Three New Medicagenic Acid Saponins from *Polygala micrantha* GUILL. & PERR.

by **Turibio Kuiate Tabopda**[a])[b]), **Anne-Claire Mitaine-Offer**[a]), **Tomofumi Miyamoto**[c]), **Chiaki Tanaka**[c]), **Bonaventure Tchaleu Ngadjui**[b]), **Jean-François Mirjolet**[d]), **Olivier Duchamp**[d]), and **Marie-Aleth Lacaille-Dubois***[a])

[a]) Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique, UMIB, UPRES-EA 3660, Faculté de Pharmacie, Université de Bourgogne, 7, Bd. Jeanne d'Arc, BP 87900, F-21079 Dijon Cedex (phone: +33-3-80393229; fax: +33-3-80393300; e-mail: m-a.lacaille-dubois@u-bourgogne.fr)

[b]) Département de Chimie Organique, Université de Yaoundé 1, BP 812 Yaoundé, Cameroun

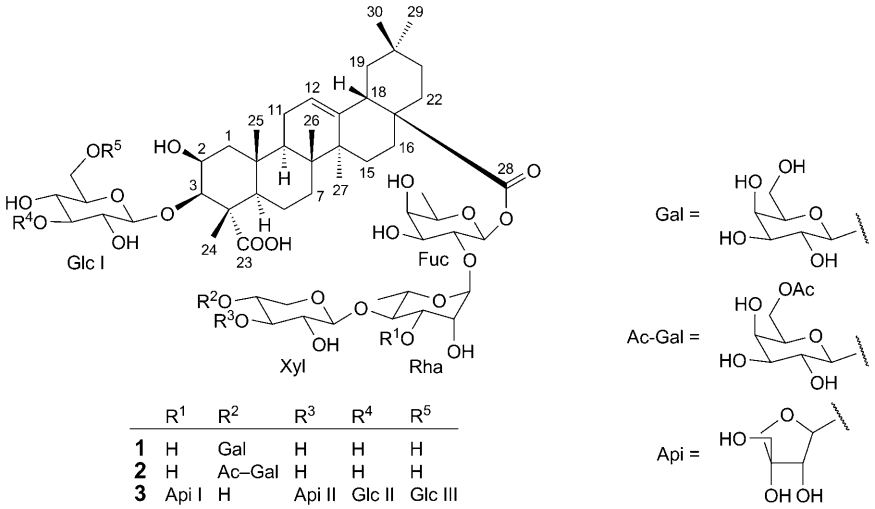
[c]) Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

[d]) *Oncodesign*, 20 rue Jean Mazen, BP 27627, F-21076 Dijon Cedex

Three new medicagenic acid saponins, micranthosides A–C (**1**–**3**), were isolated from the roots of *Polygala micrantha* GUILL. & PERR., along with six known presenegenin saponins. Their structures were elucidated on the basis of extensive 1D- and 2D-NMR experiments (^{1}H, ^{13}C, DEPT, COSY, TOCSY, NOESY, HSQC, and HMBC) and mass spectrometry as 3-*O*-*β*-D-glucopyranosylmedicagenic acid 28-[*O*-*β*-D-galactopyranosyl-(1→4)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl] ester (**1**), 3-*O*-*β*-D-glucopyranosylmedicagenic acid 28-[*O*-6-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl] ester (**2**), and 3-*O*-{*O*-*β*-D-glucopyranosyl-(1→3)-*O*-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl}medicagenic acid 28-{*O*-*β*-D-apiofuranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-[*β*-D-apiofuranosyl-(1→3)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl} ester (**3**). Compounds **1**–**3** were evaluated against HCT 116 and HT-29 human colon cancer cells, but they did not show any cytotoxicity.

Introduction. – *Polygala* is a genus of *ca.* 500 species of flowering plants belonging to the family Polygalaceae, commonly known as milkwort or snakeroot. The genus includes herbaceous perennial plants, shrubs, and has a subcosmopolitan distribution. *Polygala micrantha* GUILL. & PERR. is an annual herb up to 30 cm high, recorded from Senegal to West Cameroon. The plant is said to be used as a purge in Senegal [1]. Polygalaceae species have already been investigated, resulting in the isolation of presenegenin [2][3] and medicagenic acid glycosides [4][5], but no previous phytochemical study has been undertaken on *P. micrantha*. In continuation of our studies of saponin constituents of medicinal plants of the Polygalaceae family [6–8], we describe in this article the isolation and structure elucidation of three new medicagenic acid saponins named micranthosides A–C (**1**–**3**; *Fig. 1*) and six known presenegenin glycosides from the 70% MeOH extract of the roots of *P. micrantha*. The cytotoxicity of compounds **1**–**3** was evaluated against two human colon cancer cell lines, HT-29 and HCT 116.

