New Acylated Saponins from *Polygala myrtifolia*

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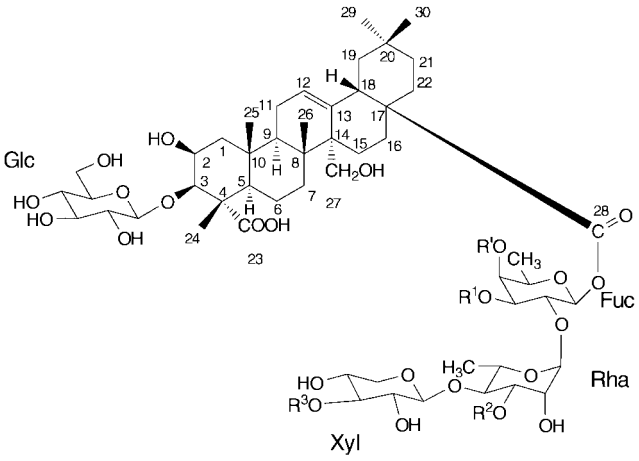
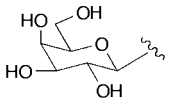
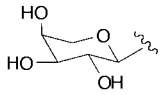
The ten new acylated presenegenin (=(2β,3β,4α)-2,3,27-trihydroxyolean-12-ene-23,28-dioic acid) glycosides **1**–**10** have been isolated by successive MPLC from the roots of *Polygala myrtifolia* L. as five inseparable mixtures of the *trans*- and *cis*-4-methoxycinnamoyl derivatives, *i.e.*, myrtifoliosides A_1/A_2 (**1/2**), B_1/B_2 (**3/4**), C_1/C_2 (**5/6**), D_1/D_2 (**7/8**), and E_1/E_2 (**9/10**). Their structures were elucidated mainly by extensive spectroscopic experiments, including 2D NMR techniques, as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[D-apio-β-D-furanosyl-(1 → 3)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-arabinopyranosyl-(1 → 3)]-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**1**) and its *cis*-isomer **2**, 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[D-apio-β-D-furanosyl-(1 → 3)]-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**3**) and its *cis*-isomer **4**, 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**5**) and its *cis*-isomer **6**, 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-D-apio-β-D-furanosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**7**) and its *cis*-isomer **8**, and 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-α-L-arabinopyranosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**9**) and its *cis*-isomer **10**.

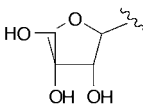
Introduction. – The Polygalaceae is a cosmopolitan plant family, consisting of approximately 1000 species in 15 genera. *Polygala myrtifolia* L., a South African medicinal herbaceous plant, is used by indigenous people to treat tuberculosis symptoms such as fever, blood in the septum, and cough [1]. Bacteriological tests showed that aqueous extracts of the green parts of the plant give doubtfully positive tests against *Staphylococcus aureus* and negative tests against *Escherichia coli* [2]. *Polygala* species have already been investigated, resulting in the isolation of presenegenin glycosides [3–6] but no previous phytochemical study has been undertaken on *P. myrtifolia.* Herein we report the isolation and structure elucidation of ten new triterpene glycosides named myrtifoliosides A_1/A_2, B_1/B_2, C_1/C_2, D_1/D_2, and E_1/E_2 (**1**–**10**), which were obtained as five inseparable mixtures of the *trans*- and *cis*-acyl derivatives from the EtOH extract of the roots of *P. myrtifolia*.

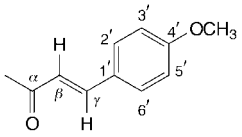
Results and Discussion. – The MeOH extract of the cortex of the roots of *P. myrtifolia* was suspended in MeOH and purified by precipitation with Et_2O, yielding a crude saponin mixture [7]. This extract was further fractionated by column chromatography (*Sephadex LH-20*) and repeated medium-pressure liquid chromatography (MPLC) over normal silica gel yielding myrtifoliosides A_1/A_2 (**1/2**) B_1/B_2 (**3/4**),

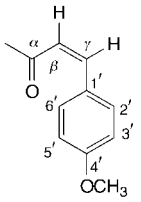



Gal


Ara


Api


S^1


S^2

	R^1	R^2	R^3	R′
Myrtifolioside A_1 **1**	Ara	Api	Gal	S^1
Myrtifolioside A_2 **2**	Ara	Api	Gal	S^2
Myrtifolioside B_1 **3**	H	Api	Gal	S^1
Myrtifolioside B_2 **4**	H	Api	Gal	S^2
Myrtifolioside C_1 **5**	H	H	Gal	S^1
Myrtifolioside C_2 **6**	H	H	Gal	S^2
Myrtifolioside D_1 **7**	H	Api	H	S^1
Myrtifolioside D_2 **8**	H	Api	H	S^2
Myrtifolioside E_1 **9**	H	Ara	H	S^1
Myrtifolioside E_2 **10**	H	Ara	H	S^2

Table 1. *^{13}C-* (150 MHz)[a]) *and ^{1}H-NMR* (600 MHz) *Data of the Aglycone Parts of* **1**–**10** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm.

