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Novel Acylated Triterpene Glycosides from *Muraltia heisteria*

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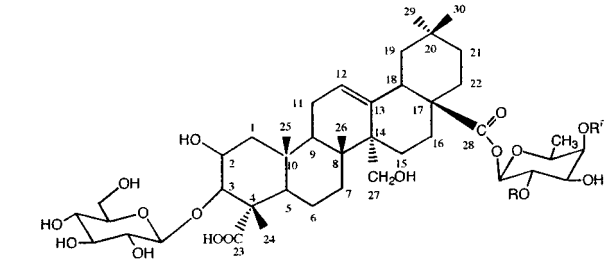
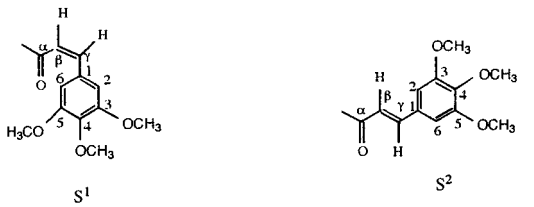
Four new acylated triterpene glycosides (**1**−**4**) have been isolated as two inseparable mixtures of the *trans*- and *cis*-3,4,5-trimethoxycinnamoyl derivatives (**1**,**2** and **3**,**4**) from the roots of *Muraltia heisteria*. The structures of these compounds were elucidated by various 1D and 2D NMR techniques, including ^{1}H and ^{13}C, COSY, NOESY, HSQC, TOCSY, and HMBC experiments and FABMS. Compounds **3** and **4** were shown to be cytotoxic in a human colon cancer cell line but did not show any ability to potentiate in vitro cisplatin cytotoxicity.

Muraltia is one of the main genera of the family of Polygalaceae, comprising about 115 species indigenous to South Africa. Although the presence of presenegenin glycosides was supposed in *Muraltia heisteria* (L.) DC., no phytochemical study has been previously realized.[1] This paper describes the isolation and structure elucidation of four new saponins (**1**−**4**), which were obtained as two inseparable mixtures of their *trans* and *cis* acylated derivatives (**1**,**2** and **3**,**4**) from the ethanolic extract of the roots of *Muraltia heisteria* (L.) DC. As part of our ongoing investigation of biologically active saponins, we also report the results of the cytotoxicity and potentiation of the cytotoxicity of cisplatin in human colon cancer cells.





1 R = R¹ R' = S²
2 R = R¹ R' = S¹
3 R = R² R' = S²
4 R = R² R' = S¹

Results and Discussion

The 80% ethanolic extract of the roots was suspended in MeOH and purified by precipitation with sulfuric diethyl ether. The resulting residue was suspended in water, dialyzed for 4 days, and lyophilized. The obtained powder was treated with charcoal and after filtration again purified by precipitation with diethyl ether, yielding a crude saponin mixture.[1] A part of this extract was separated by repeated medium-pressure liquid chromatography (MPLC) over normal Si gel, yielding **1**,**2** and **3**,**4** as two inseparable mixtures, each one giving only one spot by HPTLC but two peaks by HPLC.

The negative-ion FABMS of compounds **1**,**2** showed a quasimolecular ion peak at *m*/*z* 1749, indicating a molecular weight of 1750, compatible with a molecular formula of $C_{81}H_{122}O_{41}$. One other significant ion peak appeared at *m*/*z* 1617 $[(M - H) - 132]^-$ corresponding to the loss of one pentosyl moiety.

