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Oleanane-type glycosides from *Weigela* x Styriaca and two cultivars of *W. florida* : "Minor black" and "Brigela"

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ABSTRACT

Saponins are triterpenoid glycosides known for their vast panel of biological activities, among which their defense role against phytopathogens. The *Weigela* genus has been the subject of several studies leading to the isolation of such triterpene glycosides. Sixteen saponins were thus extracted from several *Weigela* hybrids and cultivars: *W.* x Styriaca, *W. florida* "Minor black" and *W. florida* "Brigela". Four of the molecules were never reported in the literature before: 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid, 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid, 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid, and 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid. Their full structural elucidation, reported in this article, required extensive 1D and 2D NMR experiments coupled with mass spectrometry analysis. Six molecules among the known ones were in sufficient amount to be tested for their antifungal activity against *Candida albicans,* and their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Keywords:
Weigela x Styriaca
Weigela florida "Minor Black"
Weigela florida "Brigela"
Saponins
2D-NMR

1. Introduction

The Far Eastern *Weigela* Thunb. (or *Weigelia* Schreb.) is one of the most famous genera in the Caprifoliaceae family, containing a dozen species. Over 200 cultivars were produced [1], several of which are very popular ornamental shrubs. *Weigela* species, cultivars, and hybrids, such as *W. stelzneri* [2], *W. florida* "Rumba" [3], *W.* x "kosteriana variegata" [4], *W. florida* "Pink Poppet" [5] and *W. subsessilis* [6], are known for their richness in bioactive oleanolic acid and hederagenin glycosides. These glycosides have been already tested for their cytotoxicity and anti-inflammatory effects [2], their antibody recognition capacity [3], their stimulatory activity on melanogenesis [6], and their toxicological properties [4]. Other well-known properties of saponins are their action against phytopathogens, especially fungi [7]. All these biological results usually highlight important differences, in terms of activity, depending on the nature of the aglycone and the associated sugar chain(s). In order to give additional information on the structure/activity relationship of saponins regarding these anti-phytopathogens properties, and to complete the chemotaxonomic data on the *Weigela* genus, phytochemical studies of three *Weigela* cultivars and hybrid, *W. florida* "Minor Black", *W. florida* "Brigela" and *W.* x Styriaca, were carried out.

Herein, we report the isolation of sixteen different glycosides, four of which were previously undescribed (**1-4**) and twelve (**5-16**) were already published in the literature, namely 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid (**5**) [8], 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**6**) [9], 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid (**7**) [8], 3-*O*-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid (**8**) [9], 3-*O*-α-L-arabinopyranosylhederagenin 28-*O*-β-D-xylopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl ester (loniceroside A) (**9**) [10], 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosylhederagenin 28-*O*-β-D-xylopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl ester (**10**) [2], 3-*O*-α-L-arabinopyranosylhederagenin 28-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl ester (loniceroside K)

(**11**) [6], 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid 28-*O*-β-D-xylopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl ester (**12**) [5], 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**13**) [3], 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid (**14**) [8], 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rabinopyranosyloleanolic acid (**15**) [4], and 3-*O*-β-D-xylopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid (**16**) [5].

They were obtained from an aqueous-ethanolic (35:75) extract of the roots and the leaves of *W. florida* "Brigela", and the roots of *W. florida* "Minor Black" and *W.* x Styriaca, followed by several steps of solid/liquid chromatographic methods. Their structures were established by 1D and 2D NMR analysis (^{1}H, ^{13}C, COSY, TOCSY, ROESY, HSQC, HMBC) in combination with mass spectrometry (ESIMS), and by comparison of their spectral data with literature values. From the roots of *W. florida* "Brigela", seven monodesmosidic oleanolic acid glycosides were obtained (**1, 2, 5, 6, 13-15**), together with four bidesmosidic hederagenin esters from the leaves (**9-12**). Four monodesmosidic oleanolic acid glycosides were isolated from the roots of *W. florida* "Minor Black" (**2-4, 13**) and four others from the roots of *W.* x Styriaca (**6-8, 16**).

Furthermore, six of the isolated compounds (**5-10**) were evaluated for their antifungal activity against *Candida albicans*, and their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

2. Experimental

2.1. General experimental procedures

The 1D and 2D spectra (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, TOCSY, ROESY, HSQC and HMBC) were performed using an NMR Varian INOVA 600 MHz spectrometer (Agilent Technologies) equipped with 3 mm triple resonance inverse and 3 mm dual broadband probe heads. Spectra were recorded in pyridine-d_5. Solvent signals were used as internal standard (pyridine-d_5: δ_H = 7.21, δ_C = 123.5 ppm), and all spectra were recorded at T = 35°C. Pulse sequences were taken from the Varian pulse sequence library (gCOSY; gHSQCAD and

gHMBCAD with adiabatic pulses CRISIS-HSQC and CRISIS-HMBC). TOCSY spectra were acquired using DIPSI spin-lock and 150 ms mixing time. Mixing time in ROESY experiments was 300ms. Coupling constants (*J*) were measured in Hz. HRESIMS (positive-ion mode) were carried out on a Bruker micrOTOF II spectrometer, and ESIMS (negative-ion mode) on a Finnigan LCQ Deca. A MARS 6 microwave apparatus (CEM) was used for the extractions. The vacuum liquid chromatographies (VLC) were carried out using silica gel 60 (63-200 µm, Sigma-Aldrich) and RP-18 silica gel (75-200 µm, Silicycle). Column chromatography was done with Sephadex LH-20 (550 x 20 mm, GE Healthcare Bio-Sciences AB). Medium pressure liquid chromatographies (MPLC) used silica gel 60 (15-40 µm, Merck) and RP-18 silica gel (75-200 µm, Silicycle) with a Gilson M 305 pump (25 SC head pump, M 805 manometric module) and a Büchi glass column (203 x 15 mm and 460 x 15 mm) and precolumn (110 x 15 mm). Flash chromatography was carried out using a CombiFlash Retrieve (Teledyne ISCO) with RediSep Rf normal phase silica gel columns (20-40 µm). Thin-layer chromatography (TLC, Silicycle) and high-performance thin-layer chromatography (HPTLC, Merck) were carried out on precoated silica gel plates 60 F_{254}, solvent system $CHCl_3$/MeOH/H_2O (70:30:5). The spray reagent for saponins was vanillin reagent (1% vanillin in EtOH/H_2SO_4, 50:1).

