New Acylated Presenegenin Saponins from Two Species of *Muraltia*

by **Mohamed Elbandy**[a]), **Tomofumi Miyamoto**[b]), **Clément Delaude**[c]), and **Marie-Aleth Lacaille-Dubois***[a])

[a]) Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique (UMIB EA 3660), Faculté de Pharmacie, Université de Bourgogne, 7 Bd Jeanne d'Arc, BP 87900, F-21079 Dijon Cedex

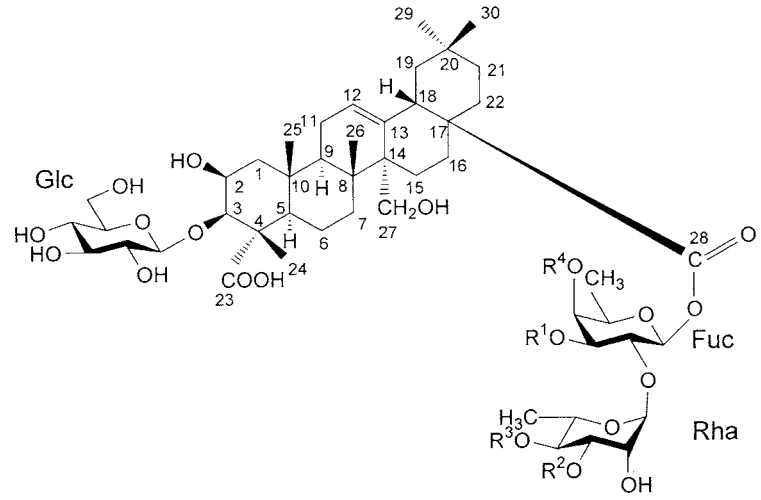
[b]) Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

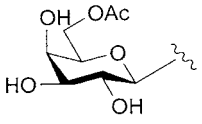
[c]) Centre de Recherche Phytochimique, Université de Liège, Institut de Chimie-B6, Sart Tilman B-4000-Liège I

Six new acylated bisdesmosidic triterpene glycosides **1**–**6** were isolated from the roots of *Muraltia heisteria* (L.) DC., as three inseparable mixtures **1/2**, **3/4**, and **5/6** of the (*E*)- and (*Z*)-3,4,5-trimethoxycinnamoyl derivatives. The compound pair **1/2** along with four known saponins were also isolated from the roots of *Muraltia satureioides* DC. Their structures were elucidated mainly by spectroscopic experiments including 2D-NMR techniques as 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*β*-D-apiofuranosyl-(1→3)-*O*-[*β*-D-xylopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[6-*O*-acetyl-*β*-D-galactopyranosyl-(1→3)]-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**1**) and its (*Z*)-isomer **2**, 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-6-*O*-acetyl-*β*-D-galactopyranosyl-(1→3)-*O*-[3-*O*-acetyl-*α*-L-rhamnopyranosyl-(1→2)]-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**3**) and its (*Z*)-isomer **4**, and 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-3-*O*-acetyl-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**5**) and its (*Z*)-isomer **6**, respectively.

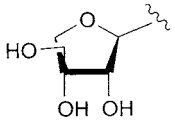
Introduction. – In a continuing program aiming at the discovery of novel natural products from the family of Polygalaceae, especially the genus *Muraltia*, we previously reported the isolation and characterization of five new medicagenic acid saponins from the roots of *Muraltia ononidifolia* [1] and of four new acylated presenegenin glycosides as two inseparable mixtures of the (*E*)- and (*Z*)-trimethoxycinnamoyl derivatives from the roots of *Muraltia heisteria* (L.) DC. [2]. As a result of our continous investigations of less polar saponin fractions of the EtOH extract of the roots of *M. heisteria*, the six additional new acylated oleanane-type triterpene saponins **1**–**6** were isolated as three inseparable mixtures **1/2**, **3/4**, and **5/6** of the (*E*)- and (*Z*)-3,4,5-trimethoxycinnamoyl derivatives. Furthermore, we investigated *Muraltia satureioides* DC., which is a shrub distributed in the Cape region in South Africa [3]. Although, this plant was reported to contain presenegenin glycosides [4], there is no detailed report on its saponins. The compound pair **1/2** together with four known saponins [2] were isolated from the EtOH extract of the roots of *M. satureiodes*. This paper describes the isolation and structure elucidation of these triterpene glycosides.