	1/2		**3/4**		**5/6**		**7/8**		**9/10**	
	δ(C)	δ(H)[b])	δ(C)	δ(H)[b])	δ(C)	δ(H)[b])	δ(C)	δ(H)[b])	δ(C)	δ(H)[b])
CH_2(1)	43.5	1.55, 2.35	43.5	1.31, 2.16	43.4	1.42, 2.20	43.5	1.41, 2.22	43.5	1.40, 2.20
CH(2)	71.0	4.92	71.0	4.90	71.3	4.71	71.0	4.72	70.5	4.72
CH(3)	86.5	4.68	86.1	4.60	86.6	4.56	86.5	4.53	86.5	4.53
C(4)	53.2		53.4		53.0		53.2		53.2	
CH(5)	51.9	2.28	52.1	2.16	52.0	2.10	51.8	2.12	51.9	2.12
CH_2(6)	20.7	1.85, 1.93	20.7	1.98, [c])	20.6	1.95, [c])	20.3	[c])	20.7	[c])
CH_2(7)	33.3	1.08, 1.26	33.1	1.00, 1.16	33.2	0.98, 1.15	33.5	0.96, 1.12	33.3	0.95, 1.13
C(8)	40.3		40.3		40.4		40.4		40.4	
CH(9)	48.8	2.25	48.8	2.21	48.8	2.10	48.5	2.10	48.7	2.13
C(10)	36.1		36.8		36.1		36.3		36.1	
CH_2(11)	22.6	1.97, 2.20	22.5	1.94, 2.16	23.6	1.95, [c])	24.0	1.97, [c])	23.0	[c])
CH(12)	127.0	5.86	126.5	5.75	127.1	5.60	127.2	5.70	127.1	5.70
C(13)	138.8		138.8		139.7		138.6		138.7	
C(14)	47.6		47.5		47.5		47.6		47.6	
CH_2(15)	23.5	1.97, 2.05	23.5	1.78, 2.01	23.6	1.74, 1.90	23.1	1.82, [c])	23.1	1.78, 1.90
CH_2(16)	23.8	2.12, 2.25	23.6	2.04, [c])	23.8	2.00, 2.08	23.9	1.98, [c])	23.8	1.98, 2.10
C(17)	46.6		46.5		46.5		46.6		46.6	
CH(18)	41.4	3.19	40.9	3.16	41.5	3.05	41.6	3.04	41.8	3.04
CH_2(19)	44.9	1.30, 1.72	45.0	1.22, 1.64	44.8	1.19, 1.58	44.9	1.18, 1.58	44.9	1.17, 1.58
C(20)	30.0		30.5		30.0		30.0		30.0	
CH_2(21)	33.7	1.95, 2.30	34.9	1.80, 2.28	33.2	1.62, 2.12	33.7	2.18, [c])	33.7	1.78, 2.20
CH_2 (22)	31.7	1.72, 1.88	31.5	1.72, 1.84	30.0	1.64, 1.78	32.0	1.61, 1.80	31.8	1.61, 1.80
C(23)	186.0		185.7		185.5		186.0		186.0	
Me(24)	14.3	1.94 (*s*)	14.2	1.92 (*s*)	14.2	1.78 (*s*)	14.5	1.78 (*s*)	14.2	1.78 (*s*)
Me(25)	16.9	1.50 (*s*)	16.8	1.48 (*s*)	17.3	1.30 (*s*)	16.0	[c])	16.0	1.40 (*s*)
Me(26)	18.4	1.19 (*s*)	19.3	0.86 (*s*)	18.2	0.90 (*s*)	18.2	0.95 (*s*)	18.3	1.00 (*s*)
CH_2(27)	64.0	3.90, 4.14	63.7	3.79, 4.10	64.0	4.05, 4.13	63.6	3.76, 4.04	63.7	3.76, 4.07
C(28)	176.3		175.9		175.8		176.5		176.4	
Me(29)	32.4	0.87 (*s*)	32.3	0.72 (*s*)	32.4	0.75 (*s*)	32.4	0.76 (*s*)	32.4	0.75 (*s*)
Me(30)	22.9	0.83 (*s*)	22.8	0.75 (*s*)	23.1	0.72 (*s*)	23.0	0.73 (*s*)	23.0	0.72 (*s*)

[a]) Multiplicities were assigned from DEPT spectra. [b]) Overlapped ^{1}H-NMR signals are reported without designated multiplicity. [c]) Not determined.

C_1/C_2 (**5/6**), D_1/D_2 (**7/8**), and E_1/E_2 (**9/10**), as five inseparable mixtures, each one of them giving only one spot by HPTLC but two peaks by HPLC.

The saponin structures were established mainly by extensive spectroscopic 1D and 2D NMR experiments (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HSQC, and HMBC, see *Tables 1–3*) and FAB-MS. All the compounds were isolated as amorphous powders, giving fluorescence quenching zones at 254 nm and violet-blue fluorescence at 365 nm on TLC without chemical treatment. On acid hydrolysis, **1**–**10** afforded the same artifactual aglycone. The comparison of the hydrolyzate sugars by TLC with standard sugars allowed the identification of glucose, galactose, xylose, rhamnose (=6-deoxymannose), apiose, arabinose, and fucose (=6-deoxygalactose) (in the case of **1/2**), glucose, galactose, xylose, rhamnose, apiose, and fucose (in the case of **3/4**), glucose, galactose, xylose, rhamnose, and fucose (in the case of **5/6**), glucose, apiose, xylose, rhamnose, and fucose (in the case of **7/8**), and glucose, xylose, arabinose, rhamnose, and fucose (in the case of **9/10**). The NMR spectroscopic data of the

