Five New Medicagenic Acid Saponins from *Muraltia ononidifolia*

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 JE 2244), 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

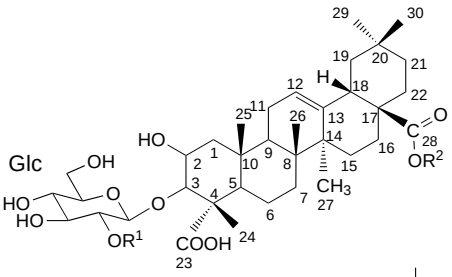
[c]) Centre de Recherche Phytochimique, Université de Liège, Institut de Chimie-B6, Sart Tilman B-4000-Liège I

Five new triterpene saponins **1**–**5** were isolated from the roots of *Muraltia ononidifolia* E. MEY along with the two known saponins 3-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]medicagenic acid 28-[*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl] ester and 3-*O*-(*β*-D-glucopyranosyl)medicagenic acid 28-[*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl] ester (medicagenic acid = (4*α*,2*β*,3*β*)-2,3-dihydroxyolean-12-ene-23,28-dioic acid). Their structures were elucidated mainly by spectroscopic experiments, including 2D-NMR techniques, as 3-*O*-(*β*-D-glucopyranosyl)medicagenic acid 28-[*O*-*β*-D-apiofuranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl] ester (**1**), 3-*O*-(*β*-D-glucopyranosyl)medicagenic acid 28-{[*O*-*β*-D-xylopyranosyl-(1→4)-*O*-[*β*-D-apiofuranosyl-(1→3)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl} ester (**2**), 3-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]medicagenic acid 28-{*O*-*β*-D-xylopyranosyl-(1→4)-*O*-[*β*-D-apiofuranosyl-(1→3)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl} ester (**3**), 3-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]medicagenic acid 28-[*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl] ester (**4**), and 3-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]medicagenic acid (**5**).

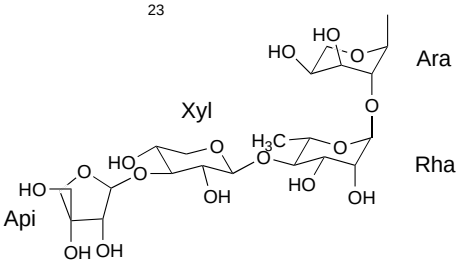
Introduction. – In continuing our studies on the genus *Muraltia* (Polygalaceae) [1], we have investigated *M. ononidifolia* E. MEY, which is an herbaceous plant indigenous to South Africa. This plant was reported to contain presenegenin glycosides [2], but no detailed phytochemical study was described. This paper deals with the isolation and structure elucidation of five new triterpene saponins **1**–**5**, in addition to two known compounds from the EtOH extract of the roots.

Results and Discussion. – The EtOH extract of the roots of *M. ononidifolia* was suspended in MeOH and purified by precipitation with Et_2O, yielding a crude saponin mixture [3]. This extract was further fractionated by column chromatography (*Sephadex LH-20*) and repeated medium-pressure liquid chromatography (MPLC) with normal silica gel, followed by semi-preparative reversed-phase HPLC to give seven saponins in pure form.

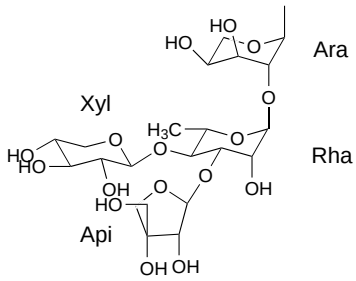
Structural elucidation of the saponins was mainly determined by 1D- and 2D-NMR experiments (^1H,^{13}C, COSY, TOCSY, NOESY, HSQC, and HMBC) and FAB-MS. Compounds **1**–**5** were isolated as amorphous powders. Acid hydrolysis of **1**–**5** afforded an aglycone, which was identified as medicagenic acid (=(4*α*,2*β*,3*β*)-2,3-dihydroxy-olean-12-ene-23,28-dioic acid) from the 1D- and 2D-NMR data of **1**–**5** (*Table 1*). Most of the signals were in good agreement with literature data [3–6]. The sugars obtained from aqueous acid hydrolysis were identified by comparison with authentic samples (TLC) as arabinose, apiose, rhamnose, glucose, and xylose in the



