Triterpene Saponins from Four Species of the Polygalaceae Family

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Twelve triterpene saponins were isolated by successive MPLC over silica gel from four species of Polygalaceae: From *Polygala ruwenzoriensis*, five new saponins **1**–**5** of which **1**–**4** as two pairs of (*E*)/(*Z*)-isomers, together with the four known compounds tenuifoline, (*E*)- and (*Z*)-senegasaponin b, (*E*)- and (*Z*)-senegin II, and polygalasaponin XXVIII, from the genus *Carpolobia*, one new saponin **6** from *C. alba* and the known arilloside (**11**) from *C. lutea*, and another new triterpene glycoside **7** from *Polygala arenaria*. Their structures were established mainly by 600-MHz 2D-NMR techniques (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, HMBC) as 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*α*-L-arabinopyranosyl-(1 → 4)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(*E*)-4-methoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**1**) and its (*Z*)-isomer **2**, 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*α*-L-arabinopyranosyl-(1 → 4)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**3**) and its (*Z*)-isomer **4**, 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-[*O*-*β*-D-galactopyranosyl-(1 → 4)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranosyl] ester (**5**), 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*α*-L-arabinopyranosyl-(1 → 3)-*O*-[*β*-D-galactopyranosyl-(1 → 4)]-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[*β*-D-apiofuranosyl-(1 → 3)]-4-*O*-acetyl-*β*-D-fucopyranosyl} ester (**6**), and 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*β*-D-galactopyranosyl-(1 → 4)-*O*-[*β*-D-glucopyranosyl-(1 → 3)]-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranosyl} ester (**7**) (presenegenin = (2*β*,3*β*,4*α*)-2,3,27-trihydroxyolean-12-ene-23,28-dioic acid).

Introduction. – In a continuation of our study on saponin constituents of plants of the Polygalaceae family to find chimiotaxonomic markers [1–7], we have examined the saponin fraction of the roots of *Polygala ruwenzoriensis* CHOD. A saponin was previously isolated from the roots, possessing presenegenin (= (2*β*,3*β*,4*α*)-2,3,27-trihydroxyolean-12-ene-23,28-dioic acid) as aglycon and glucose, galactose, xylose, arabinose, fucose (= 6-deoxygalactose), and rhamnose (= 6-deoxymannose) as sugars, as shown by acid hydrolysis [8]. We describe in this paper the isolation and structural elucidation of five new triterpene saponins **1**–**5**, of which **1**–**4** were obtained as two inseparable mixtures **1/2** and **3/4** of their (*E*)- and (*Z*)-acyl derivatives, together with the four known compounds tenuifoline [3], (*E*)- and (*Z*)-senegasaponin b [9], (*E*)- and (*Z*)-senegin II [9], and polygalasaponin XXVIII [10].

Furthermore, as part of our ongoing phytochemical studies on *Carpolobia alba* G. DON. [3], *C. lutea* G. DON. [3], and *Polygala arenaria* WILLD. [5], we report here also the isolation and structural analysis of the two new triterpene saponins **6** and **7**, isolated from *C. alba* and *P. arenaria*, respectively, while a further compound, isolated from *C. lutea*, was the known arilloside C [11].

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HO, HO, HO, OH, OH, Glc, 3, COOH, OH, 28, O, O, Me, OR[1], OR[2], Fuc, O, Me, OR[3], OH, OH, Rha

	R^1	R^2	R^3
1	OMe (4-methoxycinnamoyl, α, β, γ)	H	Ara, Xyl
2	OMe (4-methoxycinnamoyl, α, β, γ)	H	Ara, Xyl
3	OMe, OMe (3,4-dimethoxycinnamoyl, α, β, γ)	H	Ara, Xyl
4	OMe, OMe (3,4-dimethoxycinnamoyl, α, β, γ)	H	Ara, Xyl
5	H	H	Gal, Xyl
6	Ac	HO, OH, OH	Gal, Xyl, Ara
7	H	H	Gal, Xyl, Glc

Results and Discussion. – A concentrated MeOH-soluble fraction of the 80% EtOH extract of the roots of *P. ruwenzoriensis* was purified by precipitation with Et_2O and treatment with charcoal yielding a crude saponin mixture [12]. The latter was further submitted to vacuum liquid chromatography (VLC; silica gel *RP-18*) and fractionated by repeated medium-pressure liquid chromatography (MPLC; normal silica gel) yielding compounds **1**–**5**, tenuifoline, (*E*)- and (*Z*)-senegasaponin b, (*E*)- and (*Z*)-senegin II, and polygalasaponin XXVIII. Their structures were elucidated mainly by 600-MHz NMR spectroscopy, including 1D- and 2D-NMR experiments (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, HMBC) and FAB-MS.