Table 2. *^{1}H-NMR* (600 MHz) *Chemical Shifts δ of the Sugar Moieties of* **1**–**10** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments. δ* in ppm, *J* in Hz.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3-*O*-Glc										
H–C(1)	5.08	5.08	4.98	4.98	4.91	4.91	4.90	4.90	4.88	4.88
	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)	(*d*, *J* = 7.0)
H–C(2)	3.95	3.95	3.88	3.88	3.84	3.84	3.84	3.84	3.95	3.95
H–C(3)	4.26	4.26	4.25	4.25	3.96	3.96	4.10	4.10	4.08	4.08
H–C(4)	4.02	4.02	4.00	4.00	3.88	3.88	4.00	4.00	3.98	3.98
H–C(5)	3.96	3.96	3.90	3.90	3.94	3.94	3.90	3.90	3.80	3.80
CH_2(6)	4.18, 4.37	4.18, 4.37	4.10, 4.35	4.10, 4.35	4.04, 4.24	4.04, 4.24	4.02, 4.24	4.02, 4.24	4.02, 4.22	4.02, 4.22
28-*O*-Sugars; Fuc										
H–C(1)	6.00	6.00	5.99	5.99	5.89	5.89	5.84	5.84	5.90	5.90
	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)	(*d*, *J* = 7.7)
H–C(2)	4.87	4.87	4.84	4.84	4.63	4.63	4.62	4.62	4.68	4.68
H–C(3)	4.57	4.57	4.37	4.37	4.32	4.32	4.35	4.35	4.50	4.50
H–C(4)	6.02	6.03	5.94	5.97	5.63	5.62	5.61	5.60	5.63	5.65
H–C(5)	4.41	4.40	4.23	4.22	4.17	4.15	4.16	4.13	4.18	4.18
Me(6)	1.34	1.36	1.25	1.26	1.22	1.23	1.23	1.21	1.22	1.23
	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)
Rha										
H–C(1)	6.40	6.40	6.44	6.44	6.21	6.21	6.30	6.30	6.38	6.38
	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)
H–C(2)	4.91	4.91	4.92	4.92	4.62	4.62	4.77	4.77	4.62	4.62
H–C(3)	4.50	4.50	4.49	4.49	4.38	4.38	4.34	4.34	4.28	4.28
H–C(4)	4.39	4.39	4.35	4.35	4.09	4.09	4.28	4.28	4.26	4.26
H–C(5)	4.60	4.60	4.50	4.50	4.35	4.35	4.41	4.41	4.38	4.38
Me(6)	1.76	1.76	1.68	1.68	1.60	1.60	1.61	1.61	1.60	1.60
	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)	(*d*, *J* = 6.0)
Api										
H–C(1)	6.05	6.05	5.99	5.99			5.88	5.88		
	(br. *s*)	(br. *s*)	(br. *s*)	(br. *s*)			(br. *s*)	(br. *s*)		
H–C(2)	4.74	4.74	4.66	4.66			4.50	4.50		
CH_2(4)	4.14, 4.32	4.14, 4.32	4.05, 4.35	4.05, 4.35			4.10, 4.38	4.10, 4.38		
CH_2(5)	4.18, 4.18	4.18, 4.18	4.08, 4.08	4.08, 4.08			4.39, 4.39	4.39, 4.39		

Table 2 (cont.)

Xyl										
H–C(1)	5.22 ($d, J = 7.3$)	5.22 ($d, J = 7.3$)	5.13 ($d, J = 7.3$)	5.13 ($d, J = 7.3$)	4.80 ($d, J = 7.3$)	4.80 ($d, J = 7.3$)	5.09 ($d, J = 7.3$)	5.09 ($d, J = 7.3$)	5.08 ($d, J = 7.0$)	5.08 ($d, J = 7.0$)
H–C(2)	4.04	4.04	3.94	3.94	3.88	3.88	3.84	3.84	3.82	3.82
H–C(3)	4.06	4.06	3.93	3.93	3.94	3.94	3.86	3.86	3.83	3.83
H–C(4)	4.30	4.30	4.35	4.35	4.26	4.26	4.38	4.38	4.30	4.30
CH_2(5)	3.52, 4.14	3.52, 4.14	3.34, 4.08	3.34, 4.08	3.42, 4.07	3.42, 4.07	3.43, 4.05	3.43, 4.05	3.42, 4.04	3.42, 4.04
Gal										
H–C(1)	5.15 ($d, J = 7.3$)	5.15 ($d, J = 7.3$)	4.98 ($d, J = 7.3$)	4.98 ($d, J = 7.3$)	5.00 ($d, J = 7.3$)	5.00 ($d, J = 7.3$)				
H–C(2)	4.34	4.34	4.48	4.48	4.40	4.40				
H–C(3)	4.14	4.14	4.10	4.10	4.20	4.20				
H–C(4)	4.43	4.43	4.39	4.39	4.28	4.28				
H–C(5)	3.96	3.96	3.98	3.98	3.90	3.90				
CH_2(6)	4.18, 4.37	4.18, 4.37	4.10, 4.35	4.10, 4.35	4.05, 4.25	4.05, 4.25				
Ara										
H–C(1)	5.01 ($d, J = 7.7$)	5.01 ($d, J = 7.7$)							5.00 ($d, J = 7.7$)	5.00 ($d, J = 7.7$)
H–C(2)	4.54	4.54							4.38	4.38
H–C(3)	4.32	4.32							4.20	4.20
H–C(4)	4.38	4.38							4.22	4.22
CH_2(5)	3.93, 4.28	3.93, 4.28							3.77, 4.50	3.77, 4.50
Acid										
H–C(β)	6.32 ($d, J = 15.8$)	5.87 ($d, J = 13.0$)	6.30 ($d, J = 16.1$)	5.90 ($d, J = 12.8$)	6.36 ($d, J = 16.1$)	5.90 ($d, J = 12.4$)	6.35 ($d, J = 16.0$)	5.90 ($d, J = 12.4$)	6.36 ($d, J = 16.8$)	5.90 ($d, J = 12.8$)
H–C(γ)	7.83 ($d, J = 15.8$)	6.90 ($d, J = 13.0$)	7.80 ($d, J = 16.1$)	6.76 ($d, J = 12.8$)	7.77 ($d, J = 16.1$)	6.85 ($d, J = 12.4$)	7.80 ($d, J = 16.0$)	7.30 ($d, J = 12.4$)	7.80 ($d, J = 16.8$)	7.30 ($d, J = 12.8$)
H–C(2′), H–C(6′)	7.36 ($d, J = 8.8$)	7.95 ($d, J = 8.8$)	7.32 ($d, J = 8.4$)	7.90 ($d, J = 8.4$)	7.30 ($d, J = 8.8$)	7.81 ($d, J = 8.8$)	7.31 ($d, J = 8.4$)	7.88 ($d, J = 8.4$)	7.30 ($d, J = 8.8$)	7.88 ($d, J = 8.8$)
H–C(3′), H–C(5′)	7.09 ($d, J = 8.8$)	7.10 ($d, J = 8.8$)	6.95 ($d, J = 8.4$)	6.99 ($d, J = 8.4$)	6.92 ($d, J = 8.8$)	6.86 ($d, J = 8.8$)	6.92 ($d, J = 8.4$)	6.80 ($d, J = 8.4$)	6.92 ($d, J = 8.8$)	6.85 ($d, J = 8.8$)
MeO–C(4′)	3.90 (s)	3.85 (s)	3.78 (s)	3.66 (s)	3.84 (s)	3.86 (s)	3.82 (s)	3.75 (s)	3.82 (s)	3.74 (s)