2.2. Plant material

Weigela x Styriaca and *Weigela florida* "Brigela" were purchased from Jardiland® (Chenôve, France) in January and May 2016, respectively, and *Weigela florida* "Minor Black" from Botanic® (Quétigny, France) in May 2019. Voucher specimens were kept in the herbarium at the laboratory of pharmacognosy, Université de Bourgogne Franche-Comté, Dijon, France under the numbers, N° 2016/01/07, N° 2016/05/07, and N° 2019/05/16, respectively.

2.3. Extraction and isolation

The dried and powdered roots of *W. florida* "Minor Black" (7.10 g), *W. florida* "Brigela" (50.21 g), *W.* x Styriaca (38.56 g) and the dried and powdered leaves of *W. florida* "Brigela" (48.08 g), were submitted to three consecutive Microwave-Assisted Extractions (MAE)

(EtOH/H_2O, 75:35, 100 mL each). The microwave was programmed to reach 60°C in 10 min and then maintain this temperature for another 20 min.

For *W. florida* "Minor Black" (roots), after evaporation of the solvent under vacuum, 1.19 g of crude extract were dissolved in 50 mL of H_2O and successively partitioned with H_2O-saturated *n*-BuOH (50 mL each). Once evaporated, the *n*-BuOH extract (384.2 mg) was submitted to column chromatography using a Sephadex LH-20 column chromatography (MeOH 100%). Three fractions were obtained, and the first one (75.1 mg), rich in saponins, was purified by a normal phase MPLC (silica gel 60, $CHCl_3$/MeOH/H_2O, 80:20:2, 70:30:5, 2.5 mL/min), yielding compounds **2** (3.0 mg), **3** (5.5 mg), **4**, (2.3 mg), and **13** (4.3 mg).

For *W. florida* "Brigela", 4.68 g of the crude extract (11.32 g) from the roots were fractionated using a reversed phase VLC (RP-18 silica gel, gradient H_2O/EtOH 100:0, 50:50, 0:100, 300 mL each). The ethanolic fraction (585.2 mg) was purified by successive normal phase MPLC (silica gel 60, $CHCl_3$/MeOH/H_2O, 80:20:2, 70:30:5, 2.5 mL/min), yielding compounds **1** (2.1 mg), **2** (2.2 mg), **5** (3.1 mg), **6** (4.4 mg), **13** (2.6 mg), **14** (2.2 mg), **15** (1.7 mg). For the leaves, 13.96 g of crude extract (16.08 g) were used for a normal phase VLC (silica gel 60, $CHCl_3$/MeOH/H_2O, 70:30:5 60:32:7, 3 x 500 mL each). Four fractions were obtained and 137 mg of the fraction rich in saponins (fraction 2) were separated using a Sephadex LH-20 column (EtOH 100%). On the other hand, 1.00 g of crude extract were dissolved in 50 mL of H_2O and successively partitioned with H_2O-saturated *n*-BuOH (50 mL each). Once evaporated, the *n*-BuOH extract (208.3 mg) was submitted to a Sephadex LH-20 column chromatography (EtOH 100%). Then, fractions from the two different Sephadex separations were gathered based on their TLC profile (122.3 mg), and purified by successive normal phase MPLC (silica gel 60, $CHCl_3$/MeOH/H_2O, 80:20:2, 70:30:5, 2.5 mL/min), yielding compounds **9** (6.6 mg), **10** (2.2 mg), **11** (4.5 mg), **12** (3.1 mg).

For *W.* x Styriaca (roots), 5.37 g of crude extract were fractionated by several reversed phase VLC (RP-18 silica gel, gradient H_2O/EtOH 100:0, 50:50, 0:100, 350 mL each). The ethanolic fraction was submitted to successive normal phase flash chromatography ($CHCl_3$/MeOH/H_2O, 70:30:5, 9 mL/min), yielding compounds **6** (7.4 mg), **7** (4.6 mg), **8** (5 mg), **16** (3.1 mg).

*2.3.1. 3-O-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid (**1**)*

White, amorphous powder; ^{1}H and ^{13}C NMR data (600 MHz and 150 MHz, pyridine-d_5), see **Tables 1 and 2**; HR-ESIMS (positive-ion mode) *m/z* 1021.5342 $[M+Na]^+$ (calcd. for $C_{51}H_{82}NaO_{19}$, 1021.5348).

*2.3.2. 3-O-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**2**)*

White, amorphous powder; ^{1}H and ^{13}C NMR data (600 MHz and 150 MHz, pyridine-d_5), see **Tables 1 and 2**; HR-ESIMS (positive-ion mode) *m/z* 1021.5342 $[M+Na]^+$ (calcd. for $C_{51}H_{82}NaO_{19}$, 1021.5348).

*2.3.3. 3-O-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**3**)*

White, amorphous powder; ^{1}H and ^{13}C NMR data (600 MHz and 150 MHz, pyridine-d_5), see **Tables 1 and 2**; HR-ESIMS (positive-ion mode) *m/z* 1153.5748 $[M+Na]^+$ (calcd. for $C_{56}H_{90}NaO_{23}$, 1153.5771).