For all the isolated compounds, including those from *C. alba*, *P. arenaria*, and *C. lutea*, the ^{1}H- and ^{13}C-NMR signals of the prosapogenin, assigned from the 2D-NMR spectra, were in good agreement with those of tenuifoline (=3-*O*-(*β*-D-glucopyranosyl)presenegenin) commonly encountered in the Polygalaceae [1–3] [5–7]. The differences between them were located at the oligosaccharidic chain linked to C(28) of the aglycon. For the sugars characterized in this work by extensive 2D-NMR spectroscopy (*Tables 1* and *2*), the relatively large $^3J(1,2)$ values (6.9–8.4 Hz) of the glucopyranosyl (Glc), fucopyranosyl (Fuc), galactopyranosyl (Gal), xylopyranosyl (Xyl), and arabinopyranosyl (Ara) units suggested a *β*-anomeric form for Glc, Fuc, Gal, and Xyl, and an *α*-anomeric form for Ara [13]. The multiplicity of the anomeric proton of the rhamnopyranosyl (Rha) as a broad singlet (*s*) indicated an *α*-anomeric form for Rha. The common D-configuration of Fuc, Glc, Gal, and Xyl and the L-configuration of Rha and Ara were assumed, according to those most encountered among the plant glycosides in each case.

The molecular formula of compounds **1**/**2** was determined as $C_{68}H_{100}O_{30}$ by negative-ion FAB-MS which exhibited an *m/z* 1395 ($[M-H]^-$). Other fragment-ion peaks were observed at *m/z* 1263 ($[M-H-132]^-$), 1235 ($[M-H-160]^-$), and 1073 ($[M-H-160-162]^-$). These results revealed the loss of one terminal pentosyl, one 4-methoxycinnamoyl group, and one hexosyl moiety, respectively. On the basis of spectral evidence, mainly 2D-NMR, the structure of compounds **1**/**2** was elucidated as 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*α*-L-arabinopyranosyl-(1 → 4)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(*E*)-4-methoxycinnamoyl]-*β*-D-fucopyranosyl} ester[1]) and its (*Z*)-isomer, new natural triterpene glycosides.

Investigations based on the 2D-NMR spectra allowed us to identify the *(E)*- and *(Z)*-olefinic protons of a 4-methoxycinnamoyl moiety and to determine the complete assignments of all protons and C-atoms of this group (*Table 3*) which were in good agreement with [5]. This indicated that **1**/**2** was a mixture of *(E)*- and *(Z)*-4-methoxycinnamoyl triterpene glycosides. Such an (*E*)/(*Z*)-isomer behavior of the 4-methoxycinnamoyl moiety in MeOH solutions under light has often been described in many saponins from the Polygalaceae like *P. arenaria* [5] or *P. myrtifolia* [6].

The ^{1}H-NMR spectrum of **1**/**2** displayed signals for five anomeric protons at δ 6.38 (br. *s*), 5.93 (*d*, $J=8.3$ Hz), 5.09 (*d*, $J=6.9$ Hz), 4.90 (*d*, $J=7.1$ Hz), and 4.76 (*d*-like) which gave HSQC correlations with C-signals at δ 101.0, 94.0, 104.1, 103.4, and 107.0, respectively. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the ^{1}H,^{1}H-COSY, TOCSY, HSQC, and HMBC experiments (*Table 1*). After subtraction of the anomeric signals of the glucopyranosyl moi-

[1]) For systematic names, see the *Exper. Part.*

Table 1. *^{1}H-NMR Data* (600 MHz) *of the Sugar Moities of* **1**–**7** *in* (D_5)*Pyridine from 1D- and 2D-NMR Experiments*[a]). δ in ppm, *J* in Hz.