Table 3. *^{13}C-NMR* (150 MHz) *Chemical Shifts δ of the Sugar Moieties of* **1**–**10** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments. δ* in ppm. Multiplicities were assigned from DEPT spectra.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3-*O*-Glc										
CH(1)	103.6	103.6	104.1	104.1	103.7	103.7	103.7	103.7	103.6	103.6
CH(2)	74.2	74.2	74.5	74.5	74.2	74.2	74.2	74.2	74.3	74.3
CH(3)	76.3	76.3	76.4	76.4	76.2	76.2	76.2	76.2	76.3	76.3
CH(4)	70.4	70.4	70.5	70.5	70.4	70.4	70.0	70.0	70.2	70.2
CH(5)	76.8	76.8	77.2	77.2	76.0	76.0	76.7	76.7	76.8	76.8
CH_2(6)	61.4	61.4	61.5	61.5	61.4	61.4	61.4	61.4	61.4	61.4
28-*O*-Sugars; Fuc										
CH(1)	94.1	94.1	94.7	94.7	94.8	94.8	94.1	94.1	94.0	94.0
CH(2)	71.0	71.0	71.0	71.0	70.2	70.2	70.5	70.5	71.0	71.0
CH(3)	82.8	82.8	73.9	73.9	74.1	74.1	73.9	73.9	74.0	74.0
CH(4)	73.9	73.8	73.8	74.0	73.7	74.1	74.1	74.2	73.8	74.2
CH(5)	70.5	70.4	70.3	70.3	70.6	70.5	70.5	70.6	70.4	70.5
Me(6)	16.1	16.1	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Rha										
CH(1)	100.5	100.5	100.8	100.8	101.8	101.8	100.8	100.8	100.6	100.6
CH(2)	71.0	71.0	71.0	71.0	71.0	71.0	70.8	70.8	71.0	71.0
CH(3)	79.7	79.7	81.0	81.0	72.3	72.3	80.3	80.3	80.2	80.2
CH(4)	78.0	78.0	78.0	78.0	84.0	84.0	78.0	78.0	78.0	78.0
CH(5)	67.7	67.7	67.1	67.1	67.8	67.8	67.5	67.5	67.8	67.8
Me(6)	18.2	18.2	17.8	17.8	17.8	17.8	18.0	18.0	17.8	17.8
Api										
CH(1)	110.5	110.5	110.2	110.2			110.4	110.4		
CH(2)	77.2	77.2	78.0	78.0			77.4	77.4		
C(3)	79.1	79.1	79.1	79.1			78.0	78.0		
CH_2(4)	74.0	74.0	73.9	73.9			73.9	73.9		
CH_2(5)	64.1	64.1	64.5	64.5			66.7	66.7		
Xyl										
CH(1)	103.6	103.6	104.1	104.1	106.0	106.0	103.9	103.9	103.7	103.7
CH(2)	75.0	75.0	75.0	75.0	75.0	75.0	74.2	74.2	74.2	74.2
CH(3)	88.1	88.1	88.2	88.2	88.0	88.0	76.4	76.4	76.8	76.8
CH(4)	71.2	71.2	71.0	71.0	69.3	69.3	71.9	71.9	70.8	70.8
CH_2(5)	65.4	65.4	65.2	65.2	64.2	64.2	65.5	65.5	65.9	65.9
Gal										
CH(1)	105.3	105.3	105.2	105.2	105.5	105.5				
CH(2)	73.1	73.1	72.0	72.0	72.0	72.0				
CH(3)	74.0	74.0	74.0	74.0	73.2	73.2				
CH(4)	69.0	69.0	69.0	69.0	69.2	69.2				
CH(5)	76.8	76.8	76.1	76.1	75.0	75.0				
CH_2(6)	61.4	61.4	61.4	61.4	61.5	61.5				
Ara										
CH(1)	105.3	105.3							105.5	105.5
CH(2)	72.0	72.0							72.0	72.0
CH(3)	74.0	74.0							73.2	73.2
CH(4)	69.0	69.0							69.0	69.0
CH_2(5)	66.7	66.7							66.7	66.7
Acid										
C(α)	167.9	166.7	167.8	165.9	167.8	167.4	166.7	166.3	167.7	166.5
CH(β)	114.3	116.0	115.2	116.0	115.0	116.1	115.0	116.0	115.0	116.0
CH(γ)	145.6	143.8	145.0	143.4	146.0	144.0	145.4	144.0	146.0	144.0
C(1′)	126.3	127.0	126.2	127.1	126.8	127.1	126.6	127.2	126.6	127.0
CH(2′), CH(6′)	130.0	132.5	130.0	132.5	130.2	133.0	130.0	133.2	130.1	133.2
CH(3′), CH(5′)	114.2	113.6	114.0	113.2	114.8	113.5	114.4	113.8	114.2	113.8
MeO–*C*(4′)	161.2	160.4	161.0	158.8	161.5	160.4	161.4	160.5	161.4	160.5
*Me*O–C(4′)	55.2	55.0	55.5	54.8	55.2	55.0	55.2	55.0	55.2	55.0

prosapogenin of **1**–**10** obtained by alkaline hydrolysis with 5% KOH solution of **1**–**10** were in good agreement with those of tenuifolin (= 3-*O*-(*β*-D-glucopyranosyl)presenegenin (= (2*β*,3*β*,4*α*)-3-(*β*-D-glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic acid), obtained from *Muraltia heisteria* [8]. The mild alkaline hydrolysis of **1**–**10** with 1% KOH solution yielded *trans*- and *cis*-4-methoxycinnamic acid (TLC) and deacylated saponins, which were homogeneous for each inseparable mixture according to TLC and HPLC.

The negative-ion FAB-MS of **1/2** showed a quasi-molecular-ion peak at *m*/*z* 1689 ($[M-H]^-$), indicating a molecular mass of 1690, which suggested a molecular formula $C_{79}H_{118}O_{39}$. Two other significant ion peaks appeared at *m*/*z* 1557 ($[M-H-132]^-$) and 1395 ($[M-H-132-162]^-$) corresponding to the loss of one pentosyl and of one pentosyl and one hexosyl moiety, respectively. The full assignment of all the ^{1}H- and ^{13}C-NMR signals by 2D NMR experiments of **1/2** resulted in the establishment of their structures as 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*β*-D-galactopyranosyl-(1 → 3)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-[D-apio-*β*-D-furanosyl-(1 → 3)]-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[*α*-L-arabinopyranosyl-(1 → 3)]-4-*O*-(*trans*-4-methoxycinnamoyl)-*β*-D-fucopyranosyl} ester (**1**) and its *cis*-isomer **2**, two new natural compounds [3–6] [8–17].

