Triterpenoid Saponins from *Cephalaria transsylvanica*

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Two new triterpenoid saponins, transsylvanosides G and H, were isolated from *Cephalaria transsylvanica* and their structures established as 3-*O*-[*β*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl]hederagenin 28-*O*-[*β*-D-glucopyranosyl(1→4)-*β*-D-xylopyranosyl] ester (**1**) and 3-*O*-[*β*-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl-(1→2)-*β*-D-xylopyranosyl]hederagenin (**2**), respectively. A novel prosapogenin (**3**) was obtained on the alkaline hydrolysis of **1** and its structure defined as 3-*O*-[*β*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl]hederagenin. The structures of compounds **1**−**3** were established by spectral and chemical methods.

Cephalaria transsylvanica L. (*Dipsacaceae*) is an annual flowering plant with lilac flowers. It grows in the west and northwest of Turkey, central Anatolia, and south and central Europe, especially in Romania and in some parts of Russia.[1] *Cephalaria* species have been used as folk medicines for their alleviative, antiinfectant, hypothermic, and relaxant activities.[2,3] In our previous studies on this plant, we have isolated several triterpenoid saponins and triterpenoid acids.[4−8] This paper describes the isolation and structural determination of a bisdesmosidic (**1**) and a monodesmosidic (**2**) triterpenoid saponin, along with a prosapogenin (**3**), from *C. transsylvanica* (Chart 1).

Transsylvanosides G (**1**) and H (**2**) were isolated by repetitive column chromatography (CC) on Si gel from *n*-butanol-soluble fractions of a methanolic extract of the flowers of *C. transsylvanica*. Compound purification was conducted by preparative TLC. The saponins were detected by a Liebermann−Burchard test[9] and by froth formation when shaken with water. A common aglycon (**4**) was obtained by the acidic hydrolysis of **1** and **2** with 2 M HCl−MeOH (1:1), which was identified by spectroscopic and cochromatographic means.[4,10] The examination of the aqueous layers gave D-glucose, D-xylose, and L-rhamnose for **1** and D-xylose and L-rhamnose for **2** by TLC and paper chromatography using previously published solvent systems.[11,12] Analysis of the silylated sugars by GC[13] gave a glucose−xylose−rhamnose ratio of 3:2:1 for **1** and a xylose−rhamnose ratio of 2:1 for **2**.