		1/2	**3/4**	**5**	**6**	**7**
3-*O*-Glc:	H–C(1)	5.09 (*d, J* = 6.9)	5.09 (*d, J* = 6.9)	4.93 (*d, J* = 7.8)	4.97 (*d, J* = 7.8)	4.93 (*d, J* = 7.5)
	H–C(2)	3.90	3.90	3.86	3.87 (*t, J* = 7.6)	3.86 (*t, J* = 7.5)
	H–C(3)	4.21	4.21	4.15	4.19 (*t, J* = 9.2)	4.17
	H–C(4)	3.95	3.95	3.92	3.98	3.97
	H–C(5)	3.90	3.90	3.82	3.83 (br. *d, J* = 9.1)	3.82 (br. *d, J* = 9.0)
	CH_2(6)	4.10, 4.30	4.10, 4.30	4.06, 4.28	4.12, 4.30	4.11, 4.29
28-*O*-Fuc:	H–C(1)	5.93 (*d, J* = 8.3)	5.93 (*d, J* = 8.3)	5.78 (*d, J* = 8.3)	5.92 (*d, J* = 8.3)	5.86 (*d, J* = 8.1)
	H–C(2)	4.64 (*t, J* = 8.3)	4.64 (*t, J* = 8.3)	4.51 (*t, J* = 8.7)	4.63 (*t, J* = 9.0)	4.71 (*t, J* = 8.6)
	H–C(3)	4.38	4.38	4.02	4.32	4.20
	H–C(4)	5.66	5.66	3.93	5.65	3.96
	H–C(5)	4.13	4.13	3.76	4.07	3.92
	Me(6)	1.22	1.22	1.37 (*d, J* = 6.4)	1.13 (*d, J* = 6.2)	1.40
	AcO–C(4)				1.92 (*s*)	
Rha:	H–C(1)	6.38 (br. *s*)	6.38 (br. *s*)	6.23 (br. *s*)	6.16 (br. *s*)	6.58 (br. *s*)
	H–C(2)	4.70 (br. *s*)	4.70 (br. *s*)	4.64 (br. *s*)	4.67 (br. *s*)	4.68 (br. *s*)
	H–C(3)	4.48 (*dd, J* = 9.0, 2.6)	4.48 (*dd, J* = 9.0, 2.6)	4.43 (*dd, J* = 9.5, 3.0)	4.44	4.50 (*dd, J* = 9.0, 2.5)
	H–C(4)	4.14	4.14	4.09	4.14	4.14
	H–C(5)	4.41	4.41	4.29	4.40	4.38 (*dq, J* = 9.3, 5.7)
	Me(6)	1.66	1.66	1.56 (*d, J* = 5.9)	1.67 (*d, J* = 5.7)	1.55 (*d, J* = 5.7)
Xyl:	H–C(1)	4.76 (*d*-like)	4.76 (*d*-like)	4.77 (*d, J* = 7.4)	4.81 (*d, J* = 7.4)	4.81 (*d, J* = 7.8)
	H–C(2)	3.93	3.93	3.90	3.93	3.94 (*t, J* = 7.6)
	H–C(3)	3.97	3.97	3.94	3.85	3.84
	H–C(4)	3.99	3.99	4.20	4.31	4.30
	H_α–C(5)	3.39	3.39	3.37 (br. *t, J* = 10.3)	3.42 (br. *t, J* = 10.4)	3.42 (br. *t, J* = 9.7)
	H_β–C(5)	4.30	4.30	4.26	4.38	4.32
Api[b]):	H–C(1)				5.71 (br. *s*)	
	H–C(2)				4.67 (br. *s*)	
	CH_2(4)				4.28, 4.55	
	CH_2(5)				4.01 (br. *s*)	
Ara[b]):	H–C(1)	4.90 (*d, J* = 7.1)	4.90 (*d, J* = 7.1)		4.92 (*d*-like)	
	H–C(2)	4.48	4.48		4.55	
	H–C(3)	4.07	4.07		3.92	
	H–C(4)	4.28	4.28		4.12	
	H_α–C(5)	3.40	3.40		3.52 (*d, J* = 11.0)	
	H_β–C(5)	4.12	4.12		4.18	
Glc[b]):	H–C(1)					4.94 (*d, J* = 7.5)
	H–C(2)					4.02 (*t, J* = 8.5)
	H–C(3)					4.08 (*t, J* = 8.5)
	H–C(4)					3.97
	H–C(5)					3.72
	CH_2(6)					4.14, 4.20
Gal[b]):	H–C(1)			4.83 (*d, J* = 7.1)	4.80 (*d, J* = 7.4)	4.81 (*d, J* = 7.8)
	H–C(2)			4.30	4.44	4.43
	H–C(3)			4.03	3.97	3.98
	H–C(4)			4.22	4.32	4.32
	H–C(5)			4.00	3.88	3.85
	CH_2(6)			4.04, 4.12	4.18, 4.24 (*dd, J* = 11.0, 6.7)	4.10, 4.23

[a]) Overlapped signals are reported without designated multiplicity. [b]) Terminal sugar moiety.

ety of the prosapogenin, the signals of four sugars units linked to C(28) of the presenegenin remained, which were identified as Fuc, Rha, Xyl, and Ara. A HMBC correlation between δ(H) 5.93 (*d*, *J* = 8.3 Hz, Fuc H–C(1)) and δ(C) 176.7 (Agly C(28)) established a linkage between the aglycon and the fucopyranosyl moiety. The location of the 4-methoxycinnamoyl group at C(4) of Fuc (δ(H) 5.66, Fuc H–C(4)) was determined by the TOCSY and COSY experiments, starting from the anomeric H-atom at δ(H) 5.93 of Fuc (*d*, *J* = 8.3 Hz, Fuc H–C(1)). The downfield shifts observed in the HSQC spectrum for the Fuc H–C(4) and C(4) resonances at δ(H) 5.66 and δ(C) 74.8, respectively, established that the secondary-alcohol function OH–C(4) of Fuc was acylated. In the NOESY plot, a correlation between δ(H) 6.38 (br. *s*, Rha H–C(1)) and δ(H) 4.64 (*t*, *J* = 8.3 Hz, Fuc H–C(2)) revealed the (1 → 2) linkage between these two sugar moieties. Cross-peaks in the HMBC spectrum between δ(H) 4.14 (Rha H–C(4)) and δ(C) 107.0 (Xyl C(1)) and in the NOESY plot between δ(H) 4.14 (Rha H–C(4)) and δ(H) 4.76 (*d*-like, Xyl H–C(1)) indicated that the xylose was linked to the rhamnose unit by a (1 → 4) linkage. Moreover, another correlation between the C-atom at 76.6 (Xyl C(4)) and an anomeric signal at δ 4.90 (*d*, *J* = 7.1 Hz) suggested a 4-substitution of this xylose by a terminal arabinose unit, which was confirmed by a correlation in the NOESY plot between δ(H) 3.99 (Xyl H–C(4)) and δ(H) 4.90 (*d*, *J* = 7.1 Hz, Ara H–C(1)).

The negative-ion FAB-MS of compound **3/4** showed a quasi-molecular ion peak ($[M-H]^-$) at *m/z* 1425 which indicated the molecular formula as $C_{69}H_{102}O_{31}$. The fragment ion peaks at *m/z* 1235 ($[M-H-190]^-$) and 1073 ($[M-H-190-162]^-$) revealed the loss of one 3,4-dimethoxycinnamoyl moiety and one hexosyl moiety. The study of the 2D-NMR spectra of compounds **3/4** led to the establishment of their structure as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-α-L-arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]-β-D-fucopyranosyl)} ester[1]) and its (*Z*)-isomer, new natural compounds.