The ^{1}H,^{1}H COSY experiments indicated that **1/2** is a 2 : 1 mixture of a *trans*- and *cis*-4-methoxycinnamoyl-substituted triterpene/glycosides (by relative NMR and HPLC intensities). This mixture was homogeneous by HPTLC but was separated into the *trans*- and *cis*-isomers by HPLC. All attempts to separate **1/2** by semi-prep. HPLC were unsuccessful. This phenomenon of isomerization referred to the effect of light on the 4-methoxycinnamoyl group in aqueous methanolic solution, as has already been observed in (*E*)/(*Z*) mixtures of senegasaponins from *Polygala senega* [9], jenissensosides from *Silene jenisseensis* [10], atroximasaponins from *Atroxima congolana* [11], the acylated saponins from *Polygala arenaria* [6], and from *Muraltia heisteria* [8].

The HSQC spectrum of **1/2** displayed signals of five tertiary Me groups at δ 1.94 (*s*, Me(24)), 1.50 (*s*, Me(25)), 1.19 (*s*, Me(26)), 0.87 (*s*, Me(29)), and 0.83 (*s*, Me(30)) and one proton at a trisubstituted C=C bond at δ 5.86 (br. *s*), which correlated with five sp^3 C-atoms at δ 14.3, 16.9, 18.4, 32.4, and 22.9 and one sp^2 olefinic C-atom at δ 127.0, respectively. These data, together with the presence of a quaternary C-atom observed in the ^{13}C-NMR spectrum at δ 138.8, indicated that the aglycone possessed an olean-12-ene skeleton. Furthermore, its ^{1}H-NMR spectrum showed two oxygenated methine moieties at δ(H) 4.92 (H–C(2)) and 4.68 (H–C(3)) and one CH_2 group at δ(H) 3.90 and 4.14 (CH_2(27)), which gave correlations in the HSQC spectrum with ^{13}C-NMR signals at δ 71.0, 86.5, and 64.0, respectively. The ^{1}H- and ^{13}C-NMR signals of the aglycone of **1/2** were almost superimposable on those of presenegenin (= (2*β*,3*β*,4*α*)-2,3,27-trihydroxyolean-12-ene-23,28-dioic acid) [8]. The ^{1}H,^{1}H COSY experiment of **1/2** permitted us to identify the *trans*-olefinic protons of the 4-methoxycinnamoyl moiety at δ(H) 6.32 (*d*, *J* = 15.8 Hz, H–C(*β*)) and 7.83 (*d*, *J* = 15.8 Hz, H–C(*γ*)), the *cis*-olefinic protons at δ 5.87 (*d*, *J* = 13.0 Hz, H–C(*β*)) and 6.90 (*d*, *J* = 13.0 Hz, H–C(*γ*)), and the disubstituted benzene ring protons at δ 7.36 (*d*, *J* = 8.8 Hz, H–C(2′) and H–C(6′)) and 7.09 (*d*, *J* = 8.8 Hz, H–C(3′) and H–C(5′)) for the *trans*-derivative **1** and at δ 7.95 (*d*, *J* = 8.8 Hz, H–C(2′) and H–C(6′)) and 7.10 (*d*, *J* = 8.8 Hz, H–C(3′) and H–C(5′)) for the *cis*-derivative **2**. The ^{1}H-NMR spectrum of **1/2** displayed signals for seven anomeric protons at δ 6.40 (br. *s*), 6.05 (br. *s*), 6.00 (*d*, *J* = 7.7 Hz), 5.22 (*d*, *J* = 7.3 Hz), 5.15 (*d*, *J* = 7.3 Hz), 5.08 (*d*, *J* = 7.0 Hz), and 5.01 (*d*, *J* = 7.7 Hz), which correlated in the HSQC spectrum with δ(C) 100.5, 110.5, 94.1, 103.6, 105.3, 103.6, and 105.3, respectively. The ring protons of the monosaccharide residues were assigned starting from the anomeric protons by means of the COSY, TOCSY, HSQC, and HMBC NMR plots (*Table 3*), and the sequence of the oligosaccharide chains was obtained from the HMBC and NOESY experiments. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one *α*-L-rhamnopyranosyl (Rha), one *β*-D-fucopyranosyl (Fuc), one *β*-D-glucopyranosyl (Glc), one *β*-D-galactopyranosyl (Gal), one *α*-L-

arabinopyranosyl (Ara), one D-apio-β-D-furanosyl (Api), and one β-D-xylopyranosyl (Xyl) unit, whereby the common D-configuration of Fuc, Glc, Gal, Api, and Xyl and the L-configuration of Rha and Ara were assumed, according to those most encountered among the plant glycosides in each case.