Hydrolysis of **1**,**2** with 2 N TFA at 120 °C afforded an artifactual aglycon and glucose, galactose, fucose, rhamnose, arabinose, and apiose (TLC). The native aglycon was identified as presenegenin (2*β*,3*β*,27-trihydroxyolean-12-en-23,28-dioic acid) from the extensive 2D NMR data of **1**,**2**. Most of the signals were in good agreement with literature data.[2] The spectroscopic NMR data of the prosapogenin of **1**,**2** obtained by alkaline hydrolysis with 5% KOH at 120 °C of **1**,**2** were in good agreement with those of tenuifolin (3-*O*-*β*-D-glucopyranosylpresenegenin), obtained from *Polygala amarella*.[2]

The $^{1}H-^{1}H$ COSY experiment permitted us to identify the *trans*-olefinic protons of the trimethoxycinnamoyl moiety, which appeared as two doublets at *δ* 7.90 and 6.58

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Table 1. ^{13}C NMR and ^{1}H NMR Data of the Aglycons of **1**−**4** (C_5D_5N)[a−c]

		1,2		**3,4**	
position	DEPT	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H
1	CH_2	43.9	2.19,1.28	44.0	2.17,1.28
2	CH	70.0	4.57	69.9	4.59
3	CH	86.6	4.50	87.7	4.42
4	C	53.1		53.1	
5	CH	52.3	2.21	52.1	2.34
6	CH_2	21.0	1.85,1.96	21.5	1.76,1.85
7	CH_2	33.6	1.04,1.28	33.6	1.04,1.28
8	C	40.7		40.8	
9	CH	49.0	2.27	49.0	2.32
10	C	36.5		36.3	
11	CH_2	23.8	nd	23.8	nd
12	CH	127.8	5.83	127.7	5.85
13	C	139.0		139.0	
14	C	47.8		48.0	
15	CH_2	24.2	nd	24.2	nd
16	CH_2	24.0	nd	24.0	nd
17	C	46.8		47.0	
18	CH	41.8	3.14	42.0	3.16
19	CH_2	45.1	1.27,1.74	45.0	1.28,1.70
20	C	30.8		30.8	
21	CH_2	34.0	1.85,2.3	33.9	1.84,2.22
22	CH_2	32.0	1.73,1.85	32.1	1.72,1.85
23	C	180.8		180.8	
24	CH_3	14.4	1.86 (s)	14.5	1.87 (s)
25	CH_3	17.2	1.47 (s)	17.1	1.45 (s)
26	CH_3	18.7	1.08 (s)	18.5	1.05 (s)
27	CH_2	64.2	3.85,4.12	63.9	3.77,4.13
28	C	176.3		176.4	
29	CH_3	32.7	0.77 (s)	32.8	0.75 (s)
30	CH_3	23.4	0.78 (s)	23.5	0.77 (s)

[a] Multiplicities were assigned from DEPT spectra. [b] The assignments were based on the HMBC, HSQC, and DEPT experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR). [c] nd: not determined. Overlapped ^{1}H NMR signals are reported without designated multiplicity.

(1H each, J = 16.2 Hz), the *cis*-olefinic protons, which appeared as two doublets at δ 6.85 and 6.00 (1H each, J = 12.1 Hz), and the tetrasubstituted benzene ring protons (δ 6.79, 2H each, brs, for the *trans*- and the *cis*-derivatives). These findings indicated that **1** and **2** are a mixture of *trans*- and *cis*-trimethoxycinnamoyl triterpene glycosides (12:9, respectively from relative NMR and HPLC intensivities). This mixture was homogeneous by HPTLC but was separated into *trans*- and *cis*-isomers by HPLC. All attempts to separate **1** and **2** by semipreparative HPLC were unsuccessful. Such a phenomenon could be explained by the geometrical structures of the trimethoxycinnamoyl group in **1** and **2**, which showed tautomer-like behavior under light in aqueous methanolic solution. Such isomerization has already been observed in *E* and *Z* mixtures of senegasaponins[3] from *Polygala senega* and in jenissensosides from *Silene jenisseensis*.[4]

The mild alkaline hydrolysis of **1**,**2** with KOH 1% (60 min at room temperature) yielded *trans*- and *cis*-3,4,5-trimethoxycinnamic acid (TLC) and a deacylated saponin which was homogeneous according to TLC and HPLC.