Results and Discussion. – The EtOH extract of the roots of *M. heisteria* was suspended in MeOH and purified by precipitation with Et_2O yielding a crude saponin mixture [4]. This extract was further fractionated by a multiple-column-chromatography step (*Sephadex LH-20*) followed by repeated medium-pressure liquid chromatography (MPLC) on normal silica gel to give compounds **1**–**6** as three inseparable mixtures **1/2**, **3/4**, and **5/6**, each compound pair giving only one spot by HPTLC but two

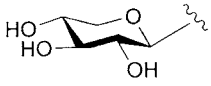




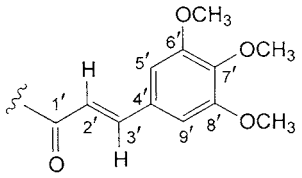
Gal Ac



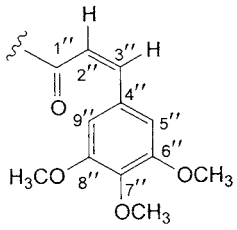
Api



Xyl



S^1



S^2

	R^1	R^2	R^3	R^4 [a)]
1	Gal Ac	Api	Xyl	S^1
2	Gal Ac	Api	Xyl	S^2
3	Gal Ac	Ac	H	S^1
4	Gal Ac	Ac	H	S^2
5	Xyl	Ac	H	S^1
6	Xyl	Ac	H	S^2

[a)] Arbitrary numbering

peaks by HPLC. In addition, by using the same methods of extraction and purification, saponins **1/2** together with four known triterpene glycosides were isolated from the EtOH extract of the roots of *M. satureioides.*

Structural elucidation of the saponins was mainly determined by spectroscopic 1D- and 2D-NMR experiments (^{1}H, ^{13}C, ^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, and HMBC; see *Tables 1–3*), HR-ESI-MS, and FAB-MS. Compounds **1/2**, **3/4**, and **5/6** were isolated as amorphous powders, which gave fluorescence-quenching zones under 254-nm and violet-blue fluorescence under 365-nm UV light on TLC without any chemical treatment. Acid hydrolysis of **1**–**6** afforded the same artifact aglycone (senegenic acid) of presenegenin (=(2β,3β,4α)-2,3,27-trihydroxyolean-12-ene-23,28-dioic acid) [5], which was identified from the 1D- and 2D-NMR data of **1**–**6** (*Table 1*). Most of the signals were in good agreement with literature data [2][5–6]. The sugars obtained from aqueous acid hydrolysis were identified by comparison with authentic

Table 1. *^{13}C-NMR* (150 MHz)[a]) *and ^{1}H-NMR* (600 MHz) *Data of the Aglycone Parts of* **1**–**6** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm.

	1/2		**3/4**		**5/6**	
	δ(C)	δ(H)[b])[c])	δ(C)	δ(H)[b])[c])	δ(C)	δ(H)[b])[c])
CH_2(1)	43.8	1.38, 2.30	44.0	1.50, 2.20	43.9	1.41, 2.24
CH(2)	70.0	4.76	69.3	4.86	69.5	4.84
CH(3)	86.2	4.71	86.6	4.74	86.2	4.72
C(4)	53.2	–	53.1	–	53.1	–
CH(5)	52.1	2.22	52.1	2.19	52.1	2.18
CH_2(6)	21.0	n.d., n.d.	21.0	n.d., n.d.	21.0	n.d., n.d.
CH_2(7)	33.3	1.08, 1.20	33.0	n.d., n.d.	33.1	n.d., n.d.
C(8)	40.4	–	40.5	–	40.3	–
CH(9)	48.8	2.20	48.9	2.24	48.9	2.22
C(10)	36.3	–	36.8	–	36.7	–
CH_2(11)	23.8	n.d., n.d.	23.8	n.d., n.d.	23.8	n.d., n.d.
CH(12)	126.8	5.74	127.9	5.82	127.7	5.80
C(13)	138.9	–	139.0	–	139.0	–
C(14)	47.6	–	47.8	–	47.8	–
CH_2(15)	24.1	n.d., n.d.	24.1	n.d., n.d.	24.1	n.d., n.d.
CH_2(16)	24.0	n.d., n.d.	24.0	n.d., n.d.	24.0	n.d., n.d.
C(17)	46.7	–	46.8	–	46.6	–
CH(18)	41.2	3.17	41.5	3.14	41.4	3.13
CH_2(19)	45.2	1.28, 1.66	45.5	n.d., 1.16	45.5	1.15, 1.60
C(20)	30.1	–	30.3	–	30.2	–
CH_2(21)	34.1	1.84, 2.34	34.0	n.d., n.d.	34.0	n.d., n.d.
CH_2(22)	31.8	1.68, 1.88	32.0	1.62, 1.82	31.9	1.61, 1.80
C(23)	181.0	–	180.8	–	180.6	–
Me(24)	14.3	1.92 (*s*)	14.8	1.90 (*s*)	14.6	1.90 (*s*)
Me(25)	17.0	1.50 (*s*)	17.1	1.47 (*s*)	17.1	1.48 (*s*)
Me(26)	18.6	1.12 (*s*)	18.7	1.03 (*s*)	18.5	1.11 (*s*)
CH_2(27)	64.1	3.97, 4.11	64.0	3.94, 4.10	63.9	3.94, 4.06
C(28)	176.5	–	176.5	–	176.3	–
Me(29)	32.4	0.82 (*s*)	32.7	0.70 (*s*)	32.5	0.81 (*s*)
Me(30)	23.0	0.84 (*s*)	23.3	0.71 (*s*)	23.4	0.82 (*s*)

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

Table 2. *^{13}C-NMR* (150 MHz) *Data of the Sugar Moieties of* **1**–**6** *in (D$_5$)Pyridine from 1D- and 2D-NMR Experiments*[a]). δ in ppm.