*2.3.4. 3-O-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**4**)*

White, amorphous powder; ^{1}H and ^{13}C NMR data (600 MHz and 150 MHz, pyridine-d_5), see **Tables 1 and 2**; HR-ESIMS (positive-ion mode) *m/z* 1285.6186 $[M+Na]^+$ (calcd. for $C_{61}H_{98}NaO_{27}$, 1285.6193).

2.4. Acid hydrolysis and GC analysis

A saponin rich fraction was hydrolyzed with 2N aq. CF_3COOH (5 mL) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 x 5 mL), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral, and then analyzed by TLC over silica gel ($CHCl_3$/MeOH/H_2O, 8:5:1) by comparison with authentic samples. Furthermore, the sugars residue was dissolved in anhydrous pyridine (100 µL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60 °C for 1 h, then 150 µL of HMDS-TMCS (hexamethyldisilazane/trimethylchlorosilane 3:1) was added, and the mixture was stirred at 60°C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under a N_2 stream. The residue was partitioned between *n*-hexane and H_2O (0.1 mL each), and the hexane layer (1 µL) was analyzed by GC [11]. The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich).

2.5. Antifungal and antibacterial assays

The yeast tested was *Candida albicans* (ATCC 90028) and the bacteria tested were *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853). The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method as recommended by the National Committee for Clinical Laboratory Standards using RPMI medium for *C. albicans* and Mueller-Hinton broth for bacteria [12,13]. Microtiter plates containing 50 µl of serial twofold dilutions of each antimicrobial agent per well were inoculated with 50 µl of a suspension of microorganisms to yield a cell density of $2.5x10^3$ CFU/ml for the yeast and 5×10^5 CFU/ml for bacteria. The tested compounds were dissolved in EtOH-H_2O mixture (50:50) and 50 µl were added to each well to obtain ten final concentrations (0.39 to 200 µg/ml). The microtiter plates were incubated at 35°C (20h for bacteria and 48h for the yeast). The MIC was the lowest concentration that prevented visible growth after incubation. One antifungal compound (fluconazole) and two antibiotics (ceftazidime and cefoxitine) were used as positive controls.

3. Results and discussion

3.1. Structural elucidation

Aqueous-ethanolic (35:75) extracts of different cultivars and hybrid of the *Weigela* genus were subjected to multiple chromatographic steps (VLC, Sephadex, MPLC, see Experimental section) and yielded sixteen saponins (**1–16**) among which four are new (**1-4**).

The structural analysis of the isolated compounds was performed by 2D NMR and mass spectrometry. For each one, the ^{1}H-NMR and ^{13}C-NMR signals of the aglycone assigned from the 2D NMR spectra were in good agreement with those of both oleanolic acid and hederagenin, commonly encountered in the *Weigela* genus. The differences were located on the osidic moieties linked to the C-3 and C-28 of the aglycone. The monosaccharides were identified by extensive 2D NMR analysis (COSY, TOCSY, ROESY, HSQC, HMBC) as arabinopyranosyl, rhamnopyranosyl, xylopyranosyl, and glucopyranosyl units. Their absolute configurations were determined to be D for glucose (Glc) and xylose (Xyl), and L for arabinose (Ara) and rhamnose (Rha) using GC analysis (see Experimental section). The relatively large $^{3}J_{\text{H-1, H-2}}$ values of the Glc, Xyl, and Ara (5.3–8.5 Hz) indicated a β anomeric orientation for Glc and Xyl, and an α anomeric orientation for Ara. For the Rha moieties, large $^{1}J_{\text{H-1, C-1}}$ values at 165–168 Hz confirmed that the anomeric protons were in an equatorial α-pyranoid form. The complete structural analysis is detailed below, only for the previously undescribed glycosides (**1–4**).

For compound **1**, the HR-ESIMS (positive-ion mode) spectrum showed a pseudo-molecular ion peak at *m/z* 1021,5342 $[M+Na]^{+}$, indicating a molecular weight of 998 g/mol and a molecular formula of $C_{51}H_{82}O_{19}$. The ^{1}H and ^{13}C spectra of the aglycone part of compound **1** (**Table 1**) were in good accordance with the signals of oleanolic acid [2–4]. This was clear in the HSQC spectrum, with signals of seven classical angular methyl groups of a triterpene skeleton at δ_C/δ_H 28.0/1.37 (s) (CH_3-23), 17.0/1.20 (s) (CH_3-24), 15.4/0.86 (s) (CH_3-25), 17.3/0.99 (s) (CH_3-26), 26.0/1.32 (s) (CH_3-27), 33.1/0.97 (s) (CH_3-29), 23.6/1.02 (s) (CH_3-30), and signals of an ethylene bond at δ_C/δ_H 122.2/5.50 (CH-12). The chemical shifts at δ_C 88.4 for C-3 and 185.4 for C-28, suggested a monodesmosidic structure with an *O*-heterosidic linkage at the C-3 position and a free carboxylic function at C-28. For the sugar part of the molecule, the ^{1}H NMR spectrum of compound **1** showed four anomeric proton signals at δ_H 4.80 (br d, J = 7.2 Hz), 4.87 (d, J = 7.6 Hz), 5.32 (d, J = 7.2 Hz) and 6.47 (br s), which gave correlations in the HSQC spectrum with their corresponding anomeric carbons at δ_C 105.8, 103.5, 106.4 and 101.3, respectively. All protons and carbons signals of the sugars were identified by 2D NMR experiments, starting

mainly by the TOCSY experiment, and led to the identification of three Xyl (Xyl I-III) and one Rha (**Table 2**). The β-D-xylopyranosyl moiety was shown to be attached on the C-3 position of the aglycone by observation of an HMBC correlation between δ_H 4.80 (Xyl I H-1) and δ_C 88.4 (C-3). Moreover, the HMBC spectrum of compound **1** displayed long-range correlations at δ_H/δ_C 6.47 (Rha H-1)/77.2 (Xyl I C-2), 5.32 (Xyl II H-1)/82.3 (Rha C-3), and 4.87 (Xyl III H-1)/76.6 (Xyl II C-4), which suggested the structure of the oligosaccharidic part as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl. The linkages were ensured by the ROESY cross-peaks at δ_H/δ_H 4.80 (Xyl I H-1)/3.34 (H-3), 6.47 (Rha H-1)/4.21 (Xyl I H-2), 5.32 (Xyl II H-1)/4.76 (Rha H-3), and 4.87 (Xyl III H-1)/4.23 (Xyl II H-4). Based on the results above, the structure of compound **1** was elucidated as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid (**Fig. 1**).