Compounds **1**,**2** were shown to contain seven sugar residues from the HSQC spectrum. The anomeric ^{1}H NMR signals at δ 6.40 (s), 6.00 (d, J = 7.7 Hz), 5.94 (brs), 5.08 (d, J = 7.3 Hz), 4.95 (d, J = 7.0 Hz), 4.81 (d, J = 7.3 Hz), and 4.80 (d, J = 7.3 Hz) give correlations with ^{13}C NMR signals at δ 101.5, 94.4, 111.1, 104.0, 104.4, 106.0, and 102.7, 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 spectra (Table 3), and the sequence of the oligosaccharidic chains was obtained from the HMBC and NOESY spectra.

Table 2. ^{13}C NMR Data of the Sugar and Acid Moieties of Compounds **1**−**4** (C_5D_5N)[a,b]

		1	**2**	**3**	**4**
3-O-Glc	1	*104.4*	*104.4*	*104.7*	*104.7*
	2	75.1	75.1	75.2	75.2
	3	77.2	77.2	77.2	77.2
	4	71.0	71.0	70.9	70.9
	5	77.9	77.9	77.6	77.6
	6	62.0	62.0	61.9	61.9
28-O-sugars					
Fuc	1	*94.4*	*94.4*	*94.4*	*94.4*
	2	*73.5*	*73.5*	*72.4*	*72.4*
	3	74.2	74.2	74.5	74.6
	4	*74.9*	*74.7*	*75.0*	*74.8*
	5	70.7	70.7	70.2	70.2
	6	16.3	16.3	16.1	16.1
Rha	1	*101.5*	*101.5*	*101.0*	*101.0*
	2	71.4	71.4	71.0	71.0
	3	*81.2*	*81.2*	72.1	72.1
	4	*78.6*	*78.6*	*85.4*	*85.4*
	5	67.6	67.6	67.5	67.5
	6	18.4	18.4	18.1	18.1
Gal	1	*102.7*	*102.7*	*102.5*	*102.5*
	2	70.2	70.2	69.7	69.7
	3	74.2	74.2	75.4	75.4
	4	69.4	69.4	*77.6*	*77.6*
	5	76.8	76.8	77.2	77.2
	6	61.7	61.7	61.4	61.4
Ara	1	*104.0*	*104.0*	*105.1*	*105.1*
	2	*77.6*	*77.6*	71.0	71.0
	3	*77.2*	*77.2*	74.0	74.0
	4	70.7	70.7	69.5	69.5
	5	68.0	68.0	68.0	68.0
Xyl	1	*106.0*	*106.0*	*106.0*	*106.0*
	2	74.9	74.9	75.4	75.4
	3	77.2	77.2	*86.1*	*86.1*
	4	71.9	71.9	69.3	69.3
	5	65.5	65.5	65.5	65.5
Api	1	*111.1*	*111.1*		
	2	77.9	77.9		
	3	79.9	79.9		
	4	74.2	74.2		
	5	64.5	64.5		
Acid	a	167.8	167.4	167.8	167.4
	b	117.3	118.3	117.4	118.3
	g	145.7	144.1	145.7	144.2
	1	130.3	130.1	131.0	130.4
	2	106.0	106.0	106.0	106.0
	3	140.6	139.7	140.7	139.9
	4	153.7	153.0	153.7	153.0
	5	140.6	139.7	140.7	139.9
	6	106.0	106.0	106.0	106.0
OMe		55.7	55.6	55.7	55.6
		55.7	55.6	55.7	55.6
		60.5	60.4	60.5	60.4

[a] ^{13}C chemical shifts of substituted residues are italicized. [b] The assignments were based on the COSY, TOCSY, NOESY, HSQC, and HMBC experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR). Multiplicities were assigned from DEPT spectra.

Evaluation of spin−spin couplings and chemical shifts allowed the identification of one α-rhamnopyranosyl (Rha), one β-fucopyranosyl (Fuc), one β-apiofuranosyl (Api), one α-arabinopyranosyl (Ara), one β-glucopyranosyl (Glc), one β-xylopyranosyl (Xyl), and one β-galactopyranosyl (Gal) units, respectively. The common D-configuration for Fuc, Gal, 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.