		1	**2**	**3**	**4**	**5**	**6**
3-*O*-Glc	CH(1)	103.9	103.9	104.5	104.5	103.8	103.8
	CH(2)	74.4	74.4	75.1	75.1	74.5	74.5
	CH(3)	76.7	76.7	77.3	77.3	77.0	77.0
	CH(4)	70.3	70.3	70.9	70.9	70.6	70.6
	CH(5)	77.0	77.0	77.6	77.6	77.3	77.3
	CH_2(6)	61.6	61.6	61.9	61.9	61.7	61.7
28-*O*-Sugars:							
Fuc	CH(1)	94.3	94.3	94.7	94.7	94.2	94.2
	CH(2)	71.1	71.1	71.6	71.6	71.2	71.2
	CH(3)	82.9	82.9	81.6	81.6	81.5	81.5
	CH(4)	74.0	74.2	74.1	74.3	74.0	74.0
	CH(5)	70.6	70.3	70.9	70.3	70.7	70.7
	Me(6)	16.7	16.5	16.7	16.6	16.7	16.7
Rha	CH(1)	100.7	100.7	101.2	101.2	101.2	101.2
	CH(2)	70.7	70.7	71.8	71.8	71.3	71.3
	CH(3)	80.2	80.2	75.1	75.1	74.8	74.8
	CH(4)	77.6	77.6	69.6	69.6	70.8	70.8
	CH(5)	67.3	67.3	67.7	67.7	67.8	67.8
	Me(6)	18.0	18.0	18.3	18.3	18.0	18.0
	AcO–C(3)	–	–	20.7, 171.5	20.6, 171.1	20.6, 171.3	20.5, 171.1
Gal	CH(1)	102.1	102.1	102.8	102.8	–	–
	CH(2)	69.8	69.8	69.9	69.9	–	–
	CH(3)	74.0	74.0	74.3	74.3	–	–
	CH(4)	69.1	69.1	69.6	69.6	–	–
	CH(5)	75.0	75.0	75.1	75.1	–	–
	CH_2(6)	63.6	63.6	63.3	63.3	–	–
	AcO–C(6)	20.6, 171.7	20.5, 171.5	20.5, 171.2	20.4, 170.9	–	–
Api	CH(1)	110.6	110.6	–	–	–	–
	CH(2)	78.0	78.0	–	–	–	–
	C(3)	79.4	79.4	–	–	–	–
	CH_2(4)	74.2	74.2	–	–	–	–
	CH_2(5)	64.5	64.5	–	–	–	–
Xyl	CH(1)	103.9	103.9	–	–	104.0	104.0
	CH(2)	74.8	74.8	–	–	74.5	74.5
	CH(3)	77.0	77.0	–	–	77.0	77.0
	CH(4)	70.3	70.3	–	–	70.4	70.4
	CH_2(5)	66.1	66.1	–	–	66.1	66.1
Acid	C(1′,1″)	166.5	166.1	167.8	167.4	166.5	166.1
	CH(2′,2″)	117.1	117.6	117.8	118.2	117.1	117.6
	CH(3′,3″)	145.4	144.1	145.8	144.6	145.4	144.1
	C(4′,4″)	130.2	130.0	130.7	130.5	130.2	130.0
	CH(5′,9′), CH(5″,9″)	105.7	105.7	106.2	106.2	105.7	105.7
	MeO–*C*(6′,8′), MeO–*C*(6″,8″)	140.2	139.4	140.6	139.8	140.2	139.4
	MeO–*C*(7′,7″)	153.3	152.6	153.7	153.0	153.3	152.6
	*Me*O–C(6′,8′), *Me*O–C(6″,8″)	55.6	55.5	56.0	55.9	55.6	55.5
	*Me*O–C(7′,7″)	60.5	60.4	60.5	60.4	60.5	60.4

[a]) Multiplicities were assigned from DEPT spectra.

Table 3. *^{1}H-NMR (600 MHz) Data of the Sugar Moieties of* **1**–**6** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