On the basis of extensive 1D and 2D NMR experiments, it can be concluded that **1/2** are bisdesmosidic saponins with one Glc at C(3) (δ(C) 86.5) of the aglycone and the six other monosaccharides linked at C(28) (δ(C) 176.3) through ester bonds. The connection of the Glc moiety at C(3) of the aglycone (Agly C(3)) was deduced by the NOESY correlation observed between the anomeric proton of Glc (Glc H–C(1)) at δ(H) 5.08 (d, J = 7.0 Hz) and the H–C(3) of the aglycone (Agly H–C(3) at δ(H) 4.68. Further confirmation was obtained by the HMBC correlation between δ(H) 5.08 (Glc, H–C(1) and δ(C) 86.5 (Agly C(3)). The HMBC and NOESY plots allowed us to establish the sequence of the sugars at C(28). A correlation in the HSQC spectrum at δ(H) 6.00 (d, J = 7.7 Hz, Fuc H–C(1))/δ(C) 94.1 (Fuc C(1)) showed that the Fuc residue was linked to the carboxylic group of the aglycon by an ester linkage. This conclusion was supported by the upfield shift of C(28) at δ(C) 176.3. The location of the 4-methoxycinnamoyl group at C(4) of Fuc (δ(H) 6.02, Fuc H–C(4)) was determined by the TOCSY and COSY experiments, starting from the anomeric H-atom at δ(H) 6.00 of Fuc (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) 6.02 and δ(C) 73.9, respectively, established that the secondary-alcohol function OH–C(4) of Fuc was acylated. The HMBC correlation between δ(H) 4.87 (Fuc H–C(2)) and δ(C) 100.5 (Rha C(1)), together with the reverse correlation between δ(H) 6.40 (br. *s*, Rha H–C(1)) and δ(C) 71.0 (Fuc C(2)), indicated that Rha was linked to Fuc by a (1 → 2) linkage. This was confirmed by a NOESY cross-peak between δ(H) 6.40 (Rha H–C(1)) and δ(H) 4.87 (Fuc H–C(2)). Other HMBCs between δ(H) 5.22 (d, J = 7.3 Hz, Xyl H–C(1)) and δ(C) 78.0 (Rha C(4)) and between δ(H) 4.39 (Rha H–C(4)) and δ(C) 103.6 (Xyl C(1)) showed that Xyl was linked to Rha by a (1 → 4) linkage. This was also confirmed by a NOESY cross-peak between δ(H) 5.22 (Xyl H–C(1)) and δ(H) 4.39 (Rha H–C(4)). The HMBC correlation between δ(H) 5.01 (d, J = 7.7 Hz, Ara H–C(1)) and δ(C) 82.8 (Fuc C(3)) indicated that Ara was linked to C(3) of Fuc. Further confirmation was obtained by a NOESY cross-peak between δ(H) 5.01 (Ara H–C(1)) and δ(H) 4.57 (Fuc H–C(3)). The HMBC between δ(H) 5.15 (d, J = 7.3 Hz, Gal H–C(1)) and δ(C) 88.1 (Xyl C(3)) together with a reverse correlation between δ(H) 4.06 (Xyl H–C(3)) and δ(C) 105.3 (Gal C(1)) established that Gal was attached to C(3) of Xyl. This was confirmed by a NOESY cross-peak between δ(H) 5.15 (Gal H–C(1)) and δ(H) 4.06 (Xyl H–C(3)). Furthermore, the NOESY cross-peak between δ(H) 6.05 (br. *s*, Api H–C(1)) and δ(H) 4.50 (Rha H–C(3)) indicated that Api was linked to Rha by a (1 → 3) linkage.

The negative-ion FAB-MS of **3/4** showed a quasi-molecular-ion peak at m/z 1557 ($[M-H]^-$), with 132 mass units less than **1/2**, indicating a molecular mass of 1558, compatible with a molecular formula of $C_{74}H_{110}O_{35}$. The assignments of all the ^{1}H- and ^{13}C-NMR signals of **3/4** were successfully carried out with 2D NMR experiments (*Tables 1–3*). Thus, the structures of **3/4** were determined as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[D-apio-β-D-furanosyl-(1 → 3)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**3**) and its *cis*-isomer **4**, two new natural compounds [3–6][8–17].

The ^{1}H- and ^{13}C-NMR spectra of **3/4** allowed identification of presenegenin as an aglycone (*Table 1*), and the presence of six monosaccharide units was suggested by the six anomeric protons at δ 6.44 (br. *s*), 5.99 (br. *s*), 5.99 (d, J = 7.7 Hz), 5.13 (d, J = 7.3 Hz), 4.98 (d, J = 7.3 Hz) and 4.98 (d, J = 7.0 Hz), which correlated in the HSQC spectrum with δ(C) 100.8, 110.2, 94.7, 104.1, 105.2, and 104.1, respectively (*Tables 2* and *3*). The ^{1}H- and ^{13}C-NMR signals of **3/4** assigned from 2D NMR spectra were almost superimposable on those of **1/2**, except for the disappearance of the signals of the Ara unit (*Tables 2* and *3*). Fuc of **3/4** showed a 1,2-diglycosylation pattern (C(2) at δ 71.0, C(3) at δ 73.9) instead of the 1,2,3-triglycosylation (C(2) at δ 71.0, C(3) at δ 82.8) seen in **1/2**. These results were in accordance with the difference in molecular mass (132 mass units) between **1/2** and **3/4**, corresponding to the loss of one pentosyl unit.

The negative-ion FAB-MS of **5/6** showed a quasi-molecular-ion peak at m/z 1425 ($[M-H]^-$), with 132 mass units less than **3/4**, indicating a molecular mass of 1426,

compatible with a molecular formula of $C_{69}H_{102}O_{31}$. On the basis of the 2D NMR data and hydrolysis, the structures of **5**/**6** were established as 3-*O*-(β-D-glucopyranosyl)-presenegenin 28-{*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**5**) and its *cis*-isomer **6**, two new natural compounds [3–6][8–17].

The ^{1}H-NMR spectrum of **5**/**6** displayed signals for five anomeric protons at δ 6.21 (br. *s*), 5.89 (*d*, *J* = 7.7 Hz), 5.00 (*d*, *J* = 7.3 Hz), 4.91 (*d*, *J* = 7.0 Hz), and 4.80 (*d*, *J* = 7.3 Hz), which correlated in the HSQC spectrum with δ(C) 101.8, 94.8, 105.5, 103.7, and 106.0, respectively. The ^{1}H- and ^{13}C-NMR signals of **5**/**6**, assigned from extensive 2D NMR analysis, were almost superimposable on those of **3**/**4**, except for the disappearance of the signals of Api seen in **5**/**6**. This was confirmed by the shifts observed in the HSQC spectrum of **5**/**6** for the Rha H–C(3) and C(3) resonance at δ(H) 4.38 and δ(C) 72.3, respectively, and for the Rha H–C(4) and C(4) at δ(H) 4.09 and δ(C) 84.0, respectively, which established the 1,4-disubstitution in **5**/**6** instead of the 1,3,4-trisubstitution of **3**/**4** (Rha H–C(3)/C(3) at δ(H) 4.49/δ(C) 81.0 and Rha H–C(4)/C(4) at δ(H) 4.35/δ(C) 78.0. These results were in accordance with the difference in molecular mass (132 mass units) between **5**/**6** and **3**/**4** corresponding to the loss of Api.