The NOESY correlation observed between the anomeric proton of Glc at δ 4.95 (d, J = 7.0 Hz) and the proton H-3 of the aglycon at δ 4.50 showed that the glucopyranosyl moiety was linked to the presenegenin unit at C-3. The correlation in the HMBC spectrum between the ^{1}H NMR signal at δ_H 6.00 (d, J = 7.7 Hz) (Fuc-1) and the ^{13}C NMR

Table 3. ^{1}H NMR Data of the Sugar and Acid Moieties of Compounds **1**−**4** (C_5D_5N)[a−c]

		1	**2**	**3**	**4**
3-O-Glc	1	*4.95* $J = 7.0$)	*4.95* (d, $J = 7.0$)	*4.95* (d, $J = 7.0$)	*4.95* (d, $J = 7.0$)
	2	3.84	3.84	3.86	3.86
	3	4.18	4.18	4.18	4.18
	4	4.00	4.00	4.00	4.00
	5	3.82	3.82	3.82	3.82
	6a	4.13	4.13	4.14	4.14
	6b	4.30	4.30	4.32	4.32
28-O-sugars					
Fuc	1	*6.00* (d, $J = 7.7$)	*6.00* (d, $J = 7.7$)	*6.02* (*d*, $J = 7.7$)	*6.02* (d, $J = 7.7$)
	2	*4.74*	*4.64*	*4.76*	*4.68*
	3	4.47	4.47	4.49	4.51
	4	*5.72*	*5.61*	*5.70*	*5.60*
	5	4.17	4.12	4.18	4.19
	6	1.29 (d, $J = 6.0$)	1.25 (d, $J = 6.0$)	1.28 (d, $J = 6.0$)	1.26 (d, $J = 6.0$)
Rha	1	*6.40* (brs)	*6.40* (brs)	*6.48* (brs)	*6.48* (brs)
	2	4.84	4.84	4.72	4.72
	3	*4.45*	*4.45*	4.51	4.51
	4	*4.32*	*4.32*	*4.19*	*4.19*
	5	4.47	4.47	4.45	4.45
	6	1.63 (d, $J = 6.0$)	1.63 (d, $J = 6.0$)	1.67 (d, $J = 6.0$)	1.67 (d, $J = 6.0$)
Gal	1	*4.80* (d, $J = 7.3$)	*4.80* (d, $J = 7.3$)	*4.81* (d, $J = 7.3$)	*4.81* (d, $J = 7.3$)
	2	4.42	4.42	4.42	4.42
	3	4.01	4.01	4.04	4.04
	4	4.38	4.38	*4.06*	*4.06*
	5	3.90	3.90	3.90	3.90
	6a	4.16	4.16	4.18	4.18
	6b	4.24	4.24	4.24	4.24
Ara	1	*5.08* (d, $J = 7.3$)	*5.08* (d, $J = 7.3$)	*5.02* (brs)	*5.02* (brs)
	2	*3.83*	*3.83*	4.31	4.31
	3	*4.15*	*4.15*	4.04	4.04
	4	4.30	4.30	4.05	4.05
	5a	nd	nd	4.23	4.23
	5b	4.33	4.33	4.32	4.32
Xyl	1	*4.81* (d, $J = 7.3$)	*4.81* (d, $J = 7.3$)	*4.82* (d, $J = 7.3$)	*4.82* (d, $J = 7.3$)
	2	3.85	3.85	3.95	3.95
	3	3.84	3.84	*3.92*	*3.92*
	4	4.38	4.38	4.35	4.35
	5a	3.42	3.42	3.42	3.42
	5b	4.37	4.37	4.37	4.37
Api	1	*5.94* (brs)	*5.94* (brs)		
	2	4.68	4.68		
	4a	4.15	4.15		
	4b	4.42	4.42		
	5a	4.05	4.05		
	5b	4.05	4.05		
Acid	β	6.58 (d, $J = 16.2$)	6.00 (d, $J = 12.1$)	6.59 (d, $J = 16.2$)	6.00 (d, $J = 12.0$)
	γ	7.90 (d, $J = 16.2$)	6.85 (d, $J = 12.1$)	7.88 (d, $J = 16.2$)	6.83 (d, $J = 12.0$)
	2, 6	6.79 (brs)	6.79 (brs)	6.78 (brs)	6.78 (brs)
OMe		3.78 (6H, s)	3.76 (6H, s)	3.72 (6H, s)	3.73 (6H, s)
		3.82 (3H, s)	3.83 (3H, s)	3.80 (3H, s)	3.82 (3H, s)