		1	**2**	**3**	**4**	**5**	**6**
3-*O*-Glc	H–C(1)	5.05 (d, J = 7.8)	5.05 (d, J = 7.8)	5.11 (d, J = 7.7)	5.11 (d, J = 7.7)	5.08 (d, J = 7.7)	5.08 (d, J = 7.7)
	H–C(2)	3.94	3.94	3.88	3.88	3.98	3.98
	H–C(3)	4.23	4.23	4.22	4.22	4.24	4.24
	H–C(4)	4.03	4.03	4.00	4.00	4.03	4.03
	H–C(5)	3.94	3.94	3.85	3.85	3.95	3.95
	CH_2(6)	4.16, 4.36	4.16, 4.36	4.11, 4.34	4.11, 4.34	4.18, 4.38	4.18, 4.38
28-*O*-Sugars:							
Fuc	H–C(1)	6.03 (br. s)	6.10 (br. s)	5.97 (br. s)	5.99 (br. s)	6.04 (br. s)	6.04 (br. s)
	H–C(2)	4.88	4.88	4.82	4.87	4.88	4.88
	H–C(3)	4.52	4.52	4.48	4.46	4.45	4.45
	H–C(4)	6.04	5.88	5.98	5.85	6.01	6.01
	H–C(5)	4.40	4.20	4.20	4.15	4.37	4.37
	Me(6)	1.47 (d, J = 6.4)	1.46 (d, J = 6.4)	1.43 (d, J = 6.1)	1.41 (d, J = 6.1)	1.56 (d, J = 6.2)	1.56 (d, J = 6.2)
Rha	H–C(1)	6.45 (br. s)	6.45 (br. s)	6.46 (br. s)	6.46 (br. s)	6.45 (br. s)	6.45 (br. s)
	H–C(2)	4.90	4.90	4.87	4.87	4.92	4.92
	H–C(3)	4.53	4.53	5.10	5.10	5.10	5.10
	H–C(4)	4.43	4.43	4.30	4.30	4.40	4.40
	H–C(5)	4.55	4.55	4.50	4.50	4.55	4.55
	Me(6)	1.74 (d, J = 6.0)	1.74 (d, J = 6.0)	1.66 (d, J = 6.0)	1.66 (d, J = 6.0)	1.72 (d, J = 6.2)	1.72 (d, J = 6.2)
	AcO–C(3)	–	–	2.07 (s, 3 H)	2.06 (s, 3 H)	2.06 (s, 3 H)	2.05 (s, 3 H)
Gal	H–C(1)	4.84 (d, J = 7.3)	4.84 (d, J = 7.3)	4.81 (d, J = 7.3)	4.81 (d, J = 7.3)	–	–
	H–C(2)	4.46	4.46	4.44	4.44	–	–
	H–C(3)	4.05	4.05	4.00	4.00	–	–
	H–C(4)	4.36	4.36	4.30	4.30	–	–
	H–C(5)	4.03	4.03	4.06	4.06	–	–
	CH_2(6)	4.73, 4.95	4.73, 4.95	4.67, 4.91	4.67, 4.91	–	–
	AcO–C(6)	2.14 (s, 3 H)	2.13 (s, 3 H)	2.05 (s, 3 H)	2.04 (s, 3 H)	–	–
Api	H–C(1)	6.06 (br. s)	6.06 (br. s)	–	–	–	–
	H–C(2)	4.76	4.76	–	–	–	–
	CH_2(4)	4.31, 4.53	4.31, 4.53	–	–	–	–
	CH_2(5)	4.14, 4.15	4.14, 4.15	–	–	–	–
Xyl	H–C(1)	5.22 (d, J = 7.3)	5.22 (d, J = 7.3)	–	–	5.24 (d, J = 7.3)	5.24 (d, J = 7.3)
	H–C(2)	3.94	3.94	–	–	3.93	3.93
	H–C(3)	4.15	4.15	–	–	4.22	4.22
	H–C(4)	3.93	3.93	–	–	4.11	4.11
	CH_2(5)	3.53, 4.13	3.53, 4.13	–	–	3.58, 4.18	3.58, 4.18
Acid	H–C(2′,2″)	6.58 (d, J = 15.9)	5.96 (d, J = 12.5)	6.50 (d, J = 15.6)	5.84 (d, J = 12.6)	6.58 (d, J = 15.8)	5.96 (d, J = 12.4)
	H–C(3′,3″)	7.90 (d, J = 15.9)	6.96 (d, J = 12.5)	7.83 (d, J = 15.6)	6.84 (d, J = 12.6)	7.90 (d, J = 15.8)	6.96 (d, J = 12.4)
	H–C(5′,9′), H–C(5″,9″)	6.80 (br. s)	6.83 (br. s)	6.75 (br. s)	6.77 (br. s)	6.80 (br. s)	6.83 (br. s)
	MeO–C(6′,8′), MeO–C(6″,8″)	3.96 (s, 6 H)	3.89 (s, 6 H)	3.77 (s, 6 H)	3.76 (s, 6 H)	3.96 (s, 6 H)	3.89 (s, 6 H)
	MeO–C(7′,7″)	3.94 (s, 3 H)	3.92 (s, 3 H)	3.83 (s, 3 H)	3.82 (s, 3 H)	3.94 (s, 3 H)	3.92 (s, 3 H)

[a]) Overlapped signals are reported without designated multiplicity.

samples (TLC) as rhamnose (=6-deoxymannose), apiose, fucose (=6-deoxygalactose), xylose, glucose, and galactose in the case of **1**/**2**, rhamnose, fucose, glucose, and galactose in the case of **3**/**4**, and rhamnose, fucose, xylose, and glucose in the case of **5**/**6**, respectively. The alkaline hydrolysis of each compound pair **1**/**2**, **3**/**4**, and **5**/**6** yielded the same prosapogenin characterized as tenuifolin (= 3-*O*-(β-D-glucopyranosyl)presenegenin) (TLC, ^{1}H- and ^{13}C-NMR), which was previously reported in this plant [2]. Mild alkaline hydrolysis of **1**–**6** yielded (*E*)/(*Z*)-3,4,5-trimethoxycinnamic acid (TLC) and a compound that presented a lower polarity than the native one (lower R_f on TLC), indicating acylation of the saponins.