After alkaline hydrolysis of **1** a prosapogenin (**3**) and the monosaccharides glucose and xylose were obtained. Qualitative and quantitative sugar analyses were carried out on the aqueous phase of the prosapogenin (**3**) obtained after acidic hydrolysis. Glucose, rhamnose, and xylose were found in the ratio 2:1:1. All these findings indicated that one glucose and one xylose were affixed at the C-28 position of the aglycon through an ester linkage. The ^{13}C-NMR data of **1** and **2** supported the proposed structures with anomeric carbon signals at δ 95.5, 101.3, 104.4, 104.7, 105.0, 106.3 ppm in the ^{13}C-NMR spectrum of **1** and that δ 101.2, 104.6, 107.4 ppm in the ^{13}C-NMR spectrum of **2**.[14−16] One of the anomeric carbon signals (δ 95.5) resonated at relatively high field in the ^{13}C-NMR spectrum of compound **1**, indicating a 28-*O*-glycosidic linkage. In the IR spectrum of **2**, C=O group absorption was observed at 1697 cm^{-1} in addition to OH and C=C absorptions (3412, 1632 cm^{-1}). The IR spectrum of **1** differed from **2** by the ester group absorption observed at 1730 cm^{-1}. The C-28 resonance of **1** appeared at *ca.* 176.5 ppm in the ^{13}C-NMR spectrum, whereas the chemical shift of the corresponding carbon in hederagenin occurred at *ca.* 180.0 ppm.[5] The presence of a 3-*O*-glycosidic linkage in **1** and **2** was inferred from downfield shifts of C-3 at δ 83.2 in **1** and δ 82.6 in **2**. In contrast, in hederagenin (**4**) this carbon signal (C-3) is observed at *ca.* 76.4 ppm.[10] Thus, compound **1** was bisdesmosidic while compound **2** was a monodesmosidic saponin. The sequence of sugars in **1** was established from its negative-ion FAB-MS spectrum which exhibited a deprotonated molecular ion peak $[M - H]^-$ at *m/z* 1367 indicating a molecular mass of 1368 Da. The molecular ion peak was observed at *m/z* 1391 $[M + Na]^+$ in the positive-ion FAB-MS. Diagnostic fragment ion peaks at *m/z* 1205 $[M - H - glc]^-$, 1073 $[M - H - glc - xyl]^-$, 911 $[M - H - 2glc - xyl]^-$, 749 $[M - H - 3glc - xyl]^-$, 603 $[M - H - 3glc - xyl - rha]^-$, and 471 $[M - H - 3glc - 2xyl - rha]^-$ were observed sequentially. The glycosidic linkages of the sugars of **1** were established after GC−MS analysis of the methylated methyl monosaccharides in which 2,3,4,6-tetra-*O*-methyl-*β*-D-glucopyranoside, methyl 2,3,6-tri-*O*-methyl-*β*-D-glucopyranoside, methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside, methyl 3,4-di-*O*-methyl-*β*-D-xylopyranoside, and methyl 2,3-di-*O*-methyl-*β*-D-xylopyranoside were obtained. The anomeric configurations of the sugars of **1** were fully defined by the ^{1}H-NMR spectral data. Thus, the anomeric proton signals at δ 6.23 (1H, d, J = 8.1 Hz, H-1 of *β*-xyl), 6.17 (1H, br s, H-1 of α-rha), 5.36 (1H, d, J = 8.3 Hz, H-1 of *β*-glc), 5.20 (1H, d, J = 7.3 Hz, H-1 of *β*-xyl), 5.12 (1H, d, J = 7.7 Hz, H-1 of *β*-glc), and 5.00 (1H, d, J = 7.5 Hz, H-1 of *β*-glc) led to the assignment of the configurations of five monosaccharide units as *β* and one as α. On the basis of all of the above evidence, the structure of **1** was

