.4, fuc 1,2 C-1 95.0, C-2 74.5, C-3 76.7, C-4 73.5, C-5 71.9, C-6 16.5, rha 1 (bound to the aglycon) C-1 104.1, C-2 72.6, C-3 72.3, C-4 74.0, C-5 70.3, C-6 17.9.

Compound 3: white, amorphous powder; mp 235−237 °C; TLC R_f 0.39 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 9.6 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 63:37); $[\alpha]^{23}_D$ 24.0° (*c* 1.3); ESIMS *m/z* 1259 $[M + Na]^+$; MS/MS of 1259 *m/z* 593 [pent + 3dhex − H_2O + $Na]^+$; ^{1}H NMR aglycon δ 0.82, 0.93, 0.99, 1.01, 1.35, 1.42 (6 × H_3), 2.98 (dd, J = 3.4/13.9 Hz, H-18), 5.37 (t, $J \sim$ 4 Hz, H-12), sugars methyl protons δ 1.25 (d, J = 6.4 Hz), 1.29 (d, J = 6.2 Hz), 1.37 (d, J = 6.5 Hz), sugar anomeric protons δ 4.48 (d, J = 7.6 Hz), 4.53 (d, J = 5.9 Hz), 5.18 (d, J = 1.2 Hz), 5.36 (d, J = 8.0 Hz), 5.41 (d, J = 1.3 Hz); ^{13}C NMR (δ) aglycon C-1 44.4, C-2 71.9, C-3 84.7, C-4 43.1, C-5 48.3, C-6 18.9, C-7 33.6, C-8 40.8, C-9 48.3, C-10 37.5, C-11 24.8, C-12 123.5, C-13 144.7, C-14 42.9, C-15 36.4, C-16 74.5, C-17 50.1, C-18 42.3, C-19 48.0, C-20 31.6, C-21 36.5, C-22 32.0, C-23 66.0, C-24 14.9, C-25 17.9, C-26 17.8, C-27 27.2, C-28

177.2, C-29 33.4, C-30 24.9, rha 1 C-1 102.5, C-2 72.2, C-3 72.2, C-4 74.0, C-5 70.0, C-6 17.8, xyl 1,3 C-1 107.1, C-2 76.5, C-3 84.0, C-4 68.8, C-5 67.2, rha 1,4 101.4, C-2 72.3, C-3 72.2, C-4 84.3, C-5 69.8, C-6 18.4, fuc 1,2 C-1 95.0, C-2 76.7, C-3 74.8, C-4 72.6, C-5 71.1, C-6 16.5, glc 1 C-1 105.4, C-2 75.3, C-3 77.7, C-4 71.0, C-5 78.1, C-6 62.2.

Compound 4: white, amorphous powder; mp 226−227 °C; TLC R_f 0.39 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 11.6 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 63:37); ESIMS see text; ^{1}H-NMR aglycon 2.12 (H-1A), 1.18 (H-1B), 4.36 (H-2), 3.63 (H-3), 1.38 (H-5), 1.64 (H-6A), 1.51 (H-6B), 1.48 (H-7A), 1.33 (H-7B), 1.62 (H-9), 2.06 (H-11A), 1.95 (H-11B), 5.33 (H-12), 2.06 (H-16A), 2.87 (H-18), 1.76 (H-19A), 1.17 (H-19B), 1.59* (H-21A), 1.41* (H-21B), 1.88* (H-22A), 1.25 (H-22B), 3.65 (H-23A), 3.25 (H-23B), 0.98 (H_3-24), 1.33 (H_3-25), 0.87 (H_3-26), 1.21 (H_3-27), 0.95 (H_3-29), H_3-30 (0.98), glc^A 5.63 (H-1), H-2 (3.86), H-3 (3.83), H-4 (3.71), glc^B 4.58 (H-1), 3.30 (H-2), glc^C 4.47 (H-1), 3.28 (H-2), rha 5.33 (H-1), H-2 (4.01), H-3 (3.68), H-4 (3.45), H-5 (3.72), H_3-6 (1.28); ^{13}C NMR (δ) aglycon C-1 44.4, C-2 71.1, C-3 83.9, C-4 43.1, C-5 48.2, C-6 18.6, C-7 33.5, C-8 40.9, C-9 49.1, C-10 37.5, C-11 24.7, C-12 123.8, C-13 145.0, C-14 43.2, C-15 28.9, C-16 24.2, C-17 48.1, C-18 42.9, C-19 47.4, C-20 31.6, C-21 34.9, C-22 33.0, C-23 65.6, C-24 14.7, C-25 17.6, C-26 18.0, C-27 26.3, C-28 178.0, C-29 33.5, C-30 24.2, glc^A C-1 95.0, C-2 77.7, C-3 87.0, C-4 69.6, C-5 78.2, C-6 62.7, glc^B C-1 104.2, C-2 75.4, C-3 77.7, C-4 71.6, C-5 78.2, C-6 62.7, glc^C C-1 105.5, C-2 75.1, C-3 77.7, C-4 71.2, C-5 78.3, C-6 62.3, rha C-1 101.7, C-2 72.0, C-3 72.2, C-4 73.7, C-5 70.6, C-6 18.2.