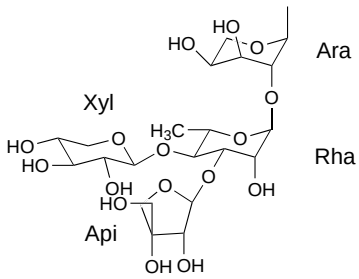
1 R^1 = H R^2 =



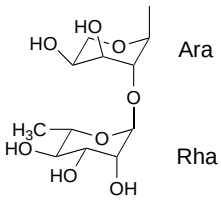
2 R^1 = H R^2 =



3 R^1 = Glc R^2 =



4 R^1 = Glc R^2 =



5 R^1 = Glc R^2 = H

Table 1. *^{13}C-NMR* (150 MHz)[a]) *and ^{1}H-NMR* (600 MHz) *Data of the Aglycone Parts of* **1**–**5** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm.

	1		**2**		**3**		**4**		**5**	
	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)	43.7	2.22, 1.24	43.8	2.22, 1.23	43.7	2.22, 1.22	43.3	2.22, 1.22	43.3	2.23, 1.26
CH(2)	69.7	4.75	69.7	4.76	70.0	4.74	69.7	4.62	70.3	4.72
CH(3)	85.6	4.58	85.6	4.60	85.6	4.64	85.0	4.51	85.0	4.66
C(4)	52.5	–	52.5	–	52.5	–	52.5	–	52.4	–
CH(5)	52.1	1.94	52.1	1.95	52.1	1.94	52.1	1.94	51.9	1.90
CH_2(6)	20.8	1.76, 1.53	20.8	1.76, 1.52	20.8	1.76, 1.53	20.2	1.62, 1.40	20.3	1.60, 1.51
CH_2(7)	32.3	1.90, 1.60	32.4	1.92, 1.60	32.3	1.90, 1.60	32.0	1.80, 1.60	32.4	1.80, 1.60
C(8)	39.9	–	40.0	–	40.5	–	39.5	–	39.5	–
CH(9)	48.3	1.65	48.4	1.68	48.3	1.62	48.0	1.57	48.0	1.64
C(10)	36.4	–	36.5	–	36.4	–	36.4	–	36.2	–
CH_2(11)	23.6	1.97, 1.86	23.7	[c])	23.6	1.97, 1.85	23.2	1.92, 1.85	23.3	1.94, 1.85
CH(12)	122.5	5.38	122.7	5.40	122.6	5.40	122.4	5.40	122.3	5.36
C(13)	143.9	–	144.0	–	144.0	–	143.7	–	144.5	–
C(14)	41.9	–	41.8	–	42.0	–	41.8	–	41.8	–
CH_2(15)	27.8	1.96, 1.16	27.8	[c]), [c])	27.8	[c]), [c])	27.8	[c]), [c])	27.6	1.96, [c])
CH_2(16)	22.8	1.95, 1.87	22.8	2.00, 1.88	22.7	1.96, 1.90	22.5	1.93, 1.86	23.0	1.93, 1.86
C(17)	47.0	–	47.1	–	47.0	–	46.8	–	46.0	–
CH(18)	41.3	3.15	41.4	3.19	41.3	3.18	41.1	3.10	41.4	3.12
CH_2(19)	45.9	1.66, 1.16	45.9	1.70, 1.20	45.8	1.66, 1.16	45.7	1.64, 1.14	46.0	1.62, 1.18
C(20)	30.5	–	30.6	–	30.5	–	30.2	–	30.4	–
CH_2(21)	33.7	1.28, 1.06	33.8	1.30, 1.08	33.7	1.29, 1.07	33.4	1.25, 1.05	33.7	1.30, 1.10
CH_2(22)	32.6	[c]), 1.34	32.7	1.57, 1.28	32.3	1.90, 1.32	32.3	1.40, 1.23	32.7	1.40, 1.23
C(23)	178.8	–	180.8	–	180.8	–	180.1	–	180.7	–
Me(24)	13.8	1.85 (*s*)	13.8	1.87 (*s*)	13.8	1.85 (*s*)	14.6	1.85 (*s*)	14.0	1.85 (*s*)
Me(25)	16.5	1.40 (*s*)	16.5	1.44 (*s*)	16.4	1.40 (*s*)	16.2	1.30 (*s*)	16.3	1.30 (*s*)
Me(26)	17.1	0.98 (*s*)	17.2	1.03 (*s*)	17.1	0.98 (*s*)	16.7	0.88 (*s*)	16.9	0.88 (*s*)
Me(27)	25.7	1.15 (*s*)	25.9	1.17 (*s*)	25.8	1.12 (*s*)	25.7	1.10 (*s*)	25.8	1.10 (*s*)
C(28)	176.2	–	176.2	–	176.1	–	176.2	–	182.0	
Me(29)	32.8	0.82 (*s*)	32.9	0.84 (*s*)	32.8	0.82 (*s*)	32.6	0.80 (*s*)	32.9	0.80 (*s*)
Me(30)	23.3	0.90 (*s*)	23.4	0.94 (*s*)	23.3	0.91 (*s*)	23.0	0.85 (*s*)	23.3	0.85 (*s*)