The ^{1}H- and ^{13}C-NMR signals of **3/4** assigned from the 2D-NMR spectra were almost superimposable on those of **1/2**, except for the acyl moiety (*Tables 1–3*). The 4-*O*-position of Fuc was substituted by an *(E)*- and a *(Z)*-3,4-dimethoxycinnamoyl group in **3** and **4**, respectively, instead of the *(E)*- and *(Z)*-4-methoxycinnamoyl group in **1/2**. The assignments of the C- and H-signals of the 3,4-dimethoxycinnamoyl units obtained by further 2D-NMR investigations (*Table 3*), were in good agreement with those described in [5].

The molecular formula of compound **5** was determined as $C_{59}H_{94}O_{29}$ by negative-ion FAB-MS which showed an *m/z* 1265 ($[M-H]^-$). Another fragment-ion peak observed at *m/z* 1103 ($[M-H-162]^-$) revealed the elimination of one terminal hexosyl moiety. Based on the study of the 2D-NMR spectra, the structure of **5** was established as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-[*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl] ester[1]).

The ^{1}H-NMR spectrum of **5** displayed signals for five anomeric protons at δ 6.23 (br. *s*), 5.78 (*d*, *J* = 8.3 Hz), 4.93 (*d*, *J* = 7.8 Hz), 4.83 (*d*, *J* = 7.1 Hz), and 4.77 (*d*, *J* = 7.4 Hz) which gave HSQC correlations with C-signals at δ 100.9, 94.2, 103.9, 103.3, and 106.3, respectively. After extensive 2D-NMR spectroscopic analysis, and subtraction of the signals assigned to Glc of the prosapogenin, the signals of four sugar units linked at C(28) remained which were identified as Fuc, Rha, Xyl, and Gal. A HMBC correlation between δ(H) 5.78 (*d*, *J* = 8.3 Hz, Fuc H–C(1)) and δ(C) 176.6 (Agly C(28)) established glycosidic ester linkage to the C(28) of the aglycon. A correlation between δ(H) 4.51 (*t*, *J* = 8.7 Hz, Fuc H–C(2)) and δ(C) 100.9 (Rha C(1)) revealed the presence of only one substitution of Fuc at C(2), a (1 → 2) linkage with Rha. HMBC Cross-peaks between δ(H) 4.77 (*d*, *J* = 7.4 Hz, Xyl H–C(1)) and δ(C) 84.9 (Rha C(4)), the reverse correlation between δ(H) 4.09 (Rha H–C(4)) and δ(C) 106.3 (Xyl C(1)), and the NOESY correlation between δ(H) 4.77 (*d*, *J* = 7.4 Hz, Xyl H–C(1)) and δ(H) 4.09 (Rha H–C(4)) indicated that the xylose unit was linked to the rhamnose moiety by a (1 → 4) linkage. Moreover, another correlation between a proton at δ(H) 4.20, which showed a HSQC cross-peak with the deshielded C-atom at δ(C) 77.0 (Xyl C-(4)), and an anomeric signal at δ(H) 4.83 (*d*, *J* = 7.1 Hz) suggested a

Table 2. *^{13}C-NMR Data* (150 MHz) *of the Sugar Moities of* **1**–**7** *in* (D_5)*Pyridine from 1D- and 2D-NMR Experiments*[a]). δ (C) in ppm.

		1/2	**3/4**	**5**	**6**	**7**
3-*O*-Glc:	CH(1)	104.1	104.1	103.9	104.8	104.8
	CH(2)	74.9	74.9	74.5	74.9	75.0
	CH(3)	77.3	77.3	77.0	77.1	77.0
	CH(4)	71.1	71.1	70.8	70.9	70.8
	CH(5)	77.8	77.8	77.2	77.6	77.5
	CH_2(6)	62.1	62.1	61.7	62.0	61.8
28-*O*-Fuc:	CH(1)	94.0	94.0	94.2	94.0	94.5
	CH(2)	73.6	73.6	73.3	70.9	71.4
	CH(3)	74.3	74.3	76.4	81.8	77.0
	CH(4)	74.8	74.8	72.5	74.0	73.0
	CH(5)	70.1	70.1	71.8	70.7	72.3
	Me(6)	16.2	16.2	16.3	16.2	16.5
	AcO–C(4)				20.4, 170.7	
Rha:	CH(1)	101.0	101.0	100.9	101.1	100.4
	CH(2)	71.2	71.2	70.9	70.9	71.2
	CH(3)	72.0	72.0	71.4	72.0	72.3
	CH(4)	85.7	85.7	84.9	85.6	86.0
	CH(5)	67.9	67.9	67.5	67.5	67.0
	Me(6)	18.2	18.2	17.6	18.1	17.8
Xyl:	CH(1)	107.0	107.0	106.3	106.3	106.2
	CH(2)	75.5	75.5	75.2	75.5	75.4
	CH(3)	75.6	75.6	75.8	86.4	86.8
	CH(4)	76.6	76.6	77.0	70.7	70.7
	CH_2(5)	65.0	65.0	64.3	65.8	65.6
Api[b]):	CH(1)				112.3	
	CH(2)				78.0	
	C(3)				80.1	
	CH_2(4)				75.1	
	CH_2(5)				64.5	
Ara[b]):	CH(1)	103.4	103.4		105.6	
	CH(2)	70.3	70.3		72.5	
	CH(3)	74.7	74.7		74.0	
	CH(4)	68.2	68.2		68.5	
	CH_2(5)	66.9	66.9		67.0	
Glc[b]):	CH(1)					105.4
	CH(2)					75.4
	CH(3)					77.5
	CH(4)					69.5
	CH(5)					78.5
	CH_2(6)					61.7
Gal[b]):	CH(1)			103.3	102.7	102.5
	CH(2)			70.7	69.5	70.0
	CH(3)			74.0	74.0	74.0
	CH(4)			69.4	69.4	69.4
	CH(5)			76.4	77.0	77.0
	CH_2(6)			61.7	61.8	61.5