Results and Discussion. – A 70% aqueous MeOH extract of the roots of *P. micrantha* was submitted to vacuum liquid chromatography (VLC) and further



	R^1	R^2	R^3	R^4	R^5
1	H	Gal	H	H	H
2	H	Ac–Gal	H	H	H
3	Api I	H	Api II	Glc II	Glc III

Fig. 1. *Compounds* **1**–**3**, *isolated from* Polygala micrantha

fractionated by repeated medium-pressure liquid chromatography (MPLC) yielding micranthosides A–C (**1**–**3**), and the known polygalasaponin XXVIII [9], polygalasaponin XXXV [10], desacylsenegasaponin B [11], polygalasaponins XLV and XLVI [12], and arilloside A [13]. Their structures were established mainly by 1D- and 2D-NMR experiments (^{1}H, ^{13}C, DEPT, COSY, TOCSY, NOESY, HSQC, and HMBC, see *Tables 1* and *2*) and mass spectrometry. ^{1}H- and ^{13}C-NMR signals of the aglycone assigned from 2D-NMR experiments of **1**–**3** were in good agreement with those of medicagenic acid (=(2β,3β,4α)-2,3-dihydroxyolean-12-ene-23,28-dioic acid; *Table 1*) [4][5]. The monosaccharides obtained by acid hydrolysis of each compound were identified by TLC comparison with standard sugars as glucose, fucose, rhamnose, xylose, and galactose in the case of **1** and **2**, and as glucose, fucose, rhamnose, xylose, and apiose in the case of **3**. The D-configuration for apiose, galactose, glucose, fucose, and xylose, and the L-configuration for rhamnose were determined by GC analysis (see *Exper. Part*). The β-orientation of the anomeric center of the glucosyl, xylosyl, and fucosyl moieties in their pyranose form was supported by the relatively large *J* values of their anomeric H-atoms ($J = 6.9–8.1$ Hz). For the rhamnosyl moiety, the large ^1J(C,H) value (165–168 Hz) and three-bond-coupled strong HMBCs from the anomeric H-atom to C(3) and C(5) (dihedral angle between H–C(1) and C(3), and between H–C(1) and C(5) *ca.* 180°) indicated that the anomeric H-atom was equatorial thus possessing an α-pyranoid anomeric form [14].

Micranthoside A (**1**) was obtained as an amorphous powder. The high-resolution electrospray-ionization mass spectrum (HR-ESI-MS; positive-ion mode) exhibited a quasimolecular-ion peak at *m*/*z* 1273.5834 ($[M + Na]^+$) consistent with the molecular formula $C_{59}H_{94}NaO_{28}$. The negative-ion-mode FAB-MS showed a quasimolecular-ion peak at *m*/*z* 1249 ($[M - H]^-$), and two other important fragment-ion peaks at *m*/*z* 1087 ($[M - H - 162]^-$) and 925 ($[M - H - 162 - 162]^-$), corresponding to the loss of one and

Table 1. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; (D_5)pyridine) *of the Aglycones of* **1**–**3** *from 1D- and 2D-NMR Experiments*[a])[b]). δ in ppm, J in Hz.