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

case of **1**–**3**, arabinose, rhamnose, and glucose in the case of **4**, and glucose in the case of **5**. The prosapogenins obtained after alkaline hydrolysis of both **1** and **2** were identical but less polar than the prosapogenin of both **3** and **4**. Saponin **5** remained intact after alkaline hydrolysis and was identical to the prosapogenin of **3** and **4**.

The negative-ion FAB-MS of **1** showed a quasi-molecular ion peak at *m/z* 1205 ($[M-H]^-$), indicating a molecular mass of 1206, compatible with a molecular formula $C_{57}H_{90}O_{27}$. Two other significant ion peaks appeared at *m/z* 1073 ($[M-H-132]^-$) and 911 ($[M-H-162]^-$) corresponding to the loss of one pentosyl and one hexosyl moiety, respectively. The assignments of all the ^{1}H- and ^{13}C-NMR signals of **1** were successfully carried out with 2D-NMR experiments (*Tables 1–3*). Thus, the structure of **1** was determined as 3-*O*-(β-D-glucopyranosyl)medicagenic acid 28-[*O*-β-D-apiofuranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl] ester (**1**), a new natural compound [4–8].

Table 2. *^{13}C-NMR* (150 MHz) *Data of the Sugar Moieties of* **1**–**5** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments*[a]). δ(C) in ppm.

		1	**2**	**3**	**4**	**5**
3-*O*-Glc	CH(1)	104.6	104.8	102.7	102.2	102.1
	CH(2)	74.7	74.7	82.5	81.1	81.5
	CH(3)	77.5	77.7	77.1	77.0	77.0
	CH(4)	70.9	71.0	70.5	70.5	70.4
	CH(5)	77.7	77.8	77.2	77.1	77.1
	CH_2(6)	62.0	62.1	61.8	61.3	61.8
T-Glc	CH(1)			105.0	104.0	104.0
	CH(2)			76.4	75.3	75.4
	CH(3)			77.0	76.6	76.8
	CH(4)			70.5	70.3	70.3
	CH(5)			77.7	77.2	77.5
	CH_2(6)			61.8	61.3	61.3
28-*O*-Sugars						
Ara	CH(1)	92.9	92.7	92.7	92.6	
	CH(2)	75.1	75.4	75.3	74.7	
	CH(3)	69.3	68.3	68.7	69.1	
	CH(4)	65.5	64.9	65.8	65.3	
	CH_2(5)	62.3	61.5	61.8	62.1	
Rha	CH(1)	100.7	100.7	100.7	100.7	
	CH(2)	71.3	71.0	71.1	71.1	
	CH(3)	72.0	81.6	81.6	71.4	
	CH(4)	83.1	77.7	77.5	72.7	
	CH(5)	68.2	68.5	68.5	70.0	
	Me(6)	18.0	18.3	18.2	17.7	
Xyl	CH(1)	106.0	104.8	104.6		
	CH(2)	74.6	75.0	75.0		
	CH(3)	84.6	77.8	77.4		
	CH(4)	68.8	70.8	70.7		
	CH_2(5)	66.3	66.7	66.5		
Api	CH(1)	110.5	111.2	111.0		
	CH(2)	77.3	77.2	77.1		
	C(3)	80.0	79.3	79.6		
	CH_2(4)	74.6	74.3	74.3		
	CH_2(5)	64.5	64.0	63.9		

[a]) Multiplicities were assigned from DEPT spectra.