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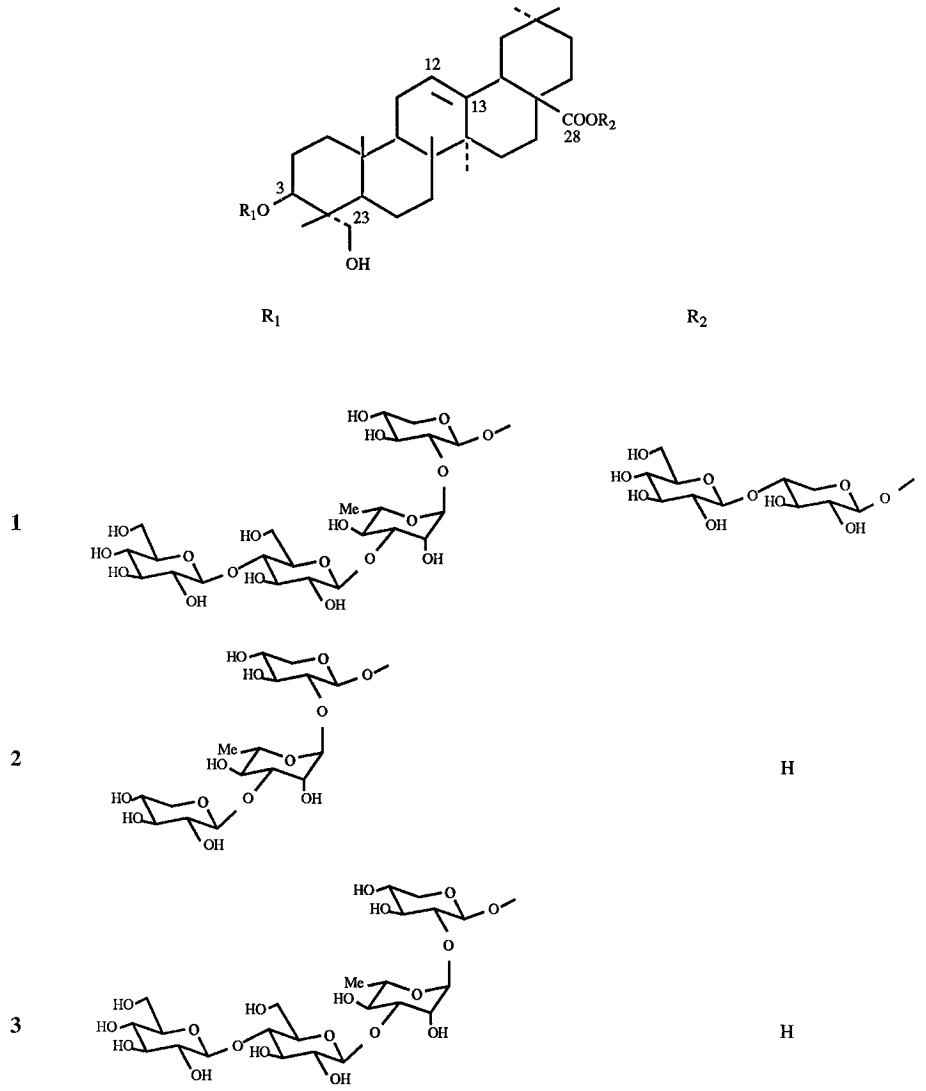
Chart 1



elucidated as 3-*O*-[*β*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl (1→2)-*β*-D-xylopyranosyl]hederagenin 28-*O*-[*β*-D-glucopyranosyl(1→4)-*β*-D-xylopyranosyl] ester.

In the negative-ion FAB-MS of **2** the sequence of the sugars was established from diagnostic ions at *m*/*z* 881 $[M - H]^-$, 749 $[M - H - xyl]^-$, 603 $[M - H - xyl - rha]^-$, and 471 $[M - H - 2xyl - rha]^-$. The molecular ion peak was confirmed by a positive-ion FAB-MS peak at *m*/*z* 905 $[M + Na]^+$. Anomeric proton signals were observed at *δ* 5.34 (1H, d, *J* = 7.0 Hz, H-1 of *β*-xyl), 5.06 (1H, d, *J* = 6.5 Hz, H-1 of *β*-xyl), and 4.91 (1H, br s, H-1 of α-rha) in the ^{1}H-NMR spectrum. Methyl 2,3,4-tri-*O*-methyl-*β*-D-xylopyranoside, methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside, and methyl 3,4-di-*O*-methyl-*β*-D-xylopyranoside were detected after methylation of the monosaccharides produced after hydrolysis and established linkage points of the units. Hence, **2** was characterized as 3-*O*-[*β*-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl]hederagenin.

The sequence of the sugars in prosapogenin **3** was observed at *m*/*z* 1073 $[M - H]^-$, 911 $[M - H - glc]^-$, 749 $[M - H - 2glc]^-$, 603 $[M - H - 2glc - rha]^-$, and 471 $[M - H - 2glc - rha - xyl]^-$. Confirmation of the molecular ion peak was obtained from the positive-ion FAB-MS peak at *m*/*z* 1097 $[M + Na]^+$. In the ^{1}H-NMR spectrum of **3**, the anomeric proton signals at *δ* 5.27 (1H, d, *J* = 7.6 Hz, H-1 of *β*-glc), 5.10 (1H, d, *J* = 6.7 Hz, H-1 of *β*-xyl), 5.03 (1H, d, *J* = 7.5 Hz, H-1 of *β*-glc), and 4.90 (1H, br s, H-1 of α-rha) led to the assignment of the anomeric configurations of the monosaccharides, which were supported by their ^{13}C-NMR signals (see Table 1). The glycosidic linkages of the sugars were established after GC−MS analysis of the methylated methyl monosaccharides as methyl 2,3,4,6-tetra-*O*-methyl-*β*-D-glucopyranoside, methyl 2,3,6-tri-*O*-methyl-*β*-D-glucopyranoside, methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside, and methyl 3,4-di-*O*-methyl-*β*-D-xylopyranoside. Compound **3** was therefore identified as 3-*O*-[*β*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl]hederagenin.