The high-resolution electrospray-ionisation (HR-ESI) MS (positive-ion mode) of **1**/**2** exhibited a quasimolecular-ion peak at *m*/*z* 1683.7002 ($[M+Na]^+$; calc. 1683.7042) consistent with the molecular formula $C_{78}H_{116}NaO_{38}$. Their negative-ion FAB-MS displayed a quasimolecular-ion peak at *m*/*z* 1659 ($[M-H]^-$), indicating a molecular mass of 1660. Other significant ion peaks appeared at *m*/*z* 1455 ($[M-H-42-162]^-$) and 679 ($[(M-H-42-162-146-146-132-132-220]^-$), corresponding to the loss of one acetyl group, one hexosyl, one tetrasaccharide consisting of two deoxyhexosyl and two pentosyl moieties, and one 3,4,5-trimethoxycinnamoyl unit, respectively. Another ion peak at *m*/*z* 517 ($[M-H-42-162-146-146-132-132-220-162]^-$) showing the loss of one additional hexosyl unit corresponded to the pseudomolecular ion of the aglycone (presenegenin) [2][6]. The full assignments of the C- and H-atoms of the 3,4,5-trimethoxycinnamoyl units obtained by 2D-NMR investigations were in good agreement with our previously reported data [2]. These findings indicated that **1**/**2** is a mixture of (*E*)/(*Z*)-3,4,5-trimethoxycinnamoyl-substituted triterpenoic acid glycosyl esters (2:1, from relative NMR and HPLC intensities). This mixture was homogeneous by HPTLC but was separated into (*E*)/(*Z*)-isomers by HPLC, but all attempts to separate **1** and **2** by semi-prep. HPLC were unsuccessful. The observed isomerization is due to the effect of light on the trimethoxycinnamoyl group in aqueous methanolic solution. Under these conditions, the geometrical-isomer structures of the trimethoxycinnamoyl moieties of **1** and **2** showed tautomer-like behavior. This phenomenon has already been observed in saponins from *Silene jenisseensis* [7], *Atroxima congolana* [8], and also in the previously reported saponins of this plant [2]. The extensive investigation of 1D- and 2D-NMR data of **1**/**2** (*Tables 1–3*) resulted in the determination of the structure as 3-*O*-(*β*-D-glucopyranosyl)presenegenin 28-{*O*-*β*-D-apiofuranosyl-(1→3)-*O*-[*β*-D-xylopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[6-*O*-acetyl-*β*-D-galactopyranosyl-(1→3)]-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**1**) and its (*Z*)-isomer **2**, two new natural compounds [9].

The ^{1}H- and ^{13}C-NMR signals corresponding to the prosapogenin part of **1**/**2** were superimposable with those previously reported [2][6] (*Tables 1–3*). The ^{1}H,^{1}H-COSY experiment of **1**/**2** permitted the identification of two acyl moieties. They appeared as 2*d* at *δ*(H) 6.58 (*J* = 15.9 Hz, H–C(2′)) and 7.90 (*J* = 15.9 Hz, H–C(3′)) for the (*E*)-olefinic protons, and at *δ* 5.96 (*J* = 12.5 Hz, H–C(2″)) and 6.96 (*J* = 12.5 Hz, H–C(3″)) for the (*Z*)-olefinic protons, respectively (*Table 3*). The ^{1}H-NMR spectrum (600 MHz, (D_5)pyridine) of **1**/**2** displayed signals for six anomeric H-atoms at *δ* 6.45 (br. *s*), 6.06 (br. *s*), 6.03 (br. *s*), 5.22 (*d*, *J* = 7.3 Hz), 5.05 (*d*, *J* = 7.8 Hz), and 4.84 (*d*, *J* = 7.3 Hz), which gave correlations with ^{13}C-NMR signals in the HSQC spectrum at *δ* 100.7, 110.6, 94.3, 103.9, 103.9, and 102.1, respectively (*Tables 2* and *3*). The ring H-atoms of the monosaccharide residues were assigned starting from the readily identifiable anomeric H-atoms by means of the ^{1}H,^{1}H-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*-rhamnopyranosyl (Rha), one *β*-apiofuranosyl (Api), one *β*-fucopyranosyl (Fuc), one *β*-xylopyranosyl (Xyl), one *β*-glucopyranosyl (Glc), and one *β*-galactopyranosyl (Gal) unit, respectively. The D-configuration for Fuc, Api, Xyl, Glc, and Gal and the L-configuration for Rha were assumed in keeping with *Massiot* and *Lavaud*'s assertion regarding these configurations commonly found for these monosaccharides in saponins [10]. From the extensive 1D- and 2D-NMR experiments and the results of alkaline hydrolysis, it can be concluded that **1**/**2** were bisdesmosidic glycosides of presenegenin, with one Glc at C(3) (*δ*(C) 86.2) of the aglycone and the other five monosaccharides linked at C(28) (*δ*(C) 176.5) through an ester bond.