The ^{1}H-NMR spectrum (600 MHz, (D_5)pyridine) of **1** displayed signals for five anomeric protons at δ 6.34 (br. *s*), 6.05 (*d*, $J = 1.8$ Hz), 5.60 (br. *s*), 4.98 (*d*, $J = 7.2$ Hz), and 4.95 (*d*, $J = 7.3$ Hz), which correlated in the HSQC spectrum with ^{13}C-NMR signals at δ 92.9, 110.5, 100.7, 104.6, and 106.0, 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-arabinopyranosyl (Ara), one β-apiofuranosyl (Api), one α-rhamnopyranosyl (Rha), one β-glucopyranosyl (Glc), and one β-xylopyranosyl (Xyl) unit. The common D-configuration for Xyl, Glc, and Api and the L-configuration for Rha and Ara were assumed, according to those most encountered among the plant glycosides in each case. From the extensive 1D- and 2D-NMR experiments, it was concluded that **1** was a bisdesmosidic saponin with one terminal Glc at C(3) (δ(C) 85.6) of the aglycone and the four other monosaccharides linked at C(28) (δ(C) 176.2) through an ester bond. The connection of the Glc moiety at C(3) of the aglycone (C(3)Agly) was deduced by the NOESY correlation observed between the

Table 3. *^{1}H-NMR* (600 MHz) *Data of the Sugar Moieties of* **1**–**5** *in (D_5)Pyridine from 1D- and 2D-NMR Experiments*[a]). δ(H) in ppm, J in Hz.

		1	**2**	**3**	**4**	**5**
3-*O*-Glc	H–C(1)	4.98 (d, J = 7.2)	5.00 (d, J = 7.7)	5.08 (d, J = 7.0)	4.98 (d, J = 7.0)	5.11 (d, J = 7.0)
	H–C(2)	3.90	3.91	4.02	4.04	4.05
	H–C(3)	4.07	4.08	4.16	4.14	4.23
	H–C(4)	4.03	4.10	4.00	3.93	3.98
	H–C(5)	3.83	3.86	3.80	3.77	3.84
	CH_2(6)	4.16, 4.38	4.17, 4.40	4.14, 4.35	4.10, 4.27	4.17, 4.41
T-Glc	H–C(1)			5.14 (d, J = 7.0)	5.14 (d, J = 7.0)	5.16 (d, J = 7.0)
	H–C(2)			3.95	3.90	3.95
	H–C(3)			4.08	4.06	4.06
	H–C(4)			3.99	3.92	4.08
	H–C(5)			3.96	3.89	3.98
	CH_2(6)			[b]), 4.24	4.08, 4.10	4.10, 4.30
28-*O*-Sugars						
Ara	H–C(1)	6.34 (br. s)	6.45 (br. s)	6.38 (br. s)	6.25 (br. s)	
	H–C(2)	4.41	4.41	4.39	4.33	
	H–C(3)	4.44	4.51	4.47	4.39	
	H–C(4)	4.32	4.35	4.32	4.31	
	CH_2(5)	3.89, 4.42	3.90, 4.49	3.89, 4.46	3.82, 4.3	
Rha	H–C(1)	5.60 (br. s)	5.49 (br. s)	5.55 (br. s)	5.56 (br. s)	
	H–C(2)	4.44	4.61	4.61	4.42	
	H–C(3)	4.42	4.39	4.37	4.30	
	H–C(4)	4.22	4.42	4.38	4.12	
	H–C(5)	4.23	4.26	4.25	4.21	
	Me(6)	1.62 (d, J = 6.0)	1.65 (d, J = 6.2)	1.62 (d, J = 6.1)	1.53 (d, J = 5.96)	
Xyl	H–C(1)	4.95 (d, J = 7.3)	5.25 (d, J = 7.7)	5.22 (d, J = 7.7)		
	H–C(2)	3.90	3.88	3.86		
	H–C(3)	3.92	4.00	4.07		
	H–C(4)	3.93	4.05	4.09		
	CH_2(5)	3.32, 4.05	3.38, 4.10	3.38, 4.10		
Api	H–C(1)	6.05 (d, J = 1.8)	5.94 (d, J = 4.4)	5.92 (d, J = 4.0)		
	H–C(2)	4.73	4.71	4.69		
	CH_2(4)	4.24, 4.65	4.13, 4.56	4.13, 4.54		
	CH_2(5)	4.05, 4.12	4.03, 4.04	4.03, 4.03		

[a]) Overlapped signals are reported without designated multiplicity. [b]) Not determined.