[a] ^{1}H NMR chemical shifts of substituted residues are italicized. [b] The assignments were based on the COSY, TOCSY, NOESY, HSQC, and HMBC experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR). [c] nd: not determined. Overlapped signals are reported without designated multiplicity. Coupling constants in Hz.

signal at δ_C 176.3 (Agly-28) proved the fucose to be attached at C-28 of the aglycon. The location of the trimethoxycinnamoyl group at Fuc-4 (δ_H 5.72) was determined by the TOCSY and COSY spectra, starting from the anomeric ^{1}H NMR signal of fucose at δ 6.00 (d, J = 7.7 Hz). The downfield shifts observed in the HSQC spectrum for the Fuc H-4/Fuc C-4 resonances at δ_H 5.72/δ_C 74.9 proved the secondary alcoholic function Fuc-4-OH to be acylated.

Other correlations between δ_H 6.40 (brs) (Rha-1) and δ_C 73.5 (Fuc-2) indicated that the rhamnose was linked to the fucose by a 1→2 linkage. This was confirmed by a reverse correlation between δ_H 4.74 (Fuc-2) and δ_C 101.5 (Rha-1) and by a NOESY cross-peak between δ_H 6.40 (brs) (Rha-1) and δ_H 4.74 (Fuc-2). Other correlations observed between δ_H 4.80 (d, J = 7.3 Hz) (Gal-1) and δ_C 81.2 (Rha-3) and between δ_H 5.08 (d, J = 7.3 Hz) (Ara-1) and δ_C 78.6 (Rha-4) indicated that the galactose and the arabinose were linked to the rhamnose by a 1→3 and 1→4 linkage, respectively. They were confirmed by a reverse correlation between δ_H 4.45 (Rha-3) and δ_C 102.7 (Gal-1) and between δ_H 4.32 (Rha-4) and δ_C 104.0 (Ara-1) and cross-peaks in the NOESY spectrum between δ_H 4.80 (d, J = 7.3 Hz) (Gal-1) and δ_H 4.45 (Rha-3) and between δ_H 5.08 (d, J = 7.3 Hz) (Ara-1) and δ_H 4.32 (Rha-4). The HMBC correlation between δ_H 4.81 (d, J = 7.3 Hz) (Xyl-1) and δ_C 77.6 (Ara-2) proved the xylose to be linked to the arabinose at C-2. It was confirmed by a reverse correlation betwwen δ_H 3.83 (Ara-2) and δ_C 106.0 (Xyl-1) and by the NOESY cross-peak between δ_H 4.81 (d, J = 7.3 Hz) (Xyl-1) and δ_H 3.83 (Ara-2). The linkage of apiose to the 3-position of arabinose was deduced by the HMBC correlation between δ_H 4.15 (Ara-3) and δ_C 111.1 (Api-1). On the basis of the above results the structure of **1** and **2** were determined as 3-*O*-β-D-glucopyranosylpresenegenin-28-*O*-[β-D-apiofuranosyl-(1→3)]-[β-D-xylopyranosyl-(1→2)]-α-L-arabinopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[4-*O*-*trans*-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranoside (**1**),

and its *cis*- isomer (**2**), respectively, two new natural compounds.[3,5−8]

