Acylated Triterpene Saponins from *Atroxima liberica* STAPF

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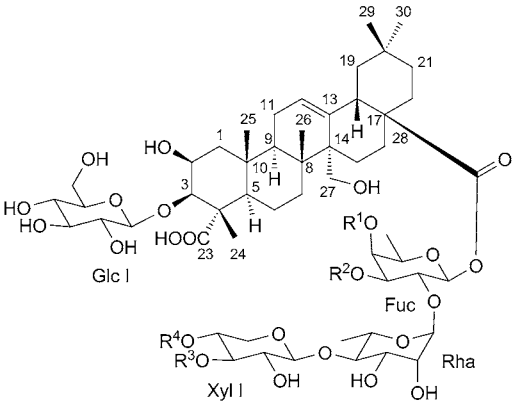
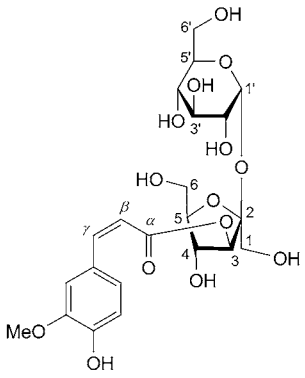
The four new acylated triterpene saponins **1**–**4**, isolated as two pairs of isomers and named libericosides A_1/A_2 and B_1/B_2, one pair of isomers **5**/**6**, the (*Z*)-isomer libericoside C_2 (**5**) being new, one new sucrose ester, atroximoside (**7**), and eight known compounds were isolated from the roots of *Atroxima liberica* by repeated MPLC and VLC on normal and reversed-phase silica gel. Their structures were elucidated on the basis of extensive 1D- and 2D-NMR studies (^{1}H- and ^{13}C-NMR, DEPT, COSY, TOCSY, NOESY, HSQC, and HMBC) and mass spectrometry as 3-*O*-*β*-D-glucopyranosylpresenegenin 28-{*O*-*α*-L-arabinopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**1**) and its (*Z*)-isomer **2**, 3-*O*-*β*-D-glucopyranosylpresenegenin 28-{*O*-*α*-L-arabinopyranosyl-(1→4)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)]-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**3**) and its (*Z*)-isomer **4**, 3-*O*-*β*-D-glucopyranosylpresenegenin 28-{*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→3)]-4-*O*-[(*Z*)-3,4-dimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**5**), and 3-*O*-[(*Z*)-feruloyl]-*β*-D-fructofuranosyl *α*-D-glucopyranoside (**7**). Compounds **1**–**6** and the known saponins **8**/**9** were evaluated against the human colon cancer cells HCT 116 and HT-29 and showed moderate to weak cytotoxicity.

Introduction. – The genus *Atroxima* (Polygalaceae), represented by trees, shrubs, herbs, or lianas (rarely) comprises six species distributed in the forests of Western and Central Africa [1]. We previously reported the isolation and structure elucidation of seven pairs of preatroxigenin (=(2*β*,3*β*,4*α*,22*β*)-2,3,22,27-tetrahydroxyolean-12-ene-23,28-dioic acid) glycosides called atroximasaponins A_1/A_2 to G_1/G_2 from the roots of *A. congolana* [2][3]. However, there is no previous study on *A. liberica*. In the course of our studies on the saponins of the Polygalaceae family to find chemotaxonomic markers, we have investigated the saponin fraction of the roots of *A. liberica* [4][5]. This plant is a liana widespread in western Africa, particularly in Cameroon. An ethnobotanical survey indicated that this species was not used in traditional medicine. This article describes the isolation and structure elucidation of four new acylated triterpene saponins, **1**–**4**, isolated as two pairs of isomers named libericosides A_1/A_2 and B_1/B_2, one pair of isomers **5**/**6**, the (*Z*)-isomer libericoside C_2 (**5**) being new, a new sucrose ester, atroximoside (**7**), and eight known compounds from the aqueous MeOH extract of the roots of *A. liberica* (*Fig.*). Compounds **1**–**6**, and the known saponins **8**/**9** were evaluated against the human colon cancer cells HCT 116 and HT-29.