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.30, 2.18	43.9	1.30, 2.18	43.9	1.31, 2.27 (*d*, J = 12.6)	44.0
H–C(2)	4.75	69.5	4.75	69.5	4.65	69.8
H–C(3)	4.56	86.4	4.56	86.4	4.62	87.0
C(4)		53.0		53.0		53.1
H–C(5)	2.02	52.1	2.02	52.1	2.03	52.0
CH_2(6)	1.77[c])	20.5	1.77[c])	20.5	1.87, 1.98	20.6
CH_2(7)	[c])	33.0	[c])	33.0	1.65, 1.71	31.9
C(8)		40.0		40.0		40.0
H–C(9)	2.20	48.6	2.20	48.6	1.74	48.1
C(10)		36.2		36.2		36.6
CH_2(11)	1.86, 2.05	23.8	1.86, 2.05	23.8	1.88, 2.01	23.8
H–C(12)	5.37 (br. *s*)	122.0	5.37 (br. *s*)	122.0	5.32 (br. *s*)	122.3
C(13)		143.8		143.8		144.1
C(14)		42.0		42.0		42.1
CH_2(15)	1.44[c])	28.2	1.44[c])	28.2	1.58, 1.88	28.4
CH_2(16)	1.96, 2.03	22.4	1.96, 2.03	22.4	1.96, 2.03	22.4
C(17)		46.6		46.6		46.8
H–C(18)	3.03 (br. *d*, J = 12.6)	41.4	3.12 (br. *d*, J = 12.0)	41.4	3.01 (br. *d*, J = 10.5)	41.6
CH_2(19)	1.14, 1.64	45.2	1.14, 1.64	45.2	1.14, 1.63	45.9
C(20)		30.4		30.4		30.2
CH_2(21)	[c])	33.4	[c])	33.4	1.01, 1.22	33.4
CH_2(22)	1.68, 1.84	31.8	1.68, 1.84	31.8	1.58, 1.65	32.0
C(23)		186.0		186.0		185.4
Me(24)	1.82 (*s*)	14.5	1.82 (*s*)	14.5	1.85 (*s*)	14.4
Me(25)	1.40 (*s*)	16.5	1.40 (*s*)	16.5	1.42 (*s*)	16.7
Me(26)	1.00 (*s*)	17.0	1.00 (*s*)	17.0	1.03 (*s*)	17.2
Me(27)	1.20 (*s*)	25.6	1.20 (*s*)	25.6	1.21 (*s*)	25.8
C(28)		176.8		176.8		176.0
Me(29)	0.69 (*s*)	32.6	0.69 (*s*)	32.6	0.77 (*s*)	32.8
Me(30)	0.75 (*s*)	23.4	0.75 (*s*)	23.4	0.72 (*s*)	23.2

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

two hexosyl units, respectively. The ^{1}H-NMR spectrum of **1** (*Table 2*) showed five anomeric-H-atom signals at δ(H) 4.81 (*d*, J = 7.4 Hz), 4.87 (*d*, J = 8.1 Hz), 5.01 (*d*, J = 7.9 Hz), 5.86 (*d*, J = 8.1 Hz), and 6.32 (br. *s*) which were correlated in the HSQC spectrum with five anomeric-C signals at δ(C) 106.3, 103.4, 104.1, 94.0, and 100.9, respectively, indicating the presence of five sugar units. The ring H- and C-atoms of the sugar moieties were assigned starting from the readily identifiable anomeric H-atoms by means of COSY, TOCSY, NOESY, HSQC, and HMBC experiments. Evaluation of spin–spin couplings and chemical shifts allowed the identification of one β-glucopyranosyl (Glc I), one β-fucopyranosyl (Fuc), one α-rhamnopyranosyl (Rha), one β-galactopyranosyl (Gal), and one β-xylopyranosyl (Xyl) unit. Analysis of HMBC data concerning the connectivities between the sugar residues and the medicagenic acid

Table 2. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; (D_5)pyridine) *of the Sugar Moieties of* **1**–**3** *from 1D- and 2D-NMR Experiments*[a])[b]). δ in ppm, J in Hz.