Compounds **3**,**4** were obtained as an inseparable mixture, giving one spot by HPTLC but two peaks by HPLC. The FABMS of compounds **3**,**4** showed a quasimolecular ion peak at *m*/*z* 1617 $[M - H]^-$, indicating a molecular weight of 1618, compatible with the molecular formula $C_{76}H_{114}O_{37}$. One significant ion peak observed at *m*/*z* 1455 $[(M - H) - 162]^-$ indicated the loss of one hexosyl moiety. The APIESMS (negative-ion mode) of **3** and **4** showed a quasimolecular ion peak at *m*/*z* 1617 $[M - H]^-$, which confirmed the proposed molecular weight.

On acid hydrolysis, **3**,**4** gave the same artifactual aglycon as in **1**,**2** and glucose, galactose, rhamnose, fucose, xylose, and arabinose, consistent with the structural assignments made. On alkaline hydrolysis, **3**,**4** gave the tenuifolin.[2] On mild alkaline hydrolysis, **3**,**4** afforded the same organic acids as in **1**,**2**, which were identified as *trans*- and *cis*-trimethoxycinnamic acids from NMR data (see Tables 2, 3).

The ^{1}H NMR and ^{13}C NMR data of **3**,**4** obtained from HSQC and HMBC, TOCSY, and NOESY spectra (Table 1) showed that the signals of the aglycon were in good agreement with those of presenegenin characterized in **1**,**2**.[5] Compounds **3** and **4** were shown to contain six sugar residues from the HSQC spectrum: the anomeric protons at δ 6.48 (brs), 6.02 (d, $J = 7.7$ Hz), 5.02 (brs), 4.95 (d, $J = 7.0$ Hz), 4.82 (d, $J = 7.3$ Hz), and 4.81 (d, $J = 7.3$ Hz) give correlations with carbon signals at δ 101.0, 94.4, 105.1, 104.7, 106.0, and 102.5, respectively. The sugar moieties were assigned mainly from ^{1}H−^{1}H DQF-COSY, TOCSY, HSQC, and HMBC experiments. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one α-rhamnopyranose (Rha), one β-fucopyranose (Fuc), one α-arabinopyranose (Ara), one β-glucopyranose (Glc), one β-xylopyranose (Xyl), and one β-galactopyranose (Gal) units. The common D-configuration for Fuc, Glc, Gal, and Xyl and the L-configuration for Rha and Ara were assumed to be those of the most commonly encountered analogues in the plant kingdom. The sequence of the sugars and their linkage to the aglycon were determined by the HMBC and NOESY experiments. The signals in the molecules **3**,**4** corresponding to the 3-*O*-β-D-glucopyranosyl moiety and the 28-*O*-α-L-rhamnopyranosyl-(1→2)-[4-*O*-*trans*-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranosyl moiety, and the *cis*-isomer form were almost superimposable to those of **1**,**2** consistent with the proposed sequence. In addition, HMBC correlations between δ_H 4.82 (d, $J = 7.3$ Hz) (Xyl-1) and δ_C 85.4 (Rha-4) indicated that the xylose was linked to the rhamnose by a 1→4 linkage. This was confirmed by a reverse correlation between δ_H 4.19 (Rha-4) and δ_C 106.0 (Xyl-1) and by a NOESY cross-peak between δ_H 4.82 (d, $J = 7.3$ Hz) (Xyl-1) and δ_H 4.19 (Rha-4). Other cross-peaks in the NOESY spectrum between δ_H 5.02 (brs) (Ara-1) and δ_H 4.06 (Gal-4) and between δ_H 4.81 (d, $J = 7.3$ Hz) (Gal-1) and δ_H 3.92 (Xyl-3) proved the arabinose to be linked to the galactose at C-4 and the galactose to the xylose at C-3, respectively. On the basis of the above results, the structures of **3** and **4** were attributed as 3-*O*-β-D-glucopyrano sylpresenegenin-28-*O*-α-L-arabinopyranosyl-(1→4)-β-D-galactopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[4-*O*-*trans*-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranoside (**3**) and its *cis*-isomer (**4**), respectively, new natural compounds.[3,5−8]