The sequence of the sugars at C(28) of the aglycone of **1**/**2** was based on HMBC and NOESY correlations. The HMBC correlation between *δ*(H) 6.03 (br. *s*, Fuc H–C(1)) and *δ*(C) 176.5 (Agly C(28)) confirmed the Fuc unit to be linked at C(28) of the aglycone. The location of the 3,4,5-trimethoxycinnamoyl group at C(4) of Fuc

(δ(H) 6.04, H–C(4)) was determined by TOCSY and COSY experiments, starting from the anomeric-proton signal of Fuc at δ 6.03 (br. *s*). The downfield shifts observed in the HSQC spectrum for the Fuc H–C(4)/C(4) resonances at δ(H) 6.04/δ(C) 74.0 established that the secondary-alcohol function OH–C(4) of Fuc was acylated. The HMBC between δ(H) 6.45 (br. *s*, Rha H–C(1)) and δ(C) 71.1 (Fuc C(2)) indicated that the Rha unit was linked to the Fuc residue by a $1 \rightarrow 2$ linkage. Further confirmation was obtained by a NOESY cross-peak between δ(H) 6.45 (br. *s*, Rha H–C(1)) and δ(H) 4.88 (Fuc H–C(2)). A NOESY cross-peak between δ(H) 4.84 (*d*, $J = 7.3$ Hz, Gal H–C(1)) and δ(H) 4.52 (Fuc H–C(3)) indicated that Gal was attached to C(3) of Fuc. The location of the acetyl group at Gal C(6) was determined by TOCSY and COSY experiments, starting from the anomeric-proton signal of Gal at δ 4.84 (*d*, $J = 7.3$ Hz). The downfield shifts observed in the HSQC spectrum for the Gal H–C(6)/Gal C(6) resonances at δ(H) 4.73, 4.95/δ(C) 63.6 established the acetylation of the primary-alcohol function OH–C(6) of Gal. A NOESY cross-peak between δ(H) 6.06 (br. *s*, Api H–C(1)) and δ(H) 4.53 (Rha H–C(3)) showed that the Api unit was attached at C(3) of Rha. HMBC Correlations between δ(H) 5.22 (*d*, $J = 7.3$ Hz, Xyl H–C(1)) and δ(C) 77.6 (Rha C(4)) and between δ(H) 4.43 (Rha H–C(4)) and δ(C) 103.9 (Xyl C(1)) suggested that Xyl was linked to C(4) of Rha. This was confirmed by a NOESY cross-peak between δ(H) 5.22 (*d*, $J = 7.3$ Hz, Xyl H–C(1)) and δ(H) 4.43 (Rha H–C(4)). This terminal Xyl was confirmed by its ^{1}H- and ^{13}C-NMR data (*Tables 2* and *3*).

The positive-ion HR-ESI-MS of **3/4** exhibited a quasimolecular-ion peak at *m/z* 1461.6336 ($[M + Na]^+$; calc. 1461.6306) consistent with the molecular formula $C_{70}H_{102}NaO_{31}$. Their negative-ion FAB-MS showed a quasimolecular-ion peak at *m/z* 1437 ($[M - H]^-$), indicating a molecular mass of 1438. Other significant ion peaks appeared at *m/z* 1395 ($[M - H - 42]^-$), 1233 ($[M - H - 42 - 162]^-$), and 517 ($[M - H - 42 - 162 - 146 - 146 - 220 - 42 - 162]^-$) corresponding to the loss of one acetyl, one hexosyl, two deoxyhexosyl, one 3,4,5-trimethoxycinnamoyl, one acetyl, and one hexosyl unit, respectively. The ion peak at *m/z* 517 corresponded to the pseudomolecular ion of presenegenin [2][6]. 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*-6-*O*-acetyl-β-D-galactopyranosyl-$(1 \rightarrow 3)$-*O*-[3-*O*-acetyl-α-L-rhamnopyranosyl-$(1 \rightarrow 2)$]-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranosyl} ester (**3**) and its (*Z*)-isomer **4**, two new natural compounds [9].

The study of the 2D-NMR spectra of **3/4** showed that the same sequence 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-6-*O*-acetyl-β-D-galactopyranosyl-$(1 \rightarrow 3)$]-*O*-[α-L-rhamnopyranosyl-$(1 \rightarrow 2)$]-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranosyl} ester and its (*Z*)-isomer already encountered in **1/2** was found in **3/4**. The difference was located at the substituents linked to the 3 and 4 positions of Rha. The presence of one acetyl group at Rha C(3) was determined by TOCSY and COSY experiments, starting from the anomeric-proton signal of Rha at δ 6.46 (br. *s*). The downfield shifts observed in the HSQC spectrum for the Rha H–C(3)/Rha C(3) resonances at δ(H) 5.10/δ(C) 75.1 established the acetylation of the secondary-alcohol function OH–C(3) of Rha. Furthermore, the acetylated Rha was terminal (δ 75.1 (C(3)), δ 69.6 (C(4)) instead of being a 1,3,4-trisubstituted Rha as observed for **1/2** (δ 81.6 (C(3)), δ 77.6 (C(4)); *Tables 2* and *3*).