For compound **2**, the HR-ESIMS (positive-ion mode) spectrum showed a pseudo-molecular ion peak at *m/z* 1021.5342 $[M+Na]^+$, indicating a molecular weight of 998 g/mol and a molecular formula of $C_{51}H_{82}O_{19}$, as compound 1. The HSQC spectrum of the sugar part showed four cross-peaks at δ_H/δ_C 4.87 (d, J = 7.6 Hz)/103.4, 4.88 (d, J = 5.3 Hz)/104.8, 5.28 (d, J = 7.0 Hz)/106.3 and 6.16 (br s)/101.2, indicating the presence of four sugar units. They were recognized as Ara, Xyl I, Xyl II and Rha (**Table 2**). All the NMR signals observed for compound **2** were similar to those of compound **1**, except for the pentosyl moiety directly linked to the C-3 of the aglycone which is an Ara in compound **2** instead of Xyl in compound **1**. This was confirmed by the HMBC correlation at δ_H/δ_C 4.88 (Ara H-1)/88.6 (C-3), and by the ROESY correlation at δ_H/δ_H 4.88 (Ara H-1)/3.31 (dd, J = 11.7, 4.1 Hz, H-3). Hence, the structure of compound **2** was established as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**Fig. 1**).

For compound **3**, the HR-ESIMS (positive-ion mode) spectrum showed a pseudo-molecular ion peak at *m/z* 1153.5748 $[M+Na]^+$, indicating a molecular weight of 1130 g/mol and a molecular formula of $C_{56}H_{90}O_{23}$. This molecular weight differs from that of compound **2** by only 132 amu, corresponding to a supplementary pentosyl group. The part of the HSQC spectrum corresponding to the osidic chain, showed cross-peaks at δ_H/δ_C 4.84 (d, J = 7.6 Hz)/103.1, 4.88 (d, J = 6.2 Hz)/104.8, 4.89 (d, J = 8,5 Hz)/103.4, 5.27 (d, J = 7.5 Hz)/106.3, and 6.16 (br s)/101.2, indicating the presence of five sugar units which were identified as Ara, Xyl I-

III and Rha. The complete assignment of the NMR data of compound **3** was achieved and the structure of a common sequence with compound **2** appeared as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**Tables 1 and 2**). The only difference between compound **2** and compound **3** was located at the Xyl II, which is terminal in **2** and substituted by a terminal osidic group at the C-4 position in **3**. Its ring protons were defined starting from the H-1 at δ_H 4.89 in the TOCSY spectrum and was identified as a β-D-xylopyranosyl moiety (Xyl III) by extensive 2D NMR analysis. This was proved by the HMBC correlation at 4.89 (Xyl III H-1)/76.6 (Xyl II C-4) and a ROESY cross-peak at δ_H/δ_H 4.89 (Xyl III H-1)/4.20 (Xyl II H-4). Thus, the structure of compound **3** was elucidated as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**Fig. 1**).

For compound **4**, the HR-ESIMS (positive-ion mode) spectrum showed a pseudo-molecular ion peak at *m/z* 1285.6186 $[M+Na]^+$, indicating a molecular weight of 1262 g/mol and a molecular formula of $C_{61}H_{98}O_{27}$. This molecular weight differs from compound **3** by only 132 amu, corresponding to a supplementary pentosyl group. The HSQC correlations at δ_H/δ_C 4.84 (d, J = 7.6 Hz)/103.1, 4.86/103.4, 4.88 (d, J = 6.2 Hz)/104.8, 4.90/103.6, 5.27 (d, J = 7.0 Hz)/106.3, and 6.16 (br s)/101.2, indicated the presence of six sugar units which were identified as Ara I, Xyl I-IV and Rha. The structural analysis of the oligosaccharidic chain linked to the C-3 position of the aglycone, revealed a common sequence between **2** and **3**, based on the COSY, ROESY, HSQC, and HMBC correlations, as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**Tables 1 and 2**). The only difference between compound **3** and **4** was located the C-4 position of the Xyl III, which was linked to a terminal monosaccharide, identified as a β-D-xylopyranosyl moiety (Xyl III) by extensive 2D NMR analysis. This was proved by the HMBC correlation at 4.90 (Xyl IV H-1)/76.6 (Xyl III C-4) and a ROESY cross-peak at δ_H/δ_H 4.90 (Xyl IV H-1)/4.27 (Xyl III H-4). Thus, the structure of compound **4** was elucidated as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyloleanolic acid (**Fig. 1**).

To conclude, the sixteen isolated glycosides possess close structures, and two structural elements can be observed. First, the presence or absence of a hydroxyl group in position 23, making the aglycon either hederagenin or oleanolic acid, respectively. Secondly, the number and

nature of the sugar moieties in the oligosaccharidic chains at position 3 and 28. It is interesting to note that molecules extracted from the roots were exclusively monodesmosides, with a single sugar chain, linked at the position 3 of oleanolic acid. On the contrary, the molecules from the leaves were bidesmosides, mostly of hederagenin, with one or two monosaccharides linked in C-3 and an additional oligosaccharidic chain in C-28. This fits what the literature already describes for the *Weigela* genus [2–6]. According to literature, and our conclusions, another characteristic feature of the monodesmosides from this genus is the sequence of the first three sugar units at C-3 of the aglycone: it can either be 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl or 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl. However, differences from what has been reported occur from the fourth sugar unit. Usually, the fourth unit is a β-D-glucopyranosyl moiety as compounds, and is often linked to two additional sugar units, creating linear or ramified sugar chains. Surprisingly, the four new monodesmosides isolated in our study possess linear chains, composed of β-D-xylopyranosyl units only from the third sugar unit.