Because triterpene saponins have been reported to potentiate the cytotoxicity of cisplatin in human colon cancer cells,[9,10] the saponins **3** and **4** were tested in an in vitro cytotoxicity assay according to the previously described technique.[9,10] In this assay, compounds **3** and **4** showed cytotoxicity for the human colon cancer cells HT 29 in the concentration range 12−50 μg/mL and did not show any potentiation of cisplatin cytotoxicity at these concentrations.

Experimental Section

General Experimental Procedures. The 1D and 2D NMR spectra (^{1}H−^{1}H COSY, TOCSY, NOESY, HSQC, and HMBC) were performed using a 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 ^{1}H and 150 MHz for ^{13}C NMR spectra). Conventional pulse sequences were used for COSY, HSQC, and HMBC. TOCSY spectra were acquired using the standard MLEV17 spin-locking sequence and 90 ms mixing time. The mixing time in the NOESY experiment was set to 500 ms.The carbon type (Me, CH_2, CH) was determined by DEPT experiments. All chemical shifts (δ) are given in ppm, and the samples were solubilized in pyridine-d_5 (δ 150.3, 155.9, 123.9). Fast-atom bombardment (FABMS) (negative-ion mode, thioglycerol matrix) was conducted on a JEOL SX 102. The APIESMS was conducted in the negative-ion mode on a micromass Quattro LS instrument. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. IR spectra (KBr disk) were recorded on a Perkin-Elmer 281 spectrophotometer. UV spectra were recorded with a Kontron, uvikon spectrophotometer 930. TLC and HPTLC employed precoated Si gel plates 60 F_{254} (Merck). The following TLC solvent systems were used: for saponins (a) $CHCl_3$−MeOH−AcOH−H_2O (15:8:3:2); for sapogenins (b) $CHCl_3$−MeOH (9:1); for monosaccharides (c) $CHCl_3$−MeOH−H_2O (8:5:1); for the acids (d) ether−toluene (1:1) saturated with AcOH 10%. Spray reagents for the saponins were Komarowsky reagent, a mixture (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and H_2SO_4 50%; for the sugars, diphenylaminephosphoric acid reagent; and for the acids the detection was by observation under UV. Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Gilson pump M 305, head pump 25 SC, manometric module M 805, Injector Rheodyne 7125, Büchi column (460 × 25 mm and 460 × 15 mm), Büchi precolumn (110 × 15 mm)]. Analytical HPLC of saponins: Gilson pumps M 305 and 306; head pump 25 SC; Injector Rheodyne 7125; Dynamic mixer 811C; manometric module 805; UV/vis, 151 Gilson detector; Merk Hitachi D 7500 integrator; column Lichrospher RP-18 (5μm) 125−4; eluent, linear gradient, 20−50% MeCN−H_2O with 0.06% TFA during 30 min; flow rate, 1 mL/min; detection wavelength, 210 nm.

Plant Material. *Muraltia heisteria* (L.) DC. was collected in July 1990 in South Africa, near the town of Cap. A voucher specimen under the reference H. Breyne no. 5420 is deposited in the Herbarium of the National Botanical Garden of Brussels, Belgium.

Extraction and Isolation. Dried, powdered roots (410 g) were macerated during 4 h with 3 L of 80% ethanol and further submitted to an ebullition for 4 h. After cooling, the ethanolic solution was filtrated and evaporated to dryness. The residue was dissolved in 400 mL of MeOH at 60 °C. After filtration, the methanolic solution was purified by precipitation with sulfuric diethyl ether (5 × 400 mL). The resulting residue was solubilized in water (400 mL) and submitted to a 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 diethyl ether, yielding a crude saponin mixture (2.39 g). One gram of this mixture was submitted to successive MPLC column chromatography on Si gel 60 (15−40 μm) using as eluent $CHCl_3$−MeOH−H_2O (65:35:10, lower phase), giving compounds **1**,**2** (18 mg) and **3**,**4** (16 mg).