The positive-ion HR-ESI-MS of **5/6** exhibited a quasimolecular-ion peak at *m/z* 1389.6060 ($[M + Na]^+$; calc. 1389.6091) consistent with the molecular formula $C_{67}H_{98}NaO_{29}$. Their negative-ion FAB-MS showed a quasimolecular-ion peak at *m/z* 1365 ($[M - H]^-$), indicating a molecular mass of 1366, with 92 mass units less than compounds **3/4**. Two other significant ion peaks appeared at *m/z* 679 ($[M - H - 132 - 146 - 146 - 220 - 42]^-$) and 517 ($[M - H - 132 - 146 - 146 - 220 - 42 - 162]^-$) corresponding to the loss of one pentosyl, two deoxyhexosyl, one 3,4,5-trimethoxycinnamoyl, one acetyl, and one hexosyl unit, respectively. Further investigation of 1D- and 2D-NMR data resulted in the determination of the structure of **5/6** as 3-*O*-(β-D-

glucopyranosyl)presenegenin 28-{*O*-3-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranosyl} ester (**5**) and its (*Z*)-isomer **6**, two new natural compounds [9].

The ^{1}H-NMR spectrum of **5/6** showed four anomeric-proton signals at δ(H) 6.45 (br. *s*), 6.04 (br. *s*), 5.24 (*d*, *J* = 7.3 Hz), and 5.08 (*d*, *J* = 7.7 Hz), which correlated in the HSQC spectrum with δ(C) 101.2, 94.2, 104.0, and 103.8, respectively. Comparison of the spectral data obtained from 2D-NMR spectra of **5/6** and **3/4** (*Tables 2* and *3*) indicated that the only difference was the nature of the monosaccharide unit at C(3) of Fuc. Compounds **5/6** were shown to possess a terminal xylopyranosyl (Xyl) unit instead of an acetylated Gal at this position in compounds **3/4**. The linkage of Xyl at C(3) of Fuc was deduced from a NOESY cross-peak between δ(H) 4.45 (Fuc H–C(3)) and δ(H) 5.24 (*d*, *J* = 7.3 Hz, Xyl H–C(1)). These results were also in accordance with the difference in molecular mass (72 mass units) between **5/6** and **3/4**.

Conclusions. – Saponins **1/2** along with four known acylated triterpene glycosides were isolated as three inseparable mixtures from *Muraltia satureioides* DC. The known compounds were identified by comparing their physical and spectral data with literature values as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-β-D-apiofuranosyl-(1 → 3)-*O*-[β-D-xylopyranosyl-(1 → 2)]-*O*-α-L-arabinopyranosyl-(1 → 4)-*O*-[β-D-galactopyranosyl-(1 → 3)]-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranosyl} ester and its (*Z*)-isomer and as 3-*O*-(β-D-glucopyranosyl)presenegenin 28-{*O*-α-L-arabinopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(*E*)-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranosyl} ester and its (*Z*)-isomer, already described in *M. heisteria* [2].

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* 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: 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, ether/toluene 1 : 1 sat. with 10% AcOH soln. (*d*); spray reagents: for saponins, *Komarowsky* reagent, 2% 4-hydroxybenzaldehyde in MeOH/50% H_2SO_4 soln. 5 : 1; for the sugars, diphenylamine/phosphoric acid reagent; for the cinnamic acids, detection by UV light (λ 254 nm). IR Spectra: KBr disc; *Perkin-Elmer-281* IR spectrophotometer; in cm^{-1}. 1D- and 2D-NMR Spectra: see [2]. High-resolution electrospray-ionization (HR-ESI) MS: *Q-TOF-1* micromass spectrometer; in *m*/*z*. Fast-atom bombardment (FAB) MS: negative ion mode; *JEOL SX-102*.

Plant Material. Muraltia heisteria (L.) DC. and *M. satureioides* DC. were collected in July 1990 in South Africa, near Capetown. Voucher specimens under the reference *H. Breyne* No. 5420 and 5446 were deposited in the Herbarium of the National Botanical Garden of Brussels, Belgium.