The negative-ion FAB-MS of **7**/**8** showed a quasi-molecular-ion peak at *m*/*z* 1395 ($[M-H]^-$), with 162 mass units less than **3**/**4**, indicating a molecular mass of 1396, compatible with a molecular formula of $C_{68}H_{100}O_{30}$. The full assignments of all the ^{1}H- and ^{13}C-NMR signals by extensive 2D NMR experiments of **7**/**8** resulted in the establishment of their structures as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-D-apio-β-D-furanosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**7**) and its *cis*-isomer **8**, two new natural compounds [3–6][8–17].

The ^{1}H- and ^{13}C-NMR spectra of **7**/**8** allowed the identification of presenegenin and five monosaccharide units by the five anomeric protons at δ(H) 6.30 (br. *s*), 5.88 (br. *s*), 5.84 (*d*, *J* = 7.7 Hz), 5.09 (*d*, *J* = 7.3 Hz), and 4.90 (*d*, *J* = 7.0 Hz) giving correlations in the HSQC spectrum with δ(C) 100.8, 110.4, 94.1, 103.9, and 103.7, respectively. Comparison of the 2D NMR signals of **7**/**8** and **3**/**4** indicated the loss of the signals of Gal at C(3) of the xylose in **3**/**4**. This was confirmed by observation of the ^{1}H- and ^{13}C-NMR data (*Tables 2* and *3*) of **7**/**8**. The xylose in **7**/**8** was terminal (C(2) at δ 74.2, and C(3) at δ 76.4) instead of the 1,3-substitution in **3**/**4** (C(2) at δ 75.0, and C(3) at δ 88.2). These results were in accordance with the difference in the molecular mass (162 mass units) corresponding to the loss of one hexosyl moiety.

Compounds **9**/**10** had the same molecular formula as **7**/**8**, $C_{68}H_{100}O_{30}$, determined from the quasi-molecular-ion peak at *m*/*z* 1395 ($[M-H]^-$) in the negative-ion FAB-MS, indicating a molecular mass of 1396. Extensive study of the 2D NMR spectra of **9**/**10** led to the establishment of their structures as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-α-L-arabinopyranosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 4)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-(*trans*-4-methoxycinnamoyl)-β-D-fucopyranosyl} ester (**9**) and its *cis*-isomer **10**, two new natural compounds [3–6][8–17].

The ^{1}H-NMR spectrum of **9**/**10** displayed signals for five anomeric protons at δ 6.38 (br. *s*), 5.90 (*d*, *J* = 7.7 Hz), 5.08 (*d*, *J* = 7.0 Hz), 5.00 (*d*, *J* = 7.7 Hz), and 4.88 (*d*, *J* = 7.0 Hz), which correlated in the HSQC spectrum with δ(C) 100.6, 94.0, 103.7, 105.5, and 103.6, respectively. Comparison of the 2D NMR spectra of **7**/**8** and **9**/**10**, as well as the results of their acid and alkaline hydrolysis revealed that the only difference between these compounds is due to the sugar linked to C(3) of the rhamnose, Ara in the case of **9**/**10** and Api in the case of **7**/**8**. This result was confirmed by the observation in the ^{1}H-NMR spectrum of **9**/**10** of one anomeric proton at δ 5.00 (*d*, *J* = 7.7 Hz for Ara H–C(1)), which correlated in the HSQC spectrum with δ(C) 105.5 (Ara C(1)), instead of an anomeric proton at δ(H) 5.88 (br. *s*, Api H–C(1)), giving a correlation in the HSQC spectrum with δ(C) 110.4 (Api C(1)).

These data corroborated the results of the literature considering the Fuc-Rha substitution at C(28) of the tenuifolin as a chemotaxonomic marker of the genus *Polygala*.

Experimental Part

General. Column Chromatography (CC): *Sephadex LH-20* (*Pharmacia*). Medium-pressure liquid chromatography (MPLC): silica gel *60* (*Merck*, 15–40 μm), *Gilson* pump *M 305*, *Büchi* column (460 × 25 mm and 460 × 15 mm), *Büchi* precolumn (110 × 15 mm). Anal. HPLC: *Gilson* pumps *M 305* and *306*; autoinjector *Gilson 234*, *UV/VIS-151 Gilson* detector; *Merck-Hitachi D-7500* integrator; column: *Dionex Vydac RP-18* (5 μm, 300 Å), 10 × 250 mm; eluent gradient: 35–60% MeCN/H_2O with 0.06% CF_3COOH; detection wavelength 210 nm. TLC and HPTLC: silica gel *60* F_{254} (*Merck*); solvent systems: for saponins, $CHCl_3$/MeOH/AcOH/H_2O 15:8:3:2 (*a*); for sapogenins, $CHCl_3$/MeOH 9:1 (*b*); for monosaccharides, $CHCl_3$/MeOH/H_2O 8:5:1 (*c*); for the acids, Et_2O/toluene 1:1 sat. with 10% AcOH (*d*); spray reagents: for saponins, *Komarowsky* reagent, a 5:1 mixture of 2% 4-hydroxybenzaldehyde in MeOH and 50% H_2SO_4 soln.; for sugars, diphenylamine/phosphoric acid reagent; for the cinnamic acids, UV-light detection. IR Spectra (KBr): *Perkin-Elmer 281* IR spectrophotometer; in cm^{-1}. 1D and 2D NMR Spectra (^1H,^1H COSY, TOCSY, NOESY, HSQC, and HMBC): *Unity-600* spectrometer at the operating frequency of 600 MHz on a *Varian Inova-600* instrument equipped with a *Sun-4 L-X* computer system (600 MHz for ^1H, 150 MHz for ^{13}C); conventional pulse sequences for COSY, HSQC, and HMBC, TOCSY by using the standard MLEV17 spin-locking sequence and 90 ms mixing time; mixing time in NOESY experiment, 500 ms, ^{13}C multiplicities by DEPT experiments; chemical shifts δ in ppm, J in Hz; (D_5)pyridine solns. (δ(C) 150.3, 155.9, 123.9). Fast-atom-bombardment (FAB) MS: negative mode; *Jeol SX-102*.