[a]) Multiplicities were assigned from DEPT spectra. [b]) Terminal sugar moiety.

4-substitution of this xylose moiety by one terminal galactose unit. Thus, **5** is the natural deacylated derivative of (*E*)- and (*Z*)-senegasaponin b and (*E*)- and (*Z*)-senegin II.

Table 3. *^{13}C-NMR* (150 MHz)[a]) *and ^{1}H-NMR Data* (600 MHz) *of the 4-Methoxycinnamoyl Moiety of* **1**/**2**, *and the 3,4-Dimethoxycinnamoyl Moiety of* **3**/**4** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments.* 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)
4-Methoxycinnamoyl:								
C(α)	167.5		[b])					
H–C(β)	115.1	6.50 (*d*, *J* = 16.5)	[b])	5.97 (*d*, *J* = 12.5)				
H–C(γ)	[b])	7.80 (*d*, *J* = 16.5)	143.7	6.85 (*d*, *J* = 12.5)				
C(1)	126.4		125.5					
H–C(2), H–C(6)	129.8	7.35 (*d*, *J* = 8.1)	132.4	7.86 (*d*, *J* = 8.1)				
H–C(3), H–C(5)	114.1	6.97 (*d*, *J* = 8.1)	113.4	6.94 (*d*, *J* = 8.1)				
C(4)	161.5		160.4					
MeO–C(4)	55.5	3.70 (*s*)	55.4	3.65 (*s*)				
3,4-Dimethoxycinnamoyl:								
C(α)					167.5		[b])	
H–C(β)					115.1	6.50 (*d*, *J* = 16.5)	[b])	5.97 (*d*, *J* = 12.5)
H–C(γ)					[b])	7.84 (*d*, *J* = 16.5)	143.7	6.85 (*d*, *J* = 12.5)
C(1)					127.5		127.5	
H–C(2)					110.4	7.00 (*s*)	114.0	7.88 (*s*)
C(3)					149.1		148.4	
C(4)					151.5		150.6	
H–C(5)					111.2	6.94 (*d*, *J* = 8.1)	111.6	6.85 (*d*, *J* = 8.1)
H–C(6)					122.7	7.03 (*d*, *J* = 8.1)	125.2	7.40 (*d*, *J* = 8.1)
MeO–C(3)					55.7	3.79 (*s*)	55.6	3.81 (*s*)
MeO–C(4)					55.7	3.79 (*s*)	55.6	3.75 (*s*)

[a]) Multiplicities were assigned from DEPT spectra. [b]) Not determined.

Comparison of the spectral data with those reported in the literature allowed us to identify the remaining compounds isolated from *P. ruwenzoriensis* as tenuifoline [3], (*E*)- and (*Z*)-senegasaponin b [9], (*E*)- and (*Z*)-senegin II [9], and polygalasaponin XXVIII [10].

From the MeOH extract of the roots of *Carpolobia alba* G. DON. and *C. lutea* G. DON, two crude saponin mixtures were obtained according to a previously described procedure [3] and saponin **6** and a known compound, arilloside C, were isolated from these saponin fractions, respectively, following repeated chromatographic steps. Arilloside C was identified by comparison of its spectral data with literature data of the same compound isolated previously from *Polygala arillata* [11].

The negative-ion FAB-MS of **6** showed a quasi-molecular-ion peak ($[M-H]^-$) at *m/z* 1571 which indicated the molecular formula as $C_{71}H_{112}O_{38}$. The fragment-ion peaks at *m/z* 1439 ($[M-H-132]^-$) and 1277 ($[M-H-132-162]^-$) revealed the loss of one terminal pentosyl moiety and one terminal hexosyl moiety. The study of the

2D-NMR spectra of **6** led to the establishment of its structure as 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*α*-L-arabinopyranosyl-(1 → 3)-*O*-[*β*-D-galactopyranosyl-(1 → 4)]-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[*β*-D-apiofuranosyl-(1 → 3)]-4-*O*-acetyl-*β*-D-fucopyranosyl} ester[1]), a new saponin.