Compound 5: white, amorphous powder; mp 209 °C; TLC R_f 0.56 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 7.4 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 65:35); ESIMS m/z 1327 [M + Na]$^+$; MS/MS of 1327 m/z 661 [pent + 3dhex + crotonic acid − H_2O + Na]$^+$; ^{1}H NMR aglycon δ 0.84, 0.93, 1.00, 1.01, 1.37, 1.43 (6 × CH_3), 2.12 (H-1A), 1.23 (H-1B), 4.37 (H-2), 3.68 (H-3), 1.67 (H-9), 1.99 (H-11A/B), 5.38 (H-12), 1.78 (H-15A), 1.49 (H-15B), 4.52 (H-16), 3.00 (dd, $J \sim$ 3.5/13.5 Hz, H-18), 2.35 (t, $J \sim$ 13.5 Hz, H-19A), 1.10 (H-19B), acid δ 7.15 (dd, H-3), 6.00 (d, H-2), 1.95 (d, H-4) (J_{H2-H3} = 15.6 Hz, J_{H3-H4} = 6.5 Hz), fucose H-1 5.50 (d, J = 8.1 Hz), H-2 4.12 (t, $J \sim$ 8.5 Hz), H-3 4.96 (dd, J = 9.7/3.2 Hz), H-4 3.85 (d, $J \sim$ 3 Hz), H-5 3.78, H-6 1.34 (d, J = 6.0 Hz), anomeric protons of the remaining sugars δ 4.47 (d, J = 7.7 Hz), 4.52 (d, J = 7.6 Hz), 5.05 (bs), 5.18 (d, J = 1.4 Hz), methyl protons of rhamnose δ 1.26 (d, J = 6.4 Hz), 1.28 (d, J = 6.4 Hz); ^{13}C NMR (δ) C-1 44.5, C-2 71.9, C-3 84.1, C-4 43.2, C-5 48.1, C-6 18.9, C-7 33.7, C-8 40.9, C-9 48.6, C-10 37.6, C-11 24.7, C-12 123.7, C-13 144.6, C-14 43.0, C-15 36.5, C-16 74.5, C-17 50.2, C-18 42.4, C-19 48.1, C-20 31.3, C-21 36.5, C-22 32.0, C-23 65.9, C-24 14.9, C-25 18.0, C-26 17.8, C-27 27.2, C-28 177.1, C-29 33.4, C-30 24.9, rha 1 C-1 102.6, C-2 72.4, C-3 72.2, C-4 74.0, C-5 70.0, C-6 17.9, xyl 1,3 C-1 107.0, C-2 76.4, C-3 84.3, C-4 69.2, C-5 67.2, rha 1,4 C-1 101.7, C-2 72.3, C-3 72.3, C-4 84.3, C-5 69.9, C-6 18.4, fuc 1,2 C-1 95.0, C-2 75.4, C-3 78.0, C-4 73.0, C-5 71.1, C-6 16.4, rha 1 (bound to the aglycon) C-1 104.2, C-2 72.4, C-3 72.3, C-4 74.0, C-5 70.4, C-6 17.9, acid C-1 168.0, C-2 123.4, C-3 147.0, C-4 18.2.