3.2. Antifungal and antibacterial assays

Compounds **5-10** (**Fig. 2**) were tested for their activity against *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The minimum inhibitory concentrations (MICs) were more than 200 µg/mL for each compound, and they were thus considered inactive. However, those negative results can provide additional information regarding their structure/activity relationship.

The antifungal or antibacterial activity of saponins is mainly linked to their ability to permeabilize membranes [14–17]. The aglycone is able to penetrate inside the membrane layers and interact with “sterol-like” elements inside. This type of interaction is more likely to happen if the genin itself is “sterol-like” [14]. Nonetheless, molecules with a triterpenic skeleton can also possess the same kind of mechanism of action; the most known examples are the hederagenin derivatives [15]. The nature of the genin is only half of the elements required for the activity. Indeed, the genin by itself often does not show any activity [7]. The length and nature of the sugar chain in glycosides play a key role in membrane permeabilization. It is suggested that the spatial orientation of the sugar chain, relatively to the aglycon, can give the whole molecule an “axe” shape or L-shape, allowing it to penetrate and disorganize membranes [15]. This can also

explain why hederagenin derivatives are working when oleanolic acid derivatives are not. The steric hindrance of the additional OH group in position 23 might rotate the sugar chain in the adequate position for this "axe" shape. None of the tested molecules possess a steroidal genin. Two of them (**9** and **10**) do have an hederagenin, but they also are bidesmosides. The activity of bidesmosides is often reported as nonexistent [7,18,19]. This might be due to the fact that, in those molecules, the genin is sandwiched between two sugar chains, making it difficult to actually reach the membrane as it should. The rest of the molecules are oleanolic acid derivatives with a single sugar chain, comporting sugar units of different nature. Some previous studies suggested that a terminal Ara unit is improving the activity [20], a feature that none of our molecules possess. According to the same authors, the nature of the pre-terminal sugar unit is also important. It might be because, depending on the combination of the two terminal sugar units, the molecule will or will not adopt an "axe" shape, thus influencing its effect on the membrane.

4. Conclusion

Sixteen (**1**-**16**) saponins, including four new ones (**1**-**4**), were extracted from *Weigela* x Styriaca, *W. florida* "Minor Black" and *W. florida* "Brigela". The structure of those molecules is in accordance with what is expected from the *Weigela* genus: in the roots are mostly found monodesmosides with an oleanolic acid as aglycon, and mostly bidesmosides with hederagenin in the leaves.

Isolated compounds that were in sufficient amount (**5**-**10**) were tested for their antifungal activity against *Candida albicans* and their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. While none of them showed any interesting effect, those results made it possible to corroborate some hypotheses that were made regarding the structure/activity relationship of saponins. Indeed, as suggested by previous articles, the number of sugar chains and nature of the genin seems to drastically change the potency of the molecules, as it probably changes the 3D conformation of the whole structure.

It will be interesting to be able to further confirm these theories by testing additional compounds, and using reliable 3D modeling software to get a grasp of how their conformation can impact their activities.

Conflict of interest

The authors have declared no conflict of interest.

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Table 1. ^{13}C and ^{1}H NMR spectroscopic data of the aglycone moieties of **1-4** in pyridine-d_5 (δ in ppm, J in Hz)

	1		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.8	0.99 m, 1.52 m	38.7	0.96 m, 1.50 m	38.7	0.96 m, 1.50 m	38.7	0.96 m, 1.51 m
2	26.7	1.89 m, 2.13 m	26.4	1.84 m, 2.10 m	26.4	1.84 m, 2.10 m	26.4	1. 86 m, 2.10 m
3	88.4	3.34	88.6	3.31 dd (11.7, 4.1)	88.6	3.30	88.7	3.31
4	39.4	–	39.4	–	39.4	–	39.4	–
5	55.9	0.84	55.8	0.83	55.8	0.82	55.8	0.84
6	18.4	1.29, 1.54 m	18.3	1.29, 1.54 m	18.3	1.39, 1.53 m	18.4	1.30, 1.53 m
7	33.1	1.28, 1.47 m	33.0	1.28, 1.49 m	33.0	1.28, 1.49 m	33.1	1.30, 1.47 m
8	39.5	–	39.5	–	39.5	–	39.5	–
9	47.9	1.68	47.9	1.67	47.9	1.67	47.9	1.67
10	36.8	–	36.8	–	36.8	–	36.9	–
11	23.6	1.93	23.6	1.92	23.6	1.92	23.7	1.92
12	122.2	5.50	122.3	5.50	122.3	5.50	121.9	5.51
13	144.8	–	144.7	–	144.6	–	144.7	–
14	41.8	–	41.8	–	41.8	–	42.0	–
15	28.1	1.22, 2.19	28.1	1.21 m, 2.15	28.1	1.21 m, 2.15	28.1	1.21 m, 2.20
16	23.5	2.01 m, 2.14	23.5	1.98 m, 2.14	23.4	1.98 m, 2.14	23.5	2.03 m, 2.13
17	46.6	–	46,6	–	46.5	–	46.6	–
18	42.0	3.31	42.0	3.30 d (11.7)	42.0	3.29	42.0	3.34
19	46.5	1.30, 1.84	46.3	1.31 d (11.7), 1.82 t (11.7)	46.3	1.29, 1.82	46.5	1.32, 1.83
20	30.8	–	30.7	–	30.7	–	30.6	–
21	34.1	1.22 m, 1.46 m	34.0	1.21 m, 1.46 m	34.0	1.21 m, 1.46 m	34.1	1.21 m, 1.44 m
22	33.0	1.84, 2.04 m	33.0	1.82, 2.03 m	32.9	1.82, 2.02 m	33.0	1.85, 2.05 m
23	28.0	1.37 s	28.0	1.30 s	28.0	1.30 s	28.0	1.30 s
24	17.0	1.20 s	16.9	1.13 s	16.9	1.12 s	16.9	1.13 s
25	15.4	0.86 s	15.4	0.85 s	15.4	0.85 s	15.4	0.85 s
26	17.3	0.99 s	17.2	0.99 s	17.2	0.99 s	17.3	1.01 s
27	26.0	1.32 s	26.0	1.33 s	26.0	1.33 s	26.0	1.33 s
28	180.4	–	Nd	–	180.4	–	Nd	–
29	33.1	0.97 s	33.1	0.98 s	33.1	0.98 s	33.2	0.97 s
30	23.6	1.02 s	23.6	1.03 s	23.6	1.02 s	23.7	1.04 s