Plant Material. The cortices of roots of *Polygala myrtifolia* L. were collected from the Democratic Republic of Congo. A voucher specimen under the reference H. Breyne No. 5427 is deposited in the *Herbarium* of the *National Botanical Garden* of Brussels, Belgium.

Extraction and Isolation. Dried powdered cortex of roots (1.920 kg) was macerated with 80% MeOH and further submitted to an ebullition for 3 h. The MeOH soln. was filtrated and evaporated. The residue was dissolved in MeOH (1.5 l). After filtration, the MeOH soln. was concentrated and purified by precipitation with Et_2O (5 l). The resulting residue was washed with Et_2O, dried, solubilized in H_2O (1.2 l) and the soln. submitted to a dialysis for 3 days and then lyophilized. After decolorization with charcoal and filtration, the residue was dissolved in MeOH and purified again by precipitation with Et_2O, yielding a crude saponin mixture (26.9 g). Of this mixture, 4 g was submitted to CC (*Sephadex LH-20*) and then to successive MPLC on silica gel *60* (15–40 μm); $CHCl_3$/MeOH/H_2O 8:5:1 and 13:7:2, lower phase) yielding **1**/**2** (10 mg), **3**/**4** (11 mg), **5**/**6** (20 mg), **7**/**8** (10 mg), and **9**/**10** (9 mg).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-β-*D*-Galactopyranosyl-(1 → 3)*-O*-β-*D*-xylopyranosyl-(1 → 4)]-O-[*D*-apio-β-*D*-furanosyl-(1 → 3)]-O-α-*L*-rhamnopyranosyl-(1 → 2)-O-[α-*L*-arabinopyranosyl-(1 → 3)]-4-O-[(2*E*)-3-(4-methoxyphenyl)-1-oxoprop-2-enyl]-β-*D*-fucopyranosyl} Ester* (= *Myrtifolioside* A_1; **1**) *and Its (2Z)-Isomer Myrtifolioside* A_2 (**2**): White amorphous powder. TLC: R_f 0.21. IR (KBr): 3405, 2927, 1736, 1635, 1605, 1514. ^1H- and ^{13}C- NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1689 ([M – H]$^-$), 1557 ([M – H – 132]$^-$), 1395 ([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 → 3)*-O*-β-*D*-xylopyranosyl-(1 → 4)-O-[*D*-apio-β-*D*-furanosyl-(1 → 3)]-O-α-*L*-rhamnopyranosyl-(1 → 2)-4-O-[(2*E*)-3-(4-methoxyphenyl)-1-oxoprop-2-enyl]-β-*D*-fucopyranosyl} Ester* (= *Myrtifolioside* B_1; **3**) *and Its (2Z)-Isomer Myrtifolioside* B_2 (**4**). White amorphous powder. TLC: R_f 0.28. IR (KBr): 3406, 2927, 1740, 1636, 1605, 1515. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1557 ([M – H]$^-$).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-β-*D*-Galactopyranosyl-(1 → 3)*-O*-β-*D*-xylopyranosyl-(1 → 4)-O-α-*L*-rhamnopyranosyl-(1 → 2)-4-O-[(2*E*)-3-(4-methoxyphenyl)-1-oxoprop-2-enyl]-β-*D*-fucopyranosyl} Ester* (= *Myrtifolioside* C_1; **5**), *and Its (2Z)-Isomer Myrtifolioside* C_2 (**6**): White amorphous powder. TLC: R_f 0.35. IR (KBr): 3406, 2926, 1740, 1636, 1604, 1514. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1425 ([M – H]$^-$).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-*D*-Apio-β-*D*-furanosyl-(1 → 3)*-O*-[β-*D*-xylopyranosyl-(1 → 4)]-O-α-*L*-rhamnopyranosyl-(1 → 2)-4-O-[(2*E*)-3-(4-methoxyphenyl)-1-oxoprop-2-enyl]-β-*D*-fucopyranosyl} Ester* (= *Myrtifolioside* D_1; **7**), *and Its (2Z)-Isomer Myrtifolioside* D_2 (**8**): White amorphous powder. TLC: R_f 0.53. IR (KBr): 3400, 2924, 1740, 1710, 1637, 1609, 1515. ^1H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1395 ([M – H]$^-$).

*(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*-xylopyranosyl-(1 → 4)]-O-α-*L*-rhamnopyranosyl-(1 → 2)-4-O-[(2E)-3-(4-methoxyphenyl)-1-oxoprop-2-enyl]-β-*D*-fucopyranosyl] Ester* (= *Myrtifolioside* E_1; **9**), *and Its (2Z)-Isomer Myrtifolioside* E_2 (**10**): White amorphous powder. TLC: R_f 0.46. IR (KBr): 3400, 2925, 1740, 1710, 1637, 1609, 1515. ^{1}H-and ^{13}C NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1395 ($[M-H]^-$).

Acid Hydrolysis. A soln. of saponin (5 mg) in H_2O (2 ml) and 2N aq. CF_3COOH (5 ml) was refluxed on a water bath for 3 h. After extraction with $CHCl_3$ (3 × 5 ml), the aq. layer was repeatedly evaporated with MeOH until neutral and then analyzed by TLC by comparison with standard sugars (solvent system *c*).

Alkaline Hydrolysis. The saponin (5 mg) was refluxed with 5% aq. KOH soln. (10 ml) for 2 h. The fraction mixture was adjusted to pH 6 with dil. HCl soln. and then extracted with H_2O-sat. BuOH (3 × 10 ml). The combined BuOH extracts were washed with H_2O and evaporated: prosapogenin.

Mild Alkaline Hydrolysis. The saponin was hydrolyzed with 1% aq. KOH soln. at r.t. After 1 h, the mixture was neutralized with dil. HCl soln. and extracted with Et_2O. The Et_2O layer gave *trans*- and *cis*-4-methoxycinnamic acids (= (2*E*)- and (2*Z*)-3-(4-methoxyphenyl)prop-2-enoic acids), which were identified by TLC (toluene/Et_2O 1:1, sat. with 10% AcOH). The aq. layer was extracted with BuOH yielding the deacylated saponin.

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