The ^{1}H- and ^{13}C-NMR signals of **6** allowed identification of seven monosaccharide units, suggested by seven anomeric protons at δ 6.16 (br. *s*), 5.92 (*d*, $J=8.3$ Hz), 5.71 (br. *s*), 4.97 (*d*, $J=7.8$ Hz), 4.92 (*d*-like), 4.81 (*d*, $J=7.4$ Hz), and 4.80 (*d*, $J=7.4$ Hz) which gave HSQC correlations with C-signals at δ 101.1, 94.0, 112.3, 104.8, 105.6, 106.3, and 102.7, respectively. After extensive 2D-NMR spectroscopic analysis and subtraction of the signals of the glucopyranosyl moiety of the prosapogenin, the signals of six sugar units linked to C(28) of the presenegenin remained, which were identified as Fuc, Rha, Xyl, Gal, Ara, and apiofuranosyl (Api). This latter was determined to be in the *β*-anomeric form by comparing its spectroscopic data with literature values [6]. Furthermore it was assumed to have the D-configuration normally found in nature. As in molecules **1**–**5**, the oligosaccharidic chain of **6** is linked to the C(28) of the aglycon by a Fuc moiety, which is substituted at the C(2) position by Rha. The difference is located at the 3-*O*-position of Fuc. A HMBC cross-peak between δ(H) 5.71 (br. *s*, Api H−C(1)) and δ(C) 81.8 (Fuc C(3)), and a NOESY correlation between δ(H) 5.71 (br. *s*, Api H−C(1)) and δ(H) 4.32 (Fuc H−C(3)) indicated that a *β*-D-apiofuranosyl moiety was linked to Fuc by a (1 → 3) linkage. Moreover, the downfield shifts observed in the HSQC spectrum for the Fuc H−C(4) and C(4) resonances at δ(H) 5.65 and δ(C) 74.0, respectively, and a signal of one Ac group at δ(H) 1.92 (*s*) established that the secondary-alcohol function OH−C(4) of Fuc was acetylated. This type of a substituted fucose unit (1, 2, 3, 4) is unusual and rarely encountered. A further examination of the HMBC spectrum showed a (1 → 4) linkage between Xyl and Rha by a correlation between δ(H) 4.81 (*d*, $J=7.4$ Hz, Xyl H−C(1)) and δ(C) 85.6 (Rha C(4)), and a reverse correlation between δ(H) 4.14 (Rha H−C(4)) and δ(C) 106.3 (Xyl C(1)). A cross-peak between δ(H) 4.80 (*d*, $J=7.4$ Hz, Gal H−C(1)) and δ(C) 70.7 (Xyl C(4)), suggested a (1 → 4) linkage between the terminal Gal and Xyl units. The substitution of the 3-*O*-position of Xyl by a terminal Ara moiety is revealed by the observation of a NOESY cross-peak between δ(H) 4.92 (*d*-like, Ara H−C(1)) and δ(H) 3.85 (Xyl H−C(3)).

After extraction of the roots of *Polygala arenaria* WILLD. with MeOH, **7** was isolated from the crude saponin mixture, obtained according to a previously described procedure [5] following repeated chromatographic steps. The molecular formula of **7** was determined as $C_{65}H_{104}O_{34}$ by negative-ion FAB-MS which revealed *m/z* 1427 ($[M-H]^-$). The fragment-ion peaks at *m/z* 1265 ($[M-H-162]^-$), 1103 ($[M-H-162-162]^-$), 971 ($[M-H-162-162-132]^-$), 825 ($[M-H-162-162-132-146]^-$), and 679 ($[M-H-162-162-132-146-146]^-$), revealed the successive loss of two hexosyl, one pentosyl, and two deoxyhexosyl moieties. The study of the 2D-NMR spectra of compound **7** led to the establishment of its structure as 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*β*-D-galactopyranosyl-(1 → 4)-*O*-[*β*-D-glucopyranosyl-(1 → 3)]-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranosyl} ester[1]), a new triterpene glycoside.

The ^{1}H- and ^{13}C-NMR signals of **7** allowed the identification of six monosaccharide units, suggested by six anomeric protons at δ 6.58 (br. *s*), 5.86 (*d*, $J=8.1$ Hz), 4.94 (*d*, $J=7.5$ Hz), 4.93 (*d*, $J=7.5$ Hz), 4.81 (*d*, $J=7.8$ Hz), and 4.81 (*d*, $J=7.8$ Hz) which gave HSQC correlations with C-signals at δ 100.4, 94.5, 105.4, 104.8, 106.2, and 102.5, respectively. After extensive 2D-NMR spectroscopic analysis of **7** and subtraction of the signals of Glc of the prosapogenin, Glc, Fuc, Rha, Xyl, and Gal were identified. As in saponin **5**, the ^{1}H- and ^{13}C-NMR signals of **7** showed an ester linkage to C(28) of the aglycon by the sequence *α*-L-rhamnopyranosyl-(1 → 2)-*O*-*β*-D-fucopyranosyl. A HMBC cross-peak between δ(H) 4.81 (*d*, $J=7.8$ Hz, Xyl H−C(1)) and δ(C) 86.0 (Rha C(4)), and the reverse correlation between δ(H) 4.14 (Rha H−C(4)) and δ(C) 106.2 (Xyl C-(1)), indicated that the xylose unit was linked to the rhamnose unit by a (1 → 4) linkage. Moreover, cross-peaks between δ(H) 4.81 (*d*, $J=7.8$ Hz, Gal H−C(1)) and δ(C) 70.7 (Xyl C(4)), and between δ(H) 4.94 (*d*, $J=7.5$ Hz, Glc H−C(1)) and δ(C) 86.8 (Xyl C(3)) suggested a (1 → 4) linkage between the terminal Gal and Xyl and a (1 → 3) linkage between the terminal Glc and Xyl. These substitutions were confirmed by NOESY correlations

between δ(H) 4.81 (*d*, *J* = 7.8 Hz, Gal H–C(1)) and δ(H) 4.30 (Xyl H–C(4)), and δ(H) 4.94 (*d*, *J* = 7.5 Hz, Glc H–C(1)) and δ(H) 3.84 (Xyl H–C(3)).