Overlapped proton signals are reported without designated multiplicity. Nd: Not determined

Table 2. ^{13}C and ^{1}H NMR spectroscopic data of the sugar moieties of **1**-**4** in pyridine-d_5 (δ in ppm, J in Hz)

	1		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
Ara-1			104.8	4.88 d (5.3)	104.8	4.88 d (6.2)	104.8	4.88 d (6.2)
2			75.3	4.56 dd (7.6. 5.9)	75.3	4.54 t (6.2)	75.3	4.56 dd (7.0, 6.4)
3			73.7	4.29	73.8	4.25	73.8	4.29 dd (7.6, 2.9)
4			68.7	4.34	68.7	4.25 m	68.7	4.33
5			65.0	3.86 d (9.4) 4.36 dd (9.4. 4.7)	65.0	3.81 dd (11.2, 1.6) 4.31 dd (11.2, 3.6)	65.0	3.86 d (10.5)
Rha-1	101.3	6.47 br s	101.2	6.16 br s	101.2	6.16 br s	101.2	6.16
2	71.4	4.98 br s	71.4	4.90 d (3.5)	71.4	4.84 br s	71.4	4.88
3	82.3	4.76	82.1	4.77 dd (9.4, 3.5)	82.0	4.66 dd (9.2, 2.8)	82.0	4.70 dd (9.4, 2.9)
4	72.4	4.46 t (9.2)	72.4	4.48 dd (10.0, 9.4)	72.4	4.44	72.3	4.48 t (9.4)
5	69.4	4.75	69.5	4.61 dq (9.4, 6.4)	69.5	4.59	69.5	4.61 dq (9.4, 5.9)
6	18.4	1.65 d (7.2)	18.2	1.58 d (6.4)	18.2	1.56 d (6.0)	18.2	1.58 d (5.9)
Xyl I-1	105.8	4.80 (7.2)	106.3	5.28 d (7.0)	106.3	5.27 d (7.5)	106.3	5.27 d (7.0)
2	77.2	4.21	74.9	4.10 dd (8.8, 7.6)	74.9	4.09 t (8.2)	74.9	4.10 t (8.2)
3	79.0	4.21	75.3	4.15	75.3	4.16	75.1	4.15
4	71.1	4.21	76.7	4.21	76.7	4.22	76.0	4.23
5	66.6	3.73 dd (10.0, 9.4) 4.36 br d (9.1)	64.4	3.64 dd (11.1, 10.6) 4.39 dd (11.7, 5.3)	64.4	3.62 dd (11.6, 10.4) 4.38	64.4	3.63, 4.39
Xyl II-1	106.4	5.32 d (7.2)	103.4	4.87 d (7.6)	103.1	4.84 d (7.6)	103.1	4.84 d (7.6)
2	74.9	4.12 t (7.2)	74.9	3.99 t (8.2)	73.4	4.00 dd (8.5, 8.1)	73.6	3.99
3	75.4	4.17	77.4	4.17	75.1	4.17	74.9	4.18
4	76.7	4.23	70.5	4.18	76.6	4.20	76.3	4.24
5	64.5	3.66 4.24 dd (11.6, 3.5)	66.9	3.69 dd (11.1, 10.0) 4.31	64.5	3.66 dd (11.3, 10.7) 4.43 dd (11.6, 5.1)	64.5	3.66, 4.43
Xyl III-1	103.5	4.87 d (7.6)			103.4	4.89 d (8,5)	103.4	4.86
2	73.5	4.00 dd (7.3, 6.9)			73.6	4.01 dd (8.8, 8.4)	73.6	4.01
3	77.4	4.17			77.4	4.15	74.5	4.20
4	70.5	4.18			70.5	4.18	76.6	4.27
5	67.0	3.70 4.32 dd (8.5, 3.5)			67.0	3.71 dd (10.9, 10.0) 4.32	64.4	3.70, 4.46
Xyl IV-1							103.6	4.90
2							73.6	4.02
3							77.3	4.18
4							70.5	4.19
5							66.9	3.71, 4.32

Overlapped proton signals are reported without designated multiplicity.