anomeric proton of Glc (H–C(1) (Glc)) at δ 4.98 (d, J = 7.2 Hz) and the H–C(3) of the aglycone at δ 4.58 (H–C(3)(Agly)). Further confirmation was obtained by the HMBC correlation between δ(H) 4.98 (d, J = 7.2 Hz) (H–C(1)(Glc)) and δ(C) 85.6 (C(3)(Agly)). The HMBC and NOESY plots allowed us to establish the sequence of the sugars at C(28). The HMBC correlation between δ(H) 6.34 (br. s, H–C(1)(Ara)) and δ(C) 176.2 (C(28)(Agly)) confirmed the Ara to be attached at C(28) of the aglycone. The HMBC correlation between δ(H) 5.60 (br. s, H–C(1)(Rha)) and δ(C) 75.1 (C(2)(Ara)) indicated that the Rha was linked to the Ara by a (1 → 2) linkage. Another HMBC correlation between δ(H) 4.95 (d, J = 7.3 Hz, H–C(1)(Xyl)) and δ(C) 83.1 (C(4)(Rha)) established that the Xyl was linked to the Rha at C(4); this was confirmed by a NOESY cross-peak between δ(H) 4.95 and δ(H) 4.22 (H–C(4)(Rha)). The linkage of Api at C(3) of Xyl was deduced by the HMBC correlation between δ(H) 6.05 (d, J = 1.8 Hz, H–C(1)(Api)) and δ(C) 84.6 (C(3)(Xyl)); this was also confirmed by a NOESY cross-peak between δ(H) 6.05 (d, J = 1.8 Hz, H–C(1)(Api)) and δ(H) 3.92 (H–C(3)(Xyl)).

Compound **2** had the same molecular formula as **1**, $C_{57}H_{90}O_{27}$, determined from the quasi-molecular ion peak at *m/z* 1205 ($[M-H]^-$) in the negative-ion FAB-MS, indicating a molecular mass of 1206. Other significant ion peaks appeared at the same *m/z* values as in **1**. Extensive study of the 2D-NMR spectra of **2** led to the establishment of its structure as 3-*O*-(*β*-D-glucopyranosyl)medicagenic acid 28-{*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-[*β*-D-apiofuranosyl-(1 → 3)]-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl} ester (**2**), a new natural compound [4–8].

The ^{1}H- and ^{13}C-NMR spectra (600 and 150 MHz, resp., (D_5)pyridine) of **2** allowed the identification of medicagenic acid as aglycone (*Table 1*) and five monosaccharide units as shown by the five anomeric protons at *δ* 6.45 (br. *s*), 5.94 (*d*, *J* = 4.4 Hz), 5.49 (br. *s*), 5.25 (*d*, *J* = 7.7 Hz), and 5.00 (*d*, *J* = 7.7 Hz), giving correlations in the HSQC spectrum with signals at *δ*(C) 92.7, 111.2, 100.7, 104.8, and 104.8, respectively. Comparison of the NMR spectra of **1** and **2** showed that Rha of **2** was 1,3,4-substituted (C(3) at *δ* 81.6, C(4) at *δ* 77.7) instead of the 1,4-substitution (C(3) at *δ* 72.0, C(4) at *δ* 83.1) seen in **1**. At the same time, **2** exhibited a terminal Xyl, which was also confirmed by its ^{1}H- and ^{13}C-NMR data (*Tables 2* and *3*). Thus, the only difference between the two compounds is the position of attachment of Api. The NOESY cross-peak between *δ*(H) 5.94 (*d*, *J* = 4.4 Hz, H–C(1)(Api)) and *δ*(H) 4.39 (C(3)(Rha)) indicated that Api was linked to C(3) of Rha.

The negative-ion FAB-MS of compound **3** showed a quasi-molecular ion peak at *m/z* 1367 ($[M-H]^-$), indicating a molecular mass of 1368, compatible with a molecular formula $C_{63}H_{100}O_{32}$, with an additional fragment at *m/z* 162 in comparison with **1** or **2**. Two other significant ion peaks appeared at *m/z* 1205 ($[M-H-162]^-$) and 1043 ($[M-H-162]^-$) corresponding to the loss of two hexosyl moieties. Further investigation of 1D and 2D spectral data resulted in the determination of the structure of **3** as 3-*O*-[*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]medicagenic acid 28-{*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-[*β*-D-apiofuranosyl-(1 → 3)]-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl} ester (**3**), a new natural compound [4–8].