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
3-*O*-Sugars:						
Glc I						
H–C(1)	5.01 (*d*, *J* = 7.9)	104.1	5.01 (*d*, *J* = 7.9)	104.2	4.94 (*d*, *J* = 6.9)	103.5
H–C(2)	3.86	74.6	3.86	74.7	3.84	74.5
H–C(3)	4.16–4.20 (*m*)	76.9	4.16–4.20 (*m*)	77.3	4.13	86.6
H–C(4)	3.96	70.7	3.96	70.5	4.10	70.8
H–C(5)	3.84	77.4	3.84	77.3	3.92	77.7
CH_2(6)	4.06, 4.35	62.0	4.06, 4.35	61.9	3.96, 4.51	69.3
Glc II						
H–C(1)					4.77 (*d*, *J* = 7.9)	104.3
H–C(2)					3.88	74.8
H–C(3)					4.14	77.3
H–C(4)					3.92	71.0
H–C(5)					3.76–3.82 (*m*)	77.7
CH_2(6)					4.15, 4.40	62.0
Glc III						
H–C(1)					5.14 (*d*, *J* = 7.6)	104.6
H–C(2)					3.91	75.5
H–C(3)					4.12	77.3
H–C(4)					4.02	71.0
H–C(5)					3.88	77.7
CH_2(6)					4.06, 4.15	62.0
28-*O*-Sugars:						
Fuc						
H–C(1)	5.86 (*d*, *J* = 8.1)	94.0	5.86 (*d*, *J* = 8.1)	94.0	5.79 (*d*, *J* = 8.1)	94.5
H–C(2)	4.58	73.6	4.58	73.6	4.63	73.2
H–C(3)	4.05	76.2	4.05	76.2	4.10	76.4
H–C(4)	3.93	72.5	3.93	72.5	3.93	73.2
H–C(5)	3.82	71.8	3.82	71.8	3.87	72.1
Me(6)	1.38 (*d*, *J* = 5.3)	16.5	1.38 (*d*, *J* = 5.1)	16.5	1.40 (*d*, *J* = 6.0)	16.5
Rha						
H–C(1)	6.32 (br. *s*)	100.9	6.32 (br. *s*)	100.9	6.45 (br. *s*)	101.1
H–C(2)	4.73	70.8	4.73	70.8	4.82	71.5
H–C(3)	4.45–4.52 (*m*)	71.6	4.45–4.52 (*m*)	71.6	4.41	81.2
H–C(4)	4.10–4.16 (*m*)	84.8	4.10–4.16 (*m*)	84.8	4.30–4.34 (*m*)	78.9
H–C(5)	4.40	67.7	4.40	67.7	4.37	67.5
Me(6)	1.60 (*d*, *J* = 5.7)	17.8	1.60 (*d*, *J* = 5.7)	17.8	1.60 (*d*, *J* = 5.7)	18.2
Xyl						
H–C(1)	4.81 (*d*, *J* = 7.4)	106.3	4.80 (*d*, *J* = 7.4)	106.3	5.05 (*d*, *J* = 7.0)	104.6
H–C(2)	3.96	75.2	3.96	75.2	3.84	74.5
H–C(3)	3.98	76.6	3.98	76.6	3.84	87.0
H–C(4)	4.27	77.0	4.27	77.0	3.94	69.0
CH_2(5)	3.35 (*t*, *J* = 9.8), 4.25	64.2	3.35 (*t*, *J* = 9.8), 4.25	64.2	3.32 (*t*, *J* = 10.0), 4.06	65.8
Gal						
H–C(1)	4.87 (*d*, *J* = 8.1)	103.4	4.82 (*d*, *J* = 8.1)	103.6		
H–C(2)	4.36	70.8	4.34	71.0		
H–C(3)	4.04	74.0	4.00	74.1		

Table 2 (cont.)

	1		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
H–C(4)	4.26	69.8	4.23	69.6		
H–C(5)	4.06	76.2	4.06	76.5		
CH_2(6)	4.08, 4.31	62.0	4.62, 4.86–4.88 (*m*)	63.6		
*Ac*O–C(6)			1.92 (*s*)	20.5, 170.8		
Api I						
H–C(1)					5.90 (*d*, *J* = 2.6)	111.1
H–C(2)					4.68	78.0
H–C(3)					–	79.3
CH_2(4)					4.26, 4.46	74.4
CH_2(5)					4.00, 4.03	64.0
Api II						
H–C(1)					5.95 (*d*, *J* = 2.6)	111.1
H–C(2)					4.78	77.0
H–C(3)					–	79.8
CH_2(4)					4.12, 4.37	74.1
CH_2(5)					4.03, 4.05	64.7

[a]) Multiplicities were assigned from DEPT spectra. [b]) Overlapped ^{1}H-NMR signals are reported without designated multiplicity.

aglycone indicated that **1** was a bisdesmoside. Correlations were observed between δ(H) 4.56 (Agly H–C(3)) and δ(C) 104.1 (Glc I C(1)) and between δ(H) 5.01 (*d*, *J* = 7.9 Hz, Glc I H–C(1)) and δ(C) 86.4 (Agly C(3)). Thus a Glc unit was *O*-linked at C(3). The remaining sugar units constituted a tetrasaccharide linked at C(28) of the aglycone through an ester bond. Interglycosidic linkages were determined by the HMBCs between δ(H) 5.86 (*d*, *J* = 8.1 Hz, Fuc H–C(1)) and δ(C) 176.8 (Agly C(28)), between δ(H) 4.58 (Fuc H–C(2)) and δ(C) 100.9 (Rha C(1)), between δ(H) 4.81 (*d*, *J* = 7.4 Hz, Xyl H–C(1)) and δ(C) 84.8 (Rha C(4)), and between δ(H) 4.87 (Gal H–C(1)) and δ(C) 77.0 (Xyl C(4)) as well as reverse HMBCs between δ(H) 6.32 (Rha H–C(1)) and δ(C) 73.6 (Fuc C(2)), between δ(H) 4.10–4.16 (Rha H–C(4)) and δ(C) 106.3 (Xyl C(1)), and between δ(H) 4.27 (Xyl H–C(4)) and δ(C) 103.4 (Gal C(1)). These linkages were also confirmed by the following NOESY correlations: Fuc H–C(2)/Rha H–C(1), Rha H–C(4)/Xyl H–C(1), and Xyl H–C(4)/Gal H–C(1). All of these data were consistent with the structure 3-*O*-β-D-glucopyranosylmedicagenic acid 28-[*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl] ester for **1**, which was called micranthoside A.