From a chemotaxonomic point of view, all the isolated saponins from these four species possessed the same sequence 3-*O*-(β-D-glucopyranosyl)presenegenin 28-[*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl] ester. This sequence presented additional substitutions of the 3-*O*- and 4-*O*-positions of the Fuc moiety. The 3-*O*-position can be substituted by either an acetyl group or an apiose unit, whereas the 4-*O*-position was acylated by either an (*E*)- and (*Z*)-4-methoxycinnamoyl, or (*E*)- and (*Z*)-3,4-dimethoxycinnamoyl group, or by an acetyl group. These results corroborated those previously described for saponins isolated from other Polygalaceae belonging to genera *Polygala* [1] [5] [6] [9] [10] [14–18], *Carpolobia* [3], *Muraltia* [2] [7], and *Securidaca* [19], and suggested that this sequence may represent a chemotaxonomic marker for the Polygalaceae family.

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*). Vacuum liquid chromatography (VLC): silica gel *RP-18* (25–40 µm; *Merck*). Medium-pressure liquid chromatography (MPLC): silica gel *60* (15–40 µm; *Merck*), *Gilson* pump *M 303*; *Büchi* glass column (460×25 mm and 460×15 mm), *Büchi* precolumn (110×15 mm). TLC and HPTLC: silica gel *60 F_{254}* (*Merck*); solvent systems: $CHCl_3$/MeOH/AcOH/H_2O 15:8:3:2 (*a*), $CHCl_3$/MeOH/H_2O 13:7:2, lower phase (*b*); spray reagent: *Komarowsky* reagent, 2% 4-hydroxybenzaldehyde in MeOH/50% H_2SO_4 soln. 5:1. Optical rotation: *AA-OR automatic* polarimeter. 1D- and 2D-NMR Spectra: see [3]. Fast-atom bombardment (FAB) MS: negative-ion mode; *Jeol SX-102*; in *m*/*z*.

Plant Material. The roots of *P. ruwenzoriensis* CHOD. were collected in the Democratic Republic of Congo, Africa, in 1981, in the forest of Nyiragongo volcano. A voucher specimen (H. Breyne n° 3939) was deposited in the Herbarium of the National Botanical Garden of Brussels, Belgium. For *C. alba* G. DON [3], *C. lutea* G. DON. [3], and *P. arenaria* WILLD. [5], as previously described.

Extraction and Isolation. For *P. ruwenzoriensis*, *C. alba*, *C. lutea*, and *P. arenaria*, crude saponin mixtures (5, 9, 8, and 5 g, resp.) were obtained according to a previously described method [12].

For *P. ruwenzoriensis*, 1.5 g of the saponin mixture was submitted to VLC (*RP-18*, H_2O containing increasing amounts of MeOH): *Fractions 1–11*. The *Fr. 7* (200 mg), rich in saponins and eluted with MeOH/H_2O 6:4, was fractionated by successive MPLC (silica gel $CHCl_3$/MeOH/H_2O 13:7:2, lower phase): **1**/**2** (5 mg), **3**/**4** (5 mg), **5** (14 mg), **6** tenuifoline (6 mg), (*E*)- and (*Z*)-senegasaponin b (4 mg), (*E*)- and (*Z*)-senegin II (4 mg), and polygalasaponin XXVIII (6 mg).

For *C. alba*, 500 mg of the saponin mixture was fractionated by MPLC (silica gel, $CHCl_3$/MeOH/H_2O 8:5:1): **6** (8 mg).

For *C. lutea*, 300 mg of the saponin mixture was fractionated by MPLC (silica gel, $CHCl_3$/MeOH/H_2O 65:40:8): arilloside C (5 mg).

For *P. arenaria*, 2 g of the saponin mixture was submitted to CC (*Sephadex LH-20*, MeOH) giving four main fractions. One of them (300 mg), rich in saponins, was fractionated by successive MPLC (silica gel, $CHCl_3$/MeOH/H_2O 13:7:2, lower phase): **7** (5 mg).

*(2β,3β,4α)-3-(β-D-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-α-L-Arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-6-deoxy-4-*O*-[(*E*)-4-methoxycinnamoyl]-β-D-galactopyranosyl} Ester* (**1**) *and Its (*Z*)-Isomer* **2**. White amorphous powder. TLC (*b*): R_f 0.34. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): for aglycon, see [3]; for sugars and acyl groups, see *Tables 1–3*. FAB-MS (neg.): 1395 ($[M-H]^-$), 1263 ($[M-H-132]^-$), 1235 ($[M-H-160]^-$), 1073 ($[M-H-160-162]^-$).