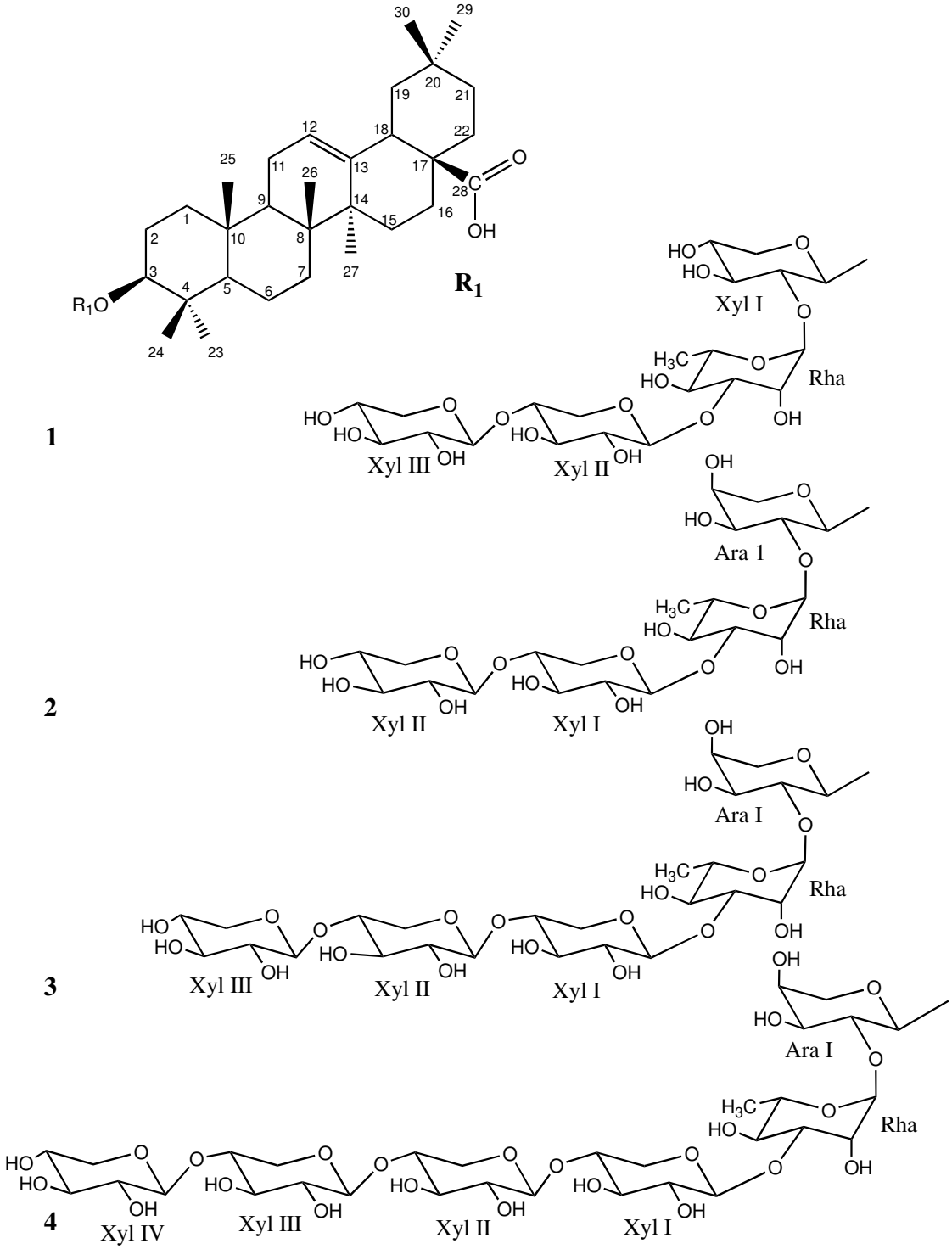


Figure 1. Structures of compounds **1-4**.