The ^{1}H- and ^{13}C-NMR spectra (600 and 150 MHz, resp., (D_5)pyridine) of **3** due to the aglycone part and the sugar moieties at C(28) (*Tables 1–3*) were almost superimposable on those of **2**, **3** differing from **2** only by the presence of an additional Glc at C(3) of the aglycone. A NOESY cross-peak was clearly detected between *δ*(H) 4.02 (H–C(2)(Glc)) and *δ*(H) 5.14 (*d*, *J* = 7.0 Hz, H–C(1)(T-Glc)), which supported a (1 → 2)-type linkage between both Glc units.

The negative-ion FAB-MS of compound **4** showed a quasi-molecular ion peak at *m/z* 1103 ($[M-H]^-$), indicating a molecular mass of 1104, compatible with the molecular formula $C_{53}H_{84}O_{24}$. Two other significant ion peaks appeared at *m/z* 779 ($[M-H-162-162]^-$) and 501 ($[M-H-132-146]^-$) indicating the respective elimination of two hexosyl, one pentosyl, and one deoxyhexosyl moieties. The full assignment of all ^{1}H- and ^{13}C-NMR signals by 2D-NMR experiments of **4** resulted in the establishment of its structure as 3-*O*-[*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]medicagenic acid 28-[*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl] ester (**4**), a new natural compound [4–8].

The ^{1}H- and ^{13}C-NMR spectra (600 and 150 MHz, resp., (D_5)pyridine) of **4** indicated that **4** was also a bisdesmosidic glycoside. Extensive NMR study showed that **4** had the same prosapogenin as in **3**. The ester glycosidic residues of **4** consisted of one each arabinopyranosyl and rhamnopyranosyl moieties. The HMBC correlation between *δ*(H) 6.25 (br. *s*, H–C(1)(Ara)) and *δ*(C) 176.2 (C(28)(Agly)) showed that the Ara was attached at C(28) of the aglycone. The NOESY cross-peak between *δ*(H) 5.56 (br. *s*, H–C(1)(Rha)) and *δ*(H) 4.33 (H–C(2)(Ara)) indicated that the Rha was linked to the Ara by a (1 → 2) linkage; this was

confirmed by a HMBC correlation between δ(H) 5.56 (br. *s*, H–C(1)(Rha)) and δ(C) 74.7 (C(2)(Ara)) (*Tables 2* and *3*).

The negative-ion FAB-MS of compound **5** showed a quasi-molecular ion peak at *m/z* 825 ($[M-H]^-$), indicating a molecular mass of 826, compatible with a molecular formula $C_{42}H_{66}O_{16}$. Another significant ion peak appeared at *m/z* 501 ($[M-H-162-162]^-$), corresponding to the loss of two hexosyl moieties. On the basis of the spectral data and hydrolyses, the structure of **5** was established as 3-*O*-[*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl]medicagenic acid (**5**), a new natural compound [4–8].

The chemical shifts of C(3) (δ 85.0) and C(28) (δ 182.0) of the aglycone in the ^{13}C-NMR spectrum (150 MHz, (D_5)pyridine) of **5** as well as the result of alkaline hydrolysis of **5** indicated that **5** was a monodesmosidic glycoside. Comparison of the NMR spectra of **5** with those of **3** and **4** revealed that the signals of the protons and C-atoms for the aglycone parts and the sugar chains at C(3) were very similar (*Tables 1–3*), indicating that **5** possessed the same aglycone and the same oligosaccharide chain at C(3) as in **3** and **4**.

The two known triterpene saponins 3-*O*-[*O*-*β*-D-glucopyranosyl-(1 → 2)-*β*-D-glucopyranosyl)medicagenic acid 28-[*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl] ester and 3-*O*-(*β*-D-glucopyranosyl)medicagenic acid 28-[*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl] ester were identified by comparing their physical and spectral data with literature values [6][9]. Since saponins were shown to possess immunomodulating activities [10][11], all new and known compounds are currently being tested *in vitro* for lymphocyte-proliferation activities.

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). Semi-prep. HPLC: *Gilson* pumps *M 305* and *306*; injector *Rheodyne 7125*, *Gilson UV/VIS-151* detector; *Merck-Hitachi D-7500* integrator; column: *Dionex Vydac RP-18* (5 μm) 300 Å, 10 × 250 mm; eluent: isocratic, 24% 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*); 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. Optical rotations: *Perkin-Elmer 241* polarimeter. IR Spectra: KBr disc; *Perkin-Elmer 281-IR* spectrophotometer; in cm^{-1}. 1D- and 2D-NMR Spectra: see [1]. Fast-atom bombardment (FAB)MS: negative-ion mode; *JEOL SX-102*.