*(2β,3β,4α)-3-(β-D-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-α-L-Arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-6-deoxy-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]-β-D-galactopyranosyl} Ester* (**3**) *and Its (*Z*)-Isomer* **4**. White amorphous powder. TLC (*b*): R_f 0.36. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): for aglycon, see [3]; for sugars and acyl groups, see *Tables 1–3*. FAB-MS (neg.): 1425 ($[M-H]^-$), 1235 ($[M-H-190]^-$), 1073 ($[M-H-190-162]^-$).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-β-*D*-Galactopyranosyl-(1 → 4)*-O*-β-*D*-xylopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-6-deoxy-β-*D*-galactopyranosyl] Ester* (**5**). White amorphous powder. $[\alpha]_D^{25} = -14$ ($c = 0.1$, MeOH). TLC (*a*): R_f 0.66. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): for aglycon, see [3]; for sugars, see *Tables 1* and *2*. FAB-MS (neg.): 1265 ($[M - H]^-$), 1103 ($[M - H - 162]^-$).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-α-*L*-Arabinopyranosyl-(1 → 3)*-O-*[β-*D*-galactopyranosyl-(1 → 4)]*-O-*β-*D*-xylopyranosyl-(1 → 4)*-O-*6-deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O-*[β-*D*-apiofuranosyl-(1 → 3)]-4*-O-*acetyl-6-deoxy-β-*D*-galactopyranosyl} Ester* (**6**). White amorphous powder. $[\alpha]_D^{25} = -20$ ($c = 0.1$, MeOH). TLC (*a*): R_f 0.17. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): for aglycon, see [3]; for sugars, see *Tables 1* and *2*. FAB-MS (neg.): 1571 ($[M - H]^-$), 1439 ($[M - H - 132]^-$), 1277 ($[M - H - 132 - 162]^-$).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-β-*D*-Galactopyranosyl-(1 → 4)*-O-*[β-*D*-glucopyranosyl-(1 → 3)]*-O-*β-*D*-xylopyranosyl-(1 → 4)*-O-*6-deoxy-α-*L*-mannopyranosyl-(1 → 2)*-O-*6-deoxy-β-*D*-galactopyranosyl} Ester* (**7**). White amorphous powder. $[\alpha]_D^{25} = -10$ ($c = 0.1$, MeOH). TLC (*a*): R_f 0.27. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): for aglycon, see [3]; for sugars, see *Tables 1* and *2*. FAB-MS (neg.): 1427 ($[M - H]^-$), 1265 ($[M - H - 162]^-$), 1103 ($[M - H - 162 - 162]^-$), 971 ($[M - H - 162 - 162 - 132]^-$), 825 ($[M - H - 162 - 162 - 132 - 146]^-$), 679 ($[M - H - 162 - 162 - 132 - 146 - 146]^-$).

REFERENCES

[1] S. Desbène, B. Hanquet, Y. Shoyama, H. Wagner, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **1999**, *62,* 923.
[2] M. Elbandy, T. Miyamoto, B. Chauffert, C. Delaude, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2002**, *65*, 193.
[3] A.-C. Mitaine-Offer, T. Miyamoto, I. A. Khan, C. Delaude, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2002**, *65,* 553.
[4] M. Elbandy, T. Miyamoto, C. Delaude, M.-A. Lacaille-Dubois, *Helv. Chim. Acta* **2002**, *85*, 2721.
[5] A.-C. Mitaine-Offer, T. Miyamoto, V. Laurens, C. Delaude, M.-A. Lacaille-Dubois, *Helv. Chim. Acta* **2003**, *86*, 2404.
[6] M. Haddad, T. Miyamoto, C. Delaude, M.-A. Lacaille-Dubois, *Helv. Chim. Acta* **2003**, *86*, 3055.
[7] M. Elbandy, T. Miyamoto, C. Delaude, M.-A. Lacaille-Dubois, *Helv. Chim. Acta* **2004**, *87*, 340.
[8] C. Delaude, Babady-Bila, R. Huls, *Bull. Soc. Roy. Sc. Liège* **1981**, *50*, 172.
[9] M. Yoshikawa, T. Murakami, T. Ueno, M. Kadoya, H. Matsuda, J. Yamahara, N. Murakami, *Chem. Pharm. Bull*. **1995**, *43*, 350.
[10] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, A. Ueno, *Chem. Pharm. Bull.* **1996**, *44*, 810.
[11] M. Ouyang, C. Yang, H. Wang, *Zhongcaoyao* **1999**, *30*, 881.
[12] C. Delaude, *Bull. Soc. Roy. Sc. Liège* **1971**, *40*, 397.
[13] Y. Mimaki, A. Yokosuka, M. Hamanaka, C. Sakuma, T. Yamori, Y. Sashida, *J. Nat. Prod.* **2004**, *67*, 1511.
[14] T. Miyase, H. Saitoh, K. Shiokawa, A. Ueno, *Chem. Pharm. Bull.* **1995**, *43*, 466.
[15] S. Sakuma, J. Shoji, *Chem. Pharm. Bull.* **1981**, *29*, 2431.
[16] S. Sakuma, J. Shoji, *Chem. Pharm. Bull.* **1981**, *30*, 810.
[17] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, H. Noguchi, *Chem. Pharm. Bull.* **1996**, *44*, 2092.
[18] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, H. Noguchi, *Phytochemistry* **1998**, *47*, 459.
[19] M. Kuroda, Y. Mimaki, Y. Sashida, M. Kitahara, M. Yamazaki, S. Yui, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 371.

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