Experimental Section

General Experimental Procedures. Melting points were determined on a electrothermal Digital melting point apparatus. Optical rotations were measured using a Schmidt-Haensch Polartronic E polarimeter. IR spectra were run as KBr pellets on a Bruker IFS-48 FT-IR spectrometer. ^{1}H and ^{13}C-NMR (APT) spectra were recorded on a Bruker AC-200 L instrument operating at 200 and 50.32 MHz, respectively, using pyridine-d_5 as solvent. Chemical shifts are expressed in ppm from

Table 1. ^{13}C-NMR Chemical Shifts of **1**−**3** (in Pyridine-d_5)

		compound		
carbon		**1**	**2**	**3**
aglycon moiety				
	C-1	38.7	38.7	38.8
	C-2	26.0	25.8	25.8
	C-3	83.2	82.6	82.6
	C-4	45.3	45.0	45.0
	C-5	47.6	47.8	47.6
	C-6	20.5	20.3	20.3
	C-7	32.9	32.9	32.9
	C-8	40.0	39.7	39.8
	C-9	48.2	48.2	48.2
	C-10	37.2	37.0	37.0
	C-11	23.9	23.5	23.6
	C-12	122.0	122.4	122.1
	C-13	144.1	144.7	144.3
	C-14	42.0	42.2	42.2
	C-15	28.4	28.5	28.4
	C-16	23.4	23.8	23.9
	C-17	47.0	46.9	46.7
	C-18	41.7	42.2	42.4
	C-19	47.1	46.8	47.0
	C-20	30.8	31.1	30.8
	C-21	34.0	34.5	34.1
	C-22	32.6	33.0	32.9
	C-23	69.5	67.2	68.4
	C-24	14.0	13.8	13.7
	C-25	16.3	16.2	16.2
	C-26	17.7	17.7	17.8
	C-27	26.2	26.3	26.3
	C-28	176.5	180.1	180.1
	C-29	33.3	33.4	33.4
	C-30	23.8	24.0	23.8
3-*O*-sugar moieties				
xyl	C-1	106.3	107.4	106.6
	C-2	78.8	78.2	78.7
	C-3	74.2	75.5	74.0
	C-4	70.8	71.0	71.0
	C-5	63.9	64.6	64.0
rha	C-1	101.3	101.2	101.3
(→^{2}xyl)	C-2	71.4	71.9	71.6
	C-3	81.0	81.0	81.0
	C-4	72.8	72.6	72.9
	C-5	69.5	69.4	69.6
	C-6	18.3	18.3	18.4
glc	C-1	104.7		104.9
(→^{3}rha)	C-2	74.8		74.0
	C-3	78.3		78.1
	C-4	78.5		78.4
	C-5	76.5		76.6
	C-6	62.2		62.6
glc	C-1	104.4		104.6
(→^{4}glc)	C-2	73.9		74.8
	C-3	77.8		77.8
	C-4	71.1		71.1
	C-5	74.9		74.0
	C-6	61.7		62.3
xyl	C-1		104.6	
(→^{3}rha)	C-2		73.9	
	C-3		75.0	
	C-4		69.6	
	C-5		63.8	
28-*O*-sugar moieties				
xyl	C-1	95.5		
	C-2	75.1		
	C-3	77.5		
	C-4	78.4		
	C-5	66.0		
glc	C-1	105.0		
(→^{4}xyl)	C-2	75.3		
	C-3	76.5		
	C-4	72.0		
	C-5	78.0		
	C-6	62.0		