Plant Material. The roots of *Muraltia ononidifolia* E. MEY. were collected in July 1990 in South Africa, near Cape Town. A voucher specimen under the reference H. Breyne No. 5458 is deposited in the Herbarium of the National Botanical Garden of Brussels, Belgium.

Extraction and Isolation. Dried powdered roots (585 g) were macerated during 4 h with 80% EtOH (3 l) and further submitted to boiling for 4 h. After cooling, the EtOH soln. was filtered and evaporated. The 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 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, yielding a crude saponin mixture (6.16 g). Of this mixture, 1 g was submitted to CC (*Sephadex L-H20*) and then to successive MPLC (silica gel *60* (15–40 μm; $CHCl_3$/MeOH/H_2O 65:35:10, lower phase), followed by semi-prep. HPLC (isocratic, 24% MeCN/H_2O with 0.06% CF_3COOH during 30 min; flow rate 3 ml/min): **1** (13 mg), **2** (12 mg), **3** (46 mg), **4** (10 mg), and **5** (9 mg).

*(4α,2β,3β)-3-(β-D-Glucopyranosyloxy)-2-hydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-β-D-Apiofuranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl] Ester* (**1**). White

amorphous powder. TLC: R_f 0.48. $[\alpha]_D^{25} = -18.4$ ($c = 0.245$, MeOH). IR (KBr): 3500–3300, 2926, 1723, 1740, 1610, 1600, 1560, 1500, 1300, 1100. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1205 ($[M-H]^-$), 1073 ($[M-H-132]^-$), 911 ($[M-H-162]^-$).

*(4α,2β,3β)-3-(β-*D*-Glucopyranosyloxy)-2-hydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-β-*D*-Xylopyranosyl-(1 → 4)*-O*-[β-*D*-apiofuranosyl-(1 → 3)]*-O*-α-*L*-rhamnopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl} Ester* (**2**). White amorphous powder. TLC: R_f 0.39. $[\alpha]_D^{25} = -26.5$ ($c = 0.25$, MeOH). IR (KBr): 3500–3300, 2927, 1723, 1740, 1710, 1636, 1580, 1500, 1420, 1260, 1090. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1205 ($[M-H]^-$), 1073 ($[M-H-132]^-$), 911 ($[M-H-162]^-$).

*(4α,2β,3β)-3-{[*O*-β-*D*-Glucopyranosyl-(1 → 2)-β-*D*-glucopyranosyl]oxy}-2-hydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-β-*D*-Xylopyranosyl-(1 → 4)*-O*-[β-*D*-apiofuranosyl-(1 → 3)]*-O*-α-*L*-rhamnopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl} Ester* (**3**). White amorphous powder. TLC: R_f 0.25. $[\alpha]_D^{25} = -20.4$ ($c = 0.245$, MeOH). IR (KBr): 3500–3300, 2927, 1723, 1740, 1710, 1636, 1580, 1500. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 1367 ($[M-H]^-$), 1205 ($[M-H-162]^-$), 1043 ($[M-H-162]^-$).

*(4α,2β,3β)-3-{[*O*-β-*D*-Glucopyranosyl-(1 → 2)-β-*D*-glucopyranosyl]oxy}-2-hydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-α-*L*-Rhamnopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl] Ester* (**4**). White amorphous powder. TLC: R_f 0.45. $[\alpha]_D^{25} = +1.96$ ($c = 0.25$, MeOH). IR (KBr): 3500–3300, 2927, 1723, 1740, 1710, 1636. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3.* FAB-MS (neg.): 1103 ($[M-H]^-$), 779 ($[M-H-162-162]^-$), 501($[M-H-132-146]^-$).

*(4α,2β,3β)-3-{[*O*-β-*D*-Glucopyranosyl-(1 → 2)-β-*D*-glucopyranosyl]oxy}-2-hydroxyolean-12-ene-23,28-dioic Acid* (**5**). White amorphous powder. TLC: R_f 0.70. $[\alpha]_D^{25} = +37.2$ ($c = 0.255$, MeOH). IR (KBr): 3405, 2926, 1700, 1636, 1076. ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1–3*. FAB-MS (neg.): 825 ($[M-H]^-$), and 501 ($[M-H-162-162]^-$).

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.

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