Micranthoside B (**2**) was obtained as an amorphous powder. The HR-ESI-MS (positive-ion mode) exhibited a quasimolecular-ion peak at *m*/*z* 1315.5940 ([*M* + Na]$^+$) consistent with the molecular formula $C_{61}H_{96}NaO_{29}$, with 42 mass units more than **1**. The negative-ion-mode FAB-MS showed a quasimolecular-ion peak at *m*/*z* 1291 ([*M* – H]$^-$), and two other important fragment-ion peaks at *m*/*z* 1249 ([*M* – H – 42]$^-$) and 1087 ([*M* – H – 42 – 162]$^-$), indicating the elimination of an Ac and a hexosyl moiety. The full assignment of all the ^{1}H- and ^{13}C-NMR signals obtained from 2D-NMR analysis showed that **2** differed from **1** only by the presence of one Ac group at

C(6) of Gal (*Table 2*). This was supported by the observation in the HSQC spectrum of deshielded signals of Gal CH_2(6) at δ(H) 4.62 and 4.86–4.88 and δ(C) 63.6 in comparison with those of the OH-unsubstituted Gal CH_2(6) of **1**. Thus, the structure of **2** was established as 3-*O*-β-D-glucopyranosylmedicagenic acid 28-[*O*-6-*O*-acetyl-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl] ester.

Micranthoside C (**3**) was obtained as an amorphous powder. The HR-ESI-MS (positive-ion mode) exhibited a quasimolecular-ion peak at *m*/*z* 1699.7197 ($[M + Na]^+$) consistent with the molecular formula $C_{75}H_{120}NaO_{41}$. The negative-ion-mode FAB-MS showed a quasimolecular-ion peak at *m*/*z* 1675 ($[M - H]^-$), and important fragment-ion peaks at *m*/*z* 1513 ($[M - H - 162]^-$), 1381 ($[M - H - 162 - 132]^-$), and 1057 ($[M - H - 162 - 132 - 162 - 162]^-$) indicating the elimination of one hexosyl, one pentosyl, and two hexosyl moieties, respectively. The ^{1}H-NMR spectrum of **3** showed eight anomeric-H-atom NMR signals at δ(H) 4.77 (*d*, *J* = 7.9 Hz), 4.94 (*d*, *J* = 6.9 Hz), 5.05 (*d*, *J* = 7.0 Hz), 5.14 (*d*, *J* = 7.6 Hz), 5.79 (*d*, *J* = 8.1 Hz), 5.90 (*d*, *J* = 2.6 Hz), 5.95 (*d*, *J* = 2.6 Hz), and 6.45 (br. *s*, 1 H) which gave correlations, in the HSQC spectrum with eight anomeric-C signals at δ(C) 104.3, 103.5, 104.6, 104.6, 94.5, 111.1, 111.1, and 101.1, respectively, indicating the presence of eight sugar units. From the extensive 1D- and 2D-NMR experiments, these sugar units were identified as three β-glucopyranosyl (Glc I, Glc II, and Glc III), one β-fucopyranosyl (Fuc), one α-rhamnopyranosyl (Rha), one β-xylopyranosyl (Xyl), and two β-apiofuranosyl moieties (Api I and Api II). Comparison of the C-atom chemical shifts of the sugar units thus assigned with those of the literature [4–7] taking into account the known effects of *O*-glycosylation, indicated that **3** contained two terminal Glc (Glc II and Glc III), two terminal Api (Api I and Api II), a C(3)-substituted Xyl, a C(3)- and C(4)-disubstituted Rha, a C(3)- and C(6)-disubstituted Glc (Glc I), and a C(2)-substituted Fuc unit. The downfield shift of Rha C(3) from δ(C) 71.6 in **1** to 81.2 in **3** and the upfield shift of Rha C(4) from δ(C) 84.8 in **1** to 78.9 in **3**, suggested Rha to be C(3)- and C(4)-disubstituted [7]. This was also confirmed by the HMBC spectrum (*Fig. 2*) showing long-range correlations between δ(H) 6.45 (br. *s*, Rha H–C(1)) and δ(C) 81.2 (Rha C(3)), 73.2 (Fuc C(2)), and 67.5 (Rha C(5)), and between δ(H) 4.30–4.34 (Rha H–C(4)) and δ(C) 104.6 (Xyl C(1)), 81.2 (Rha C(3)), and 67.5 (Rha C(5)). Further analysis of NOESY cross-peaks between δ(H) 5.90 (Api I H–C(1)) and δ(H) 4.41 (Rha C(3)) and between δ(H) 5.05 (Xyl H–C(1)) and δ(H) 4.30–4.34 (Rha H–C(4)) established that Api I and Xyl were linked to Rha by a (1 → 3) and (1 → 4) linkage, respectively. Moreover, the NOESY correlations between δ(H) 5.95 (Api II H–C(1)) and δ(H) 3.84 (Xyl H–C(3)) and between δ(H) 3.84 (Xyl H–C(3)) and δ(H) 5.05 (Xyl H–C(1)) established that Xyl C(3) was substituted by Api II. Thus, it was revealed that one Fuc, one Rha, one Xyl, and two Api (Api I and Api II) were attached to Agly C(28) and that Agly C(3) was linked to three Glc residues. The connection of the Glc moieties at Agly C(3) was deduced by the NOESY and HMBC data. The terminal Glc II and Glc III moieties were ascertained to be linked to C(3) and C(6), respectively, of the inner Glc I by NOESY correlations between δ(H) 4.77 (Glc II H–C(1)) and δ(H) 4.13 (Glc I H–C(3)), and between δ(H) 5.14 (Glc III H–C(1)) and δ(H) 3.96 and 4.51 (Glc I CH_2(6)). Furthermore, NOESY correlations between δ(H) 4.94 (Glc I H–C(1)), 4.13 (Glc I H–C(3)), and 4.62 (Agly H–C(3)) established that the trisaccharide structure