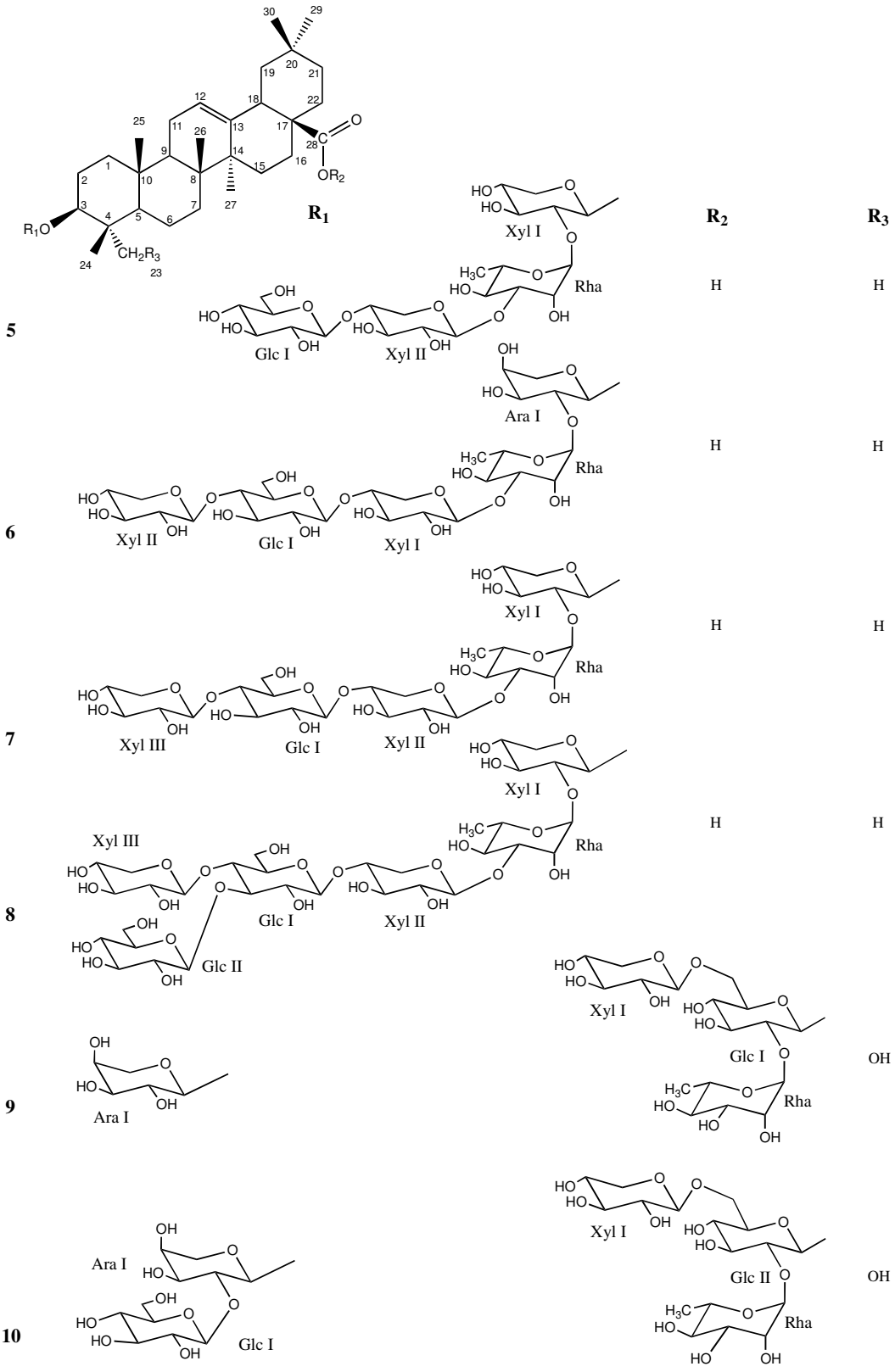


Figure 2. Structures of compounds **5-10**

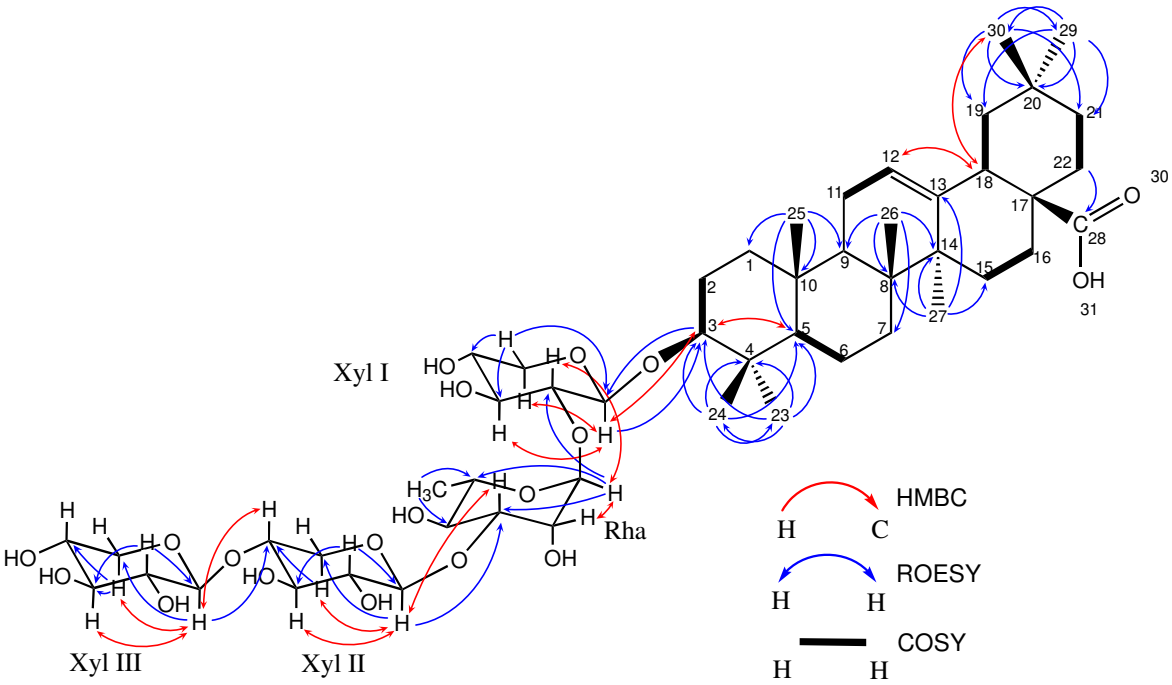
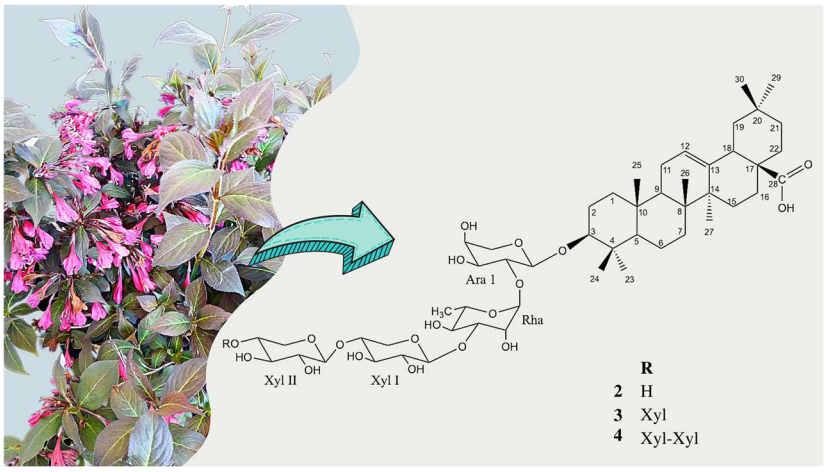


Figure 3. Key HMBC, COSY and ROESY correlations in compound **1**


OH
HO
Ara 1
O
Rha
H3C
HO
RO
HO
OH
Xyl II
Xyl I
R
2 H
3 Xyl
4 Xyl-Xyl