Compound 6: white, amorphous powder; mp 215 °C; TLC R_f 0.56 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 7.3 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 65:35); ESIMS m/z 1311 [M + Na]$^+$; MS/MS of 1311 m/z 661 [pent + 3dhex + crotonic acid − H_2O + Na]$^+$; ^{1}H NMR, aglycon δ 0.85, 0.93, 0.96, 1.01, 1.38, 1.44 (6 × CH_3), 2.08 (H-1A), 1.26 (H-1B), 4.25 (H-2), 3.73 (H-3), 1.69 (H-9), 2.00 (H-11A/B), 5.38 (H-12), 1.76 (H-15A), 1.50 (H-15B), 4.52 (H-16), 3.00 (dd, $J \sim$ 3.5/13.5 Hz, H-18), 2.35 (t, J = 13.5 Hz, H-19A), 1.09 (H-19B), acid δ 7.11 (dd, H-3), 6.04 (dd, H-2), 1.98 (dd, H-4) (J_{H2-H3} = 15.5 Hz, J_{H2-H4} = 1.3 Hz, J_{H3-H4} = 6.5 Hz), sugar anomeric protons δ 4.53 (d, J = 7.8 Hz), 4.91 (bs), 5.18 (bs), 5.34 (bs), 5.43 (d, J = 8.2 Hz), sugar methyl protons δ1.09 (d, J = 6.2 Hz), 1.28 (d, J = 6.1 Hz), 1.29 (d, J = 6.1 Hz), 1.37 (d, J = 6.6 Hz); ^{13}C NMR (δ), aglycon C-1 45.1, C-2 71.9, C-3 82.5, C-4 43.5, C-5 48.0, C-6 19.0, C-7 33.7, C-8 40.9, C-9 48.5, C-10 37.8, C-11 24.7, C-12 123.5, C-13 144.7, C-14 43.0, C-15 36.5, C-16 74.7, C-17 50.3, C-18 42.4, C-19 48.1, C-20 31.3, C-21 36.4, C-22 31.9, C-23 65.8, C-24 14.9, C-25 18.0, C-26 18.0, C-27 27.2, C-28 177.3, C-29 33.4, C-30 25.0, acid C-1 168.0, C-2 123.3, C-3 147.0, C-4 18.2, rha 1 C-1 102.5, C-2 72.3, C-3 72.3, C-4 74.0, C-5 70.0, C-6 18.0, xyl 1,3 C-1 107.1, C-2 76.4, C-3 84.3, C-4 68.9, C-5 67.2, rha 1,4 C-1 101.7, C-2 72.3, C-3 72.3, C-4 84.5, C-5 69.9, C-6 18.4, fuc 1,2 C-1 95.0, C-2 75.6, C-3 74.7, C-4 74.9, C-5 71.2, C-6 16.6, rha 1 (bound to the aglycon) C-1 104.1, C-2 72.3, C-3 72.3, C-4 74.0, C-5 70.3, C-6 17.9.

Compound 7: white, amorphous powder; mp 221−222 °C; $[\alpha]^{22}_D$ −25.6° (c 1.09); TLC, R_f 0.52 ($CHCl_3$−MeOH−H_2O 7:4:1); HPLC t_R 10.7 min (LiChrosorb RP-18, 7 μm, 250 mm × 4 mm i.d., MeOH−H_2O 65:35); ESIMS, m/z 1327 [M + Na]$^+$; MS/MS of 1327 m/z 661 [pent + 3dhex − H_2O + crotonic acid + Na]$^+$; ^{1}H NMR aglycon δ 0.85, 0.93, 1.00, 1.01, 1.35, 1.43 (6 × CH_3), 2.13 (dd, $J \sim$ 3/12 Hz, H-1A), 1.22 (H-1B), 4.37 (d, J = 3.0 Hz, H-2), 3.68 (H-3), 1.67 (H-9), 2.00 (H-11A/B), 5.39 (t, $J \sim$ 3 Hz, H-12), 1.76 (H-15A), 1.50 (H-15B), 4.52 (H-16), 3.00 (dd, J = 3.4/13.7 Hz, H-18), 2.34 (t, J = 13.7 Hz, H-19A), 1.11 (H-19B), acid δ 7.10 (dd, H-3), 6.04 (dd, H-2), 1.97 (dd, H-4) (J_{H2-H3} = 15.5 Hz, J_{H2-H4} = 1.5 Hz, J_{H3-H4} = 6.8 Hz), sugar anomeric protons δ4.48 (d, J = 7.3 Hz), 4.53 (d, J = 7.5 Hz), 5.18 (d, J = 1.2 Hz), 5.33 (d, J = 1.3 Hz), 5.44 (d, J = 8.0 Hz), sugar methyl protons δ 1.09 (d, J = 6.3 Hz), 1.29 (d, J = 6.2 Hz), 1.37 (d, J = 6.2 Hz); ^{13}C NMR (δ) aglycon C-1 44.5, C-2 71.9, C-3 84.1, C-4 43.7, C-5 48.6, C-6 18.9, C-7 33.7, C-8 40.9, C-9 48.7, C-10 37.6, C-11 24.6, C-12 123.5, C-13 144.7, C-14 43.0, C-15 36.6, C-16 74.9, C-17 50.3, C-18 42.4, C-19 48.0, C-20 31.3, C-21 36.4, C-22 32.0, C-23 66.0, C-24 14.9, C-25 18.0, C-26 17.9, C-27 27.2, C-28 177.3, C-29 33.4, C-30 25.0, acid C-1 168.0, C-2 123.3, C-3 147.0, C-4 18.2, rha 1 C-1 102.5, C-2 72.3, C-3 72.3, C-4 74.0, C-5 69.9, C-6 17.8, xyl 1,3 C-1 107.1, C-2 76.4, C-3 84.4, C-4 68.9, C-5 67.2, rha 1,4 C-1 101.7, C-2 72.2, C-3 72.2, C-4 84.6, C-5 70.0, C-6 18.4, fuc 1,2 C-1 95.0, C-2 75.7, C-3 74.7, C-4 74.9, C-5 71.2, C-6 16.6, glc 1 (bound to the aglycon) C-1 105.4, C-2 75.4, C-3 77.7, C-4 71.1, C-5 78.1, C-6 62.3.

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