was attached at C(3) of the aglycone. In the HMBC spectrum, the correlation between δ(H) 4.77 (Glc II H–C(1)) and δ(C) 86.6 (Glc I C(3)) confirmed the (1 → 3) linkage between Glc II and Glc I. Accordingly, the structure of **3** was formulated as 3-*O*-{*O*-*β*-D-glucopyranosyl-(1 → 3)-*O*-[*β*-D-glucopyranosyl-(1 → 6)]-*β*-D-glucopyranosyl}medicagenic acid 28-{*O*-*β*-D-apiofuranosyl-(1 → 3)-*O*-*β*-D-xylopyranosyl-(1 → 4)-*O*-[*β*-D-apiofuranosyl-(1 → 3)]-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranosyl} ester. To the best of our knowledge, this is the first saponin with a trisaccharide moiety attached at C(3) of the aglycone isolated from the Polygalaceae family. On the contrary, saponins with two (1 → 2)-linked glucose units at C(3) of the aglycone have been isolated from many Polygalaceae species [6].

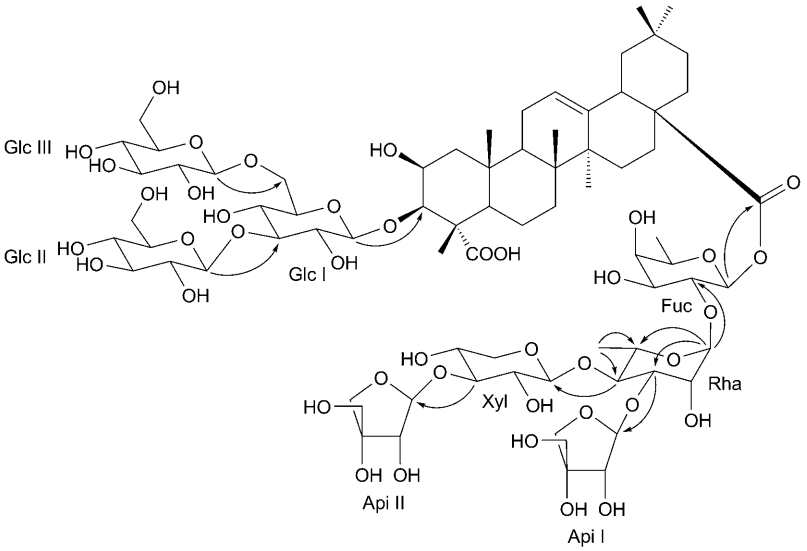


Fig. 2. *Selective HMBCs of* **3**

Compounds **1**–**3** were evaluated against two human colon cancer cells, HCT 116 and HT-29, by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method [15] with paclitaxel as positive control, and all of them were inactive at the concentration of 100 μM.

The authors are grateful to the *Conseil Régional de Bourgogne* for financial support.