Extraction and Isolation. Dried powdered roots of *M. heisteria* (410 g) and of *M. satureoides* (189 g) were macerated separately during 4 h with 3 l of 80% EtOH and further submitted to boiling for 4 h. After cooling, the EtOH soln. was filtered and evaporated. Each residue was dissolved in MeOH (400 ml) at 60°. After filtration, the MeOH soln. was purified by precipitation with Et_2O (5 × 400 ml). The resulting residue was solubilized in H_2O (400 ml) and the soln. submitted to dialysis for 4 days and then lyophilized. After decolorization with charcoal and filtration, the residue was dissolved in MeOH and purified again by precipitation with Et_2O: crude saponin mixture (2.39 g and 1.10 g) from *M. heisteria* and *M. satureioides*, resp. Of these mixtures, 588 mg (from *M. heisteria*) and 226 mg (from *M. satureioides*) were individually further fractionated by CC (*Sephadex LH-20*) and then submitted to successive MPLC (silica gel *60* (15–40 μm), $CHCl_3$/MeOH/H_2O 65 : 35 : 10, lower phase): **1/2** (7 mg), **3/4** (10 mg), and **5/6** (6 mg) from *M. heisteria* and **1/2** (12 mg) from *M. satureioides*.

(2β,3β,4α)-3-O-*(β*-D-*Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-[*O-*β*-D-*Apiofuranosyl-(1 → 3)*-O-*[β*-D-*xylopyranosyl-(1 → 4)]*-O-*6-deoxy-α*-L-*mannopyranosyl-(1 → 2)*-O-*[6*-O-*acetyl-β*-

D-*galactopyranosyl-(1 → 3)]-6-deoxy-4*-O-*[(2*E*)-3-(3,4,5-trimethoxyphenyl)-1-oxoprop-2-enyl]-β*-D-*galacto-pyranosyl] Ester* (**1**) *and Its (2*Z*)-Isomer* **2**. White amorphous powder. TLC: R_f 0.39. IR (KBr): 3405, 2926, 1750 and 1740, 710, 1634, 1610, 1560. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. HR-ESI-MS (pos.): 1683.7002 ($[M+Na]^+$, $C_{78}H_{116}O_{38}Na^+$; calc. 1683.7042). FAB-MS (neg.): 1659 ($[M-H]^-$), 1455 ($[M-H-42-162]^-$), 679 ($[(M-H-42-162-146-146-132-132-220]^-$), 517 ($[M-H-42-162-146-146-132-132-220-162]^-$).

(2β,3β,4α)-3-O-*(β*-D-*Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-6*-O*-Acetyl-β*-D-*galactopyranosyl-(1 → 3)*-O-*[3*-O*-*acetyl-6-deoxy-α*-L-*mannopyranosyl-(1 → 2)]-6-deoxy-4*-O-*[(2*E*)-3-(3,4,5-trimethoxyphenyl)-1-oxoprop-2-enyl]-β*-D-*galactopyranosyl] Ester* (**3**) *and Its (2*Z*)-Isomer* **4**. White amorphous powder. TLC: R_f 0.45. IR (KBr): 3406, 2927, 1723, 1740, 1710, 1636, 1580, 1420. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. HR-ESI-MS (pos.): 1461.6336 ($[M+Na]^+$, $C_{70}H_{102}O_{31}Na^+$; calc. 1461.6306). FAB-MS (neg.): 1437 ($[M-H]^-$), 1395 ($[M-H-42]^-$), 1233 ($[M-H-42-162]^-$) and 517 ($[M-H-42-162-146-146-220-42-162]^-$).

(2β,3β,4α)-3-O-*(β*-D-*Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-3*-O-*Acetyl-6-deoxy-α*-L-*mannopyranosyl-(1 → 2)*-O-*[β*-D-*xylopyranosyl-(1 → 3)]-6-deoxy-4*-O-*[(2*E*)-3-(3,4,5-trimethoxy-phenyl)-1-oxoprop-2-enyl]-β*-D-*galactopyranosyl] Ester* (**5**) *and Its (2*Z*)-Isomer* **6**. White amorphous powder. TLC: R_f 0.47. IR (KBr): 3404, 2927, 1723, 1740, 1710, 1636, 1580, 1500, 1420. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. HR-ESI-MS (pos.): 1389.6060 ($[M+Na]^+$, $C_{67}H_{98}O_{29}Na^+$; calc. 1389.6091). FAB-MS (neg.): 1365 ($[M-H]^-$), 679 ($[M-H-132-146-146-220-42]^-$), 517 ($[M-H-132-146-146-220-42-162]^-$).

Acid Hydrolysis. A soln. of each saponin pair (2 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 (*c*) by comparison with standard sugars.

Alkaline Hydrolysis. Each saponin pair (2 mg) was refluxed with 5% aq. KOH soln. (10 ml) for 2 h. The 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 tenuifolin (= 3-*O*-(β-D-glucopyranosyl)presenegenin).

Mild Alkaline Hydrolysis. Each saponin pair (2 mg) was hydrolyzed with 1% aq. KOH soln. (10 ml) at r.t. After 1 h, the mixture was neutralized with dil. HCl soln. and extracted with Et_2O (3×5 ml). The Et_2O layer gave (*E*)- and (*Z*)-3,4,5-trimethoxycinnamic acids (=(2*E*)- and (2*Z*)-3-(3,4,5-trimethoxyphenyl)prop-2-enoic acids), which were identified by TLC. The aq. layer was extracted with H_2O-sat. BuOH (3×10 ml): deacylated saponin.

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