TMS as internal standard. FAB-MS spectra were obtained in the negative- and positive-ion modes. Poly(ethylene glycol) was used as a matrix on a VG 20-250 quadrupole. The same mass spectrometer was used to record EI MS spectra (70 eV ion beam energy, 200 °C ion source). GC and GC-MS analyses were carried out using a Hewlett-Packard GC (5870)−MSD (5917) combined system (column, 1 μm × 0.25 mm × 30 m, SPB-5; He, temperature 130−280 °C, 3° min^{-1}). Si gel 60 (Merck 7743) and Si gel 60 (Merck 7747) were used for CC and preparative TLC, respectively. TLC was performed on precoated Si gel 60 (Merck 5554) plates by spraying with 10% H_2SO_4 solution. Sugar analysis was carried out on precoated Cellulose F (Merck 5574) plates. Spots were visualized by spraying aniline phthalate reagent followed by heating. For paper chromatography Schleicher and Schüll 2043-b chromatography paper was used in the descending mode. The following chromatographic solvent systems were used: A, $CHCl_3$−MeOH−H_2O (65:25:10); B, $CHCl_3$−MeOH−H_2O (65:35:10); C, $CHCl_3$−MeOH−H_2O (65:42:10); D, $CHCl_3$−MeOH (15:1); E, $CHCl_3$−EtOH (97:3); F, EtOAc−pyridine−H_2O (2:1:2); G, *n*-BuOH−EtOH−H_2O (2:1:1 and 4:1:5). For solvent systems A−C, the lower phases were utilized.

Plant Material. The plant material was collected from Bornova, İzmir, Turkey, in July 1994. A voucher specimen is deposited (No. 7435) in the Herbarium of Ege University.

Extraction and Isolation. The air-dried and powdered flowers (1.5 kg) of *C. transsylvanica* were extracted with 80% MeOH. The dried MeOH residue was washed with hexane, $CHCl_3$, and Me_2CO to remove nonglycosidic substances. The remaining residue was dissolved in H_2O and extracted with *n*-BuOH. The butanol layer was evaporated under reduced pressure at *ca*. 40 °C. The crude mixture (10 g) was chromatographed repetitively over Si gel columns using solvent systems A−C. Fractions were purified by preparative TLC using solvent system B to afford two new saponins, **1** (83 mg) and **2** (65 mg), as amorphous powders.

Transsylvanoside G [3-*O*-[*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl (1→3)-α-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl]hederagenin 28-*O*-[*β*-D-glucopyranosyl(1→4)-*β*-D-xylopyranosyl] ester] (1): amorphous powder; $[\alpha]^{25}_D$ − 86.1° (*c* 2.35, MeOH), IR (KBr) ν_{max} 3400 (OH), 1730 (COOR), 1630 (C=C) cm^{-1}; ^{1}H NMR (pyridine-d_5, 200 MHz) δ 6.23 (1H, d, J = 8.1 of β-xyl ester), 6.17 (1H, br s, H-1 of α-rha), 5.36 (1H, d, J = 8.3 Hz, H-1 of terminal β-glc), 5.20 (1H, d, J = 7.3 Hz, H-1 of β-xyl), 5.12 (1H, d, J = 7.7 Hz, H-1 of terminal β-glc ester), 5.00 (1H, d, J = 7.5 Hz, H-1 of inner β-glc), 5.40 (1H, br s, H-12), 3.18 (1H, m, H-3), 1.55 (3H, d, J = 5.7 Hz, Me of rha), 1.14 (3H, s, Me), 1.11 (3H, s, Me), 1.09 (3H, s, Me), 0.99 (3H, s, Me), 0.89 (3H, s, Me), 0.86 (3H, s, Me); ^{13}C NMR (pyridine-d_5, 50.32 MHz), see Table 1; negative-ion FAB-MS *m*/*z* 1367 [M − H]$^-$, 1205 [M − H − glc]$^-$, 1073 [M − H − glc − xyl]$^-$, 911 [M − H − 2glc − xyl]$^-$, 749 [M − H − 3glc − xyl]$^-$, 603 [M − H − 3glc − xyl − rha]$^-$, 471 [M − H − 3glc − 2xyl − rha]$^-$.