Experimental Part

General. Column chromatography (CC)/medium-pressure liquid chromatography (MPLC): silica gel *60* (SiO_2; 15–40 μm; *Merck*), *RP-18* (*Silicycle*; 75–200 μm), *Gilson* pump *M 305*, *Büchi* column (460 × 25 mm and 460 × 15 mm), *Büchi* precolumn (110 × 15 mm). TLC and HP-TLC: SiO_2 plates *60* F_{254} (*Merck*); solvent systems: $CHCl_3$/MeOH/H_2O 80:20:2, 70:30:5, and 60:32:7; spray reagent: vanillin reagent (2% mixture of conc. H_2SO_4 soln. and 1% vanillin in EtOH). Optical rotation: *Perkin-Elmer-241* polarimeter. GC Analysis: *Thermoquest* gas chromatograph; *DB-1701* cap. column (30 m × 0.25 mm (i.d.); *J & W Scientific*); detection by FID; detector temp. 250°, injection temp. 230°, initial temp. maintained at 80° for 5 min and then raised to 270° at 15°/min; carrier gas He. 1D- and 2D-NMR

Spectra (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, and HMBC): *Varian-Unity-Inova*-600 spectrometer, equipped with a *Sun-4L-X* computer system; at 600 (^{1}H) and 150 MHz (^{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, 135.9, and 123.9). FAB-MS: *Jeol-SX-102* spectrometer; neg.-ion mode; glycerol matrix; in m/z. HR-ESI-MS: micromass *Quattro-LS* instrument; neg.-ion mode; in m/z.

Plant Material. The roots of *P. micrantha* were collected in the village Bangoua near Bangangté City located in the Ndé division of the Western highlands of Cameroon in July 2009, and identified by *P. Nana*, botanist at the National Herbarium of Cameroon (NHC), Yaoundé, where a voucher specimen (57326 HNC) was deposited.

Extraction and Isolation. Dried and finely powdered roots of *P. micrantha* (180 g) were extracted three times under reflux in MeOH/H_2O 7:3 (1500 ml) for 3 h. After evaporation, a dark residue of 25 g was obtained. A fraction (10 g) of this extract was submitted to VLC (*RP-18*, MeOH/H_2O gradient (each eluent 250 ml): *Frs. 1–6*. *Fr. 6* (MeOH/H_2O 50:50; 1.6 g) was fractionated by MPLC (SiO_2, $CHCl_3$/MeOH/H_2O 80:20:2, 70:30:5, and 60:32:7) and MPLC (*RP-18*, MeOH/H_2O 40:60 → 100:0)): **1** (8 mg), **2** (7.7 mg), **3** (35 mg), arilloside A (11 mg), polygalasaponin XXXV (5.7 mg), desacylsenegasaponin B (4.3 mg), polygalasaponin XXVIII (4.5 mg), and polygalasaponins XLV and XLVI (9.3 mg).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2-hydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-β-*D*-Galactopyranosyl-(1 → 4)*-O*-β-*D*-xylopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-6-deoxy-β-*D*-galactopyranosyl] Ester* (**1**): White amorphous powder. $[\alpha]_D^{25} = +1.76$ ($c = 0.43$, MeOH). ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1* and *2*. HR-ESI-MS (pos.): 1273.5834 ($[M+Na]^+$, $C_{59}H_{94}NaO_{28}^+$; calc. 1273.5829). FAB-MS (neg.): 1249 ($[M-H]^-$), 1087 ($[M-H-162]^-$), 925 ($[M-H-162-162]^-$).

*(2β,3β,4α)-3-(β-*D*-Glucopyranosyloxy)-2-hydroxyolean-12-ene-23,28-dioic Acid 28-[*O*-6-*O*-Acetyl-β-*D*-galactopyranosyl-(1 → 4)*-O*-β-*D*-xylopyranosyl-(1 → 4)*-O*-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-6-deoxy-β-*D*-galactopyranosyl] Ester* (**2**): White amorphous powder. $[\alpha]_D^{25} = -1.21$ ($c = 0.43$, MeOH). ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1* and *2*. HR-ESI-MS (pos.): 1315.5940 ($[M+Na]^+$, $C_{61}H_{96}NaO_{29}^+$; calc. 1315.5935). FAB-MS (neg.): 1291 ($[M-H]^-$), 1249 ($[M-H-42]^-$), 1087 ($[M-H-42-162]^-$).

*(2β,3β,4α)-3-[*O*-β-*D*-Glucopyranosyl-(1 → 3)-O-[β-*D*-glucopyranosyl-(1 → 6)]-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-[β-*D*-apiofuranosyl-(1 → 3)]-O-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-6-deoxy-β-*D*-galactopyranosyl] Ester* (**3**): White amorphous powder. $[\alpha]_D^{25} = -6.52$ ($c = 0.43$, MeOH). ^{1}H- and ^{13}C-NMR ((D_5)pyridine): *Tables 1* and *2*. HR-ESI-MS (pos.): 1699.7197 ($[M+Na]^+$, $C_{75}H_{120}NaO_{41}^+$; calc. 1699.7203). FAB-MS (neg.): 1675 ($[M-H]^-$), 1513 ($[M-H-162]^-$), 1381 ($[M-H-162-132]^-$), 1057 ($[M-H-162-132-162-162]^-$).