Compounds 1 and 2: white amorphous powder; $[\alpha]^{20}_D$ −4.3° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 311, 230 nm; IR (KBr)

ν_{max} 3500−3300 (OH), 2926 (CH), 1750 and 1740 (CO ester groups), 1710 (CO carboxylic acid), 1634 (C=C), 1610, 1600, 1560, 1500, 1300, 1100 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 3 and 2; long-range correlations in the HMBC spectrum used for defining the aglycon, as previously reported;[2] negative FABMS (thioglycerol matrix) *m/z* 1749 $[M - H]^-$, 1617 $[(M - H) - 132]^-$; TLC R_f 0.26 (system a); blue spots by spraying with Komarowsky reagent; HPLC t_R 19.72; 20.36 min; eluent, linear gradient, 20−50% MeCN−H_2O with 0.06% TFA during 30 min.; flow rate, 1 mL/min; detection wavelength, 210 nm.

Compounds 3 and 4: white amorphous powder; $[\alpha]^{20}_D$ −13.4° (*c* 0.08, MeOH); UV (MeOH) λ_{max} 310, 230 nm; IR (KBr) ν_{max} 3500−3300 (OH), 2927 (CH), 1723 and 1740 (CO ester groups), 1710 (CO carboxylic acid), 1636 (C=C), 1580, 1500, 1420, 1260, 1090 cm^{-1}; ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 3 and 2; negative FABMS (thioglycerol matrix) *m/z* 1617 $[M - H]^-$, 1455 $[(M - H) - 162]^-$; APIESMS (negative-ion mode) *m/z* 1617 $[M - H]^-$; TLC R_f 0.34 (system a); blue spots by spraying with Komarowsky reagent; t_R 21.18; 21.74 min; eluent, linear gradient, 20−50% MeCN−H_2O with 0.06% TFA during 30 min; flow rate, 1 mL/min; detection wavelength, 210 nm.

Acid Hydrolysis. A solution of saponin (5 mg) in H_2O (2 mL) and 2 N aqueous CF_3COOH (5 mL) was refluxed on a water bath for 3 h. After extraction with $CHCl_3$ (3 × 5 mL), the aqueous layer was repeatedly evaporated to dryness 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% aqueous KOH (10 mL) for 2 h. The fraction mixture was adjusted to pH 6 with dilute HCl and then extracted with H_2O-saturated *n*-BuOH (3 × 10 mL). The combined BuOH extracts were washed (H_2O). Evaporation of the solvent yielded the prosapogenin, which was identified as tenuifolin (TLC, ^{13}C NMR) in comparison with an authentic sample.

Mild Alkaline Hydrolysis. The saponin was hydrolyzed with KOH 1% at room temperature. After 1 h, the mixture was neutralized with dilute HCl and extracted with Et_2O. The Et_2O layer gave *trans*- and *cis*-trimethoxycinnamic acids, which were identified by TLC. The aqueous layer was extracted with *n*-BuOH, yielding the deacylated saponin.

Bioassay. The potentiation of the in vitro cisplatin cytotoxicity in a human colon cancer cell line was evaluated according to the method of Assem et al.[9,10] HT 29 cells (2×10^4 per well) were seeded in 96-well culture plates and cultured 2 days before treatment. Saponins were dissolved immediatly before use in a mixture of DMSO and absolute ethanol, then diluted at different concentrations in serum-free Ham's F-10 medium. Cells were treated 3 h with saponins alone or associated to 10 μg/mL cisplatin. After treatment, cells were washed twice with Ham's F-10 and cultured again for 7 days in drug-free culture medium. Cell survival was measured by the Crystal Violet colorimetric assay.

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