Transsylvanoside H [3-*O*-[*β*-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl]hederagenin] (2): amorphous powder; $[\alpha]^{15}_D$ +69.6° (*c* 1.26, MeOH); IR ν_{max} (KBr) 3412 (OH), 1697 (COOH), 1632 (C=C) cm^{-1}; ^{1}H NMR (pyridine-d_5, 200 MHz) δ 5.34 (1H, d, J = 7.0 Hz, H-1 of β-xyl), 5.06 (1H, d, J =

6.5 Hz, H-1 of terminal β-xyl), 4.91 (1H, br s, H-1 of α-rha), 5.45 (1H, br s, H-12), 3.26 (1H, m, H-3), 1.50 (3H, d, J = 5.8 Hz, Me of rha), 1.23 (3H, s, Me), 1.12 (3H, s, Me), 1.00 (3H, s, Me), 0.96 (3H, s, Me), 0.91 (3H, s, Me), 0.85 (3H, s, Me); ^{13}C NMR (pyridine-d_5, 50.32 MHz), see Table 1; negative-ion FAB-MS m/z 881 [M − H]$^-$, 749 [M − H − xyl]$^-$, 603 [M − H − xyl − rha]$^-$, 471 [M − H − 2xyl − rha]$^-$.

Alkaline Hydrolysis of Compounds 1 and 2. Compounds **1** and **2** (25 mg each) were dissolved separately in MeOH (5 mL). After dry methanolic $NaOCH_3$ was added up to pH 12−13, the solutions were left overnight at room temperature. The reaction mixtures were neutralized with 2 M HCl and then concentrated to dryness *in vacuo*. The residues were extracted with *n*-BuOH. Hydrolysis of **1** afforded the prosapogenin **3** (20 mg), while the compound **2** was not affected by hydrolysis. The aqueous layer of compound **1** was analyzed by TLC, paper chromatography, and GC (solvent systems C, F, G), affording glucose and xylose in a 1:1 ratio.

3-*O*-[β-D-Glucopyranosyl(1→4)-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl]hederagenin (3): amorphous powder; $[\alpha]^{27}_D$ +16.8° (*c* 1.07, MeOH); IR (KBr) ν_{max} 3393 (OH), 1630 (COOH), 1693 (C=C) cm^{-1}; ^{1}H NMR (pyridine-d_5, 200 MHz) δ 5.27 (1H, d, J = 7.6 Hz, H-1 of β-glc), 5.10 (1H, d, J = 6.7 Hz, H-1 of β-xyl), 5.03 (1H, d, J = 7.5 Hz, H-1 of β-glc), 4.90 (1H, br s, H-1 of α-rha), 5.43 (1H, br s, H-12), 3.24 (1H, m, H-3), 1.57 (3H, d, J = 5.8 Hz, Me of rha), 1.16 (6H, s, 2 × Me), 1.02 (3H, s, Me), 1.00 (3H, s, Me), 0.84 (3H, s, Me), 0.82 (3H, s, Me); ^{13}C NMR (pyridine-d_5, 50.32 MHz), see Table 1; negative-ion FAB-MS m/z 1073 [M − H]$^-$, 911 [M − H − glc]$^-$, 749 [M − H − 2glc]$^-$, 603 [M − H − 2glc − rha]$^-$, 471 [M − H − 2glc − rha − xyl]$^-$.