Acid Hydrolysis and GC Analysis. Each compound (3 mg) was hydrolyzed with 2N aq. CF_3COOH (5 ml) for 3 h at 95°. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatedly concentrated with MeOH until neutral, and then analyzed by TLC (SiO_2, $CHCl_3$/MeOH/H_2O 8:5:1) by comparison with authentic samples. Furthermore, the residue of the sugars was dissolved in anh. pyridine (100 µl), and L-cysteine methyl ester hydrochloride (0.06 mol/l) was added. The mixture was stirred at 60° for 1 h, then 150 µl of HMDS/Me_3SiCl (hexamethyldisilazane/trimethylchlorosilane 3:1) was added, and the mixture was stirred at 60° for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under an N_2 stream. The residue was partitioned between hexane (1 ml) and H_2O (0.1 ml), and the hexane layer (1 µl) was analyzed by GC [16]. D-Glucose, D-galactose, D-xylose, L-rhamnose, and D-fucose were detected from **1** and **2** by co-injection of the hydrolysate with standard silylated samples with t_R 18.60, 17.20, 13.45, 13.15, and 12.17 min, respectively. Identification of D-glucose, D-xylose, L-rhamnose, D-fucose, and D-apiose was carried out for **3**, giving peaks at t_R 18.64, 13.47, 13.15, 12.12 min, and 11.55, resp.

MTT Cytotoxicity Assay. The bioassay was carried out according to the method described by *Carmichael* and co-workers [15] with two human colorectal cancer cell lines (HCT 116 and HT-29) provided by the *Oncodesign*, Dijon, France. Paclitaxel was used as pos. control, and exhibited IC_{50} values of 2.40 and 4.27 nM against HCT 116 and HT-29, resp.

REFERENCES

[1] J. M. Dalziel, in 'The Useful Plants of W. Tropical Africa, Crown Agents for the Colonies', London, 1937, p. 27.
[2] M. Haddad, T. Miyamoto, C. Delaude, M. A. Lacaille-Dubois, *Helv. Chim. Acta* **2003**, *86*, 3055.
[3] A. C. Mitaine-Offer, T. Miyamoto, V. Laurens, C. Delaude, M. A. Lacaille-Dubois, *Helv. Chim. Acta* **2003**, *86*, 2404.
[4] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, A. Ueno, *Chem. Pharm. Bull.* **1996**, *44*, 173.
[5] M. Elbandy, T. Miyamoto, C. Delaude, M. A. Lacaille-Dubois, *Helv. Chim. Acta* **2002**, *85*, 2721.
[6] M. A. Lacaille-Dubois, A. C. Mitaine-Offer, *Phytochem. Rev.* **2005**, *4*, 139.
[7] A. C. Mitaine-Offer, N. Pénez, T. Miyamoto, C. Delaude, J. F. Mirjolet, O. Duchamp, M. A. Lacaille-Dubois, *Phytochemistry* **2010**, *71*, 90.
[8] G. Timité, A. C. Mitaine-Offer, T. Miyamoto, M. Ramezani, A. Rustaiyan, J. F. Mirjolet, O. Duchamp, M. A. Lacaille-Dubois, *Magn. Res. Chem.* **2010**, *48*, 370.
[9] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, A. Ueno, *Chem. Pharm. Bull.* **1996**, *44*, 810.
[10] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, H. Noguchi, *Chem. Pharm. Bull.* **1996**, *44*, 2092.
[11] M. Yoshikawa, T. Murakami, T. Ueno, M. Kadoya, H. Matsuda, J. Yamahara, N. Murakami, *Chem. Pharm. Bull.* **1995**, *43*, 350.
[12] D. Zhang, T. Miyase, M. Kuroyanagi, K. Umehara, H. Noguchi, *Phytochemistry* **1998**, *47*, 459.
[13] M. Ouyang, C. Yang, H. Wang, *Zhongcaoyao* **1998**, *30*, 881.
[14] Z. Jia, K. Koike, T. Nikaido, *J. Nat. Prod.* **1998**, *61*, 1368.
[15] J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna, J. B. Mitchell, *Cancer Res.* **1987**, *47*, 936.
[16] S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.* **1987**, *35*, 501.

Received August 31, 2010