Acid Hydrolysis of Compounds 1−3. Solutions of compounds (20 mg each) in 80% MeOH−benzene (1:1) (5 mL) were refluxed for 6 h at 95 °C after addition of 2 M HCl (5 mL) separately. The organic layers were evaporated *in vacuo*. Water was added to each reaction mixture, and the aglycon was extracted with $CHCl_3$. Each $CHCl_3$ extract was purified on a Si gel column using solvent system D, thereby affording the same aglycon. Thus, hederagenin (3β,23-dihydroxy-Δ^{12}-oleanen-28-carboxylic acid) was obtained from **1**, **2**, and **3** (9, 7, and 6 mg), respectively: mp 327 °C,[4,5] $[\alpha]^{25}_D$ + 77.1° (*c* 1.02, MeOH).[4,5]

The aqueous layers were combined and neutralized with a saturated solution of Na_2CO_3. The products were compared with standard sugars on TLC (solvent system C) and descending paper chromatography (solvent systems F and G) giving D-glucose, D-xylose, and L-rhamnose from **1**, D-xylose, and L-rhamnose from **2**, and D-glucose, D-xylose, and L-rhamnose from **3**. The sugar mixtures were silylated with trimethylchlorosilane and hexamethyldisilazane in dry pyridine for 1 h at 60 °C. Analysis of the silylated sugars by GC gave glucose−xylose−rhamnose (3:2:1 ratio) from **1**, xylose−rhamnose (2:1 ratio) from **2**, and glucose−xylose−rhamnose (2:1:1 ratio) from **3**.

Methylation of Compounds 1−3. Each compound (10 mg) was methylated using Ag_2O (0.15 g) and CH_3I (0.75 mL) in dry DMF. This procedure was repeated twice. Each crude permethylated product was purified by preparative TLC using solvent system E, with 9, 10, and 8 mg, respectively, obtained from **1**, **2**, and **3**. The permethylated saponins were methanolyzed for 5 h with 5% anhydrous methanolic HCl at 80 °C. The methylated methyl monosaccharides were identified by comparison with authentic samples. The GC−MS analysis of compound **1** gave methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (t_R 18.5 min), m/z 187, 149, 101, 88 (100), 75, 73, 71; methyl 2,3,6-tri-*O*-methyl-β-D-glucopyranoside (t_R 20.0 min), m/z 161, 149, 101, 88 (100), 74, 58, 43; methyl 2,3-di-*O*-methyl-β-D-xylopyranoside (t_R 11.6 min), m/z 161, 129, 101, 88 (100), 75, 74, 58; methyl 3,4-di-*O*-methyl-β-D-xylopyranoside (t_R 14.8 min), m/z 161, 101, 88, 75, 74 (100), 58; and methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside (t_R 12.9 min), m/z 161, 101, 88 (100), 74, 43. Compound **2** gave the following methylated methyl pyranosides: methyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside (t_R 19.5 min), m/z 176, 161, 130, 101, 88 (100), 75, 58; methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside (t_R 12.8 min), m/z 161, 101, 88 (100), 74, 43; and methyl 3,4-di-*O*-methyl-β-D-xylopyranoside (t_R 14.7 min), m/z 161, 101, 88, 75, 74 (100), 58. Compound **3** afforded methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (t_R 18.5 min) m/z 187, 149, 101, 88 (100), 75, 73, 71; methyl 2,3,6-tri-*O*-methyl-β-D-glucopyranoside (t_R 20.0 min), m/z 161, 149, 101, 88 (100), 74, 58, 43; methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside (t_R 12.8 min), m/z 161, 101, 88 (100), 74, 43; and methyl 3,4-di-*O*-methyl-β-D-xylopyranoside (t_R 14.7 min), m/z 161, 101, 88, 75, 74 (100), 58.

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