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		R^1	R^2	R^3	R^4
1	Libericoside A1	(*E*)-DMC	H	Ara	H
2	Libericoside A2	(*Z*)-DMC	H	Ara	H
3	Libericoside B1	(*E*)-DMC	R'	H	Ara
4	Libericoside B2	(*Z*)-DMC	R'	H	Ara
5	Libericoside C2	(*Z*)-DMC	Glc II Ac	H	H
6	Securioside A	(*E*)-DMC	Glc II Ac	H	H
8		(*E*)-DMC	Glc II	H	H
9		(*Z*)-DMC	Glc II	H	H

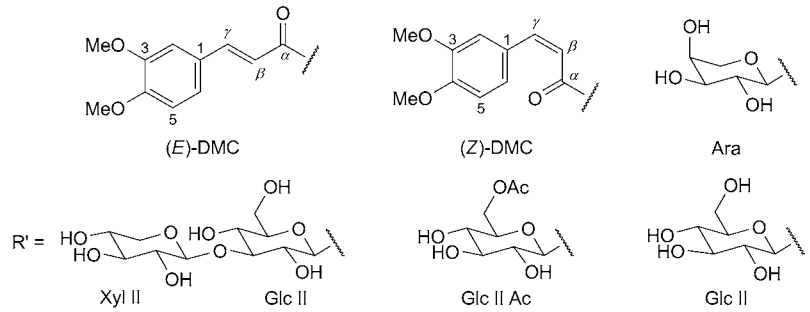

Figure. *Compounds* **1**–**9**, *isolated from* Atroxima liberica STAPF

Results and Discussion. – The MeOH/H_2O 7:3 extract of the roots of *A. liberica* was submitted to vacuum liquid chromatography (VLC) and fractionated by repeated medium-pressure liquid chromatography (MPLC) yielding compounds **1**–**5**, the known saponins securioside A (**6**) [6] and **8**/**9** [7], the new sucrose ester **7**, as well as five known sucrose esters, sibiricose A5 [8], 3′,6-di-*O*-sinapoylsucrose [8], 3′,6-di-*O*-feruloylsucrose [9], 6-*O*-sinapoyl-3′-*O*-feruloylsucrose [10], and 6-*O*-feruloyl-3′-*O*-sinapoylsucrose [10] (sinapic acid = 3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid, ferulic acid = 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid). Their structures were established mainly by spectroscopic 1D- and 2D-NMR experiments (^{1}H- and, ^{13}C-NMR, COSY, TOCSY, NOESY, HSQC, HMBC; *Tables 1*–*4*) and mass spectrometry. Compounds **1**–**5** were obtained as white amorphous powders. ^{1}H- and ^{13}C-NMR

signals of the prosapogenin assigned from the 2D-NMR spectra of each pair of isomers (*Tables 1–3*) were in good agreement with those of tenuifolin (3-*O*-β-D-glucopyranosylpresenegenin). Presenegenin (=(2β,3β,4α)-2,3,27-trihydroxyolean-12-ene-23,28-dioic acid) is commonly encountered in the Polygalaceae family [11–13]. The difference between these newly isolated compounds was located at the oligosaccharide chain linked to C(28) of the aglycon. The monosaccharides obtained by acid hydrolysis of each pair of isomers were identified by comparison on TLC with standard sugars as glucose, fucose (=6-deoxygalactose), rhamnose (=6-deoxymannose), xylose, and arabinose (in the case of **1**–**4**), and as glucose, fucose, rhamnose, and xylose in the case of **5**. The D-configuration for fucose, xylose, and glucose, and the L-configuration for rhamnose and arabinose were determined by GC analysis (see *Exper. Part*). In the 1D-

Table 1. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; C_5D_5N) *of the Aglycone Moieties of* **1**–**5**[a])[b]). δ in ppm, *J* in Hz.

	1/2		**3/4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	1.40, 2.28	43.8	1.40, 2.24	44.1	1.35, 2.18	43.8
H–C(2)	4.58	69.8	4.61	69.8	4.60	69.8
H–C(3)	4.66	87.0	4.63	86.9	4.57	86.6
C(4)	–	53.4	–	53.8	–	53.1
H–C(5)	2.34	52.1	2.24	52.2	2.17	51.8
CH_2(6)	1.88[c])	21.4	1.76, 1.88	21.2	1.80	21.0
CH_2(7)	0.96[c])	33.6	1.00, 1.20	33.8	1.00, 1.12	33.5
C(8)	–	40.6	–	41.0	–	40.7
H–C(9)	2.29	48.5	2.29	48.8	2.20	48.7
C(10)	–	37.0	–	37.4	–	37.3
CH_2(11)	1.92, 2.05	24.1	1.91, 2.11	24.4	[c]), 2.06	23.4
H–C(12)	5.81	127.4	5.76	127.3	5.72	127.4
C(13)	–	139.1	–	138.9	–	139.0
C(14)	–	47.7	–	47.8	–	47.7
CH_2(15)	2.10[c])	24.1	[c]), [c])	24.0	1.80, 2.00	24.0
CH_2(16)	2.10[c])	24.1	[c]), [c])	23.9	1.84, 2.04	24.0
C(17)	–	46.8	–	47.0	–	46.8
H–C(18)	3.20	41.7	3.16	42.0	3.10	41.8
CH_2(19)	1.30, 1.71	45.0	1.27, 1.70	45.8	1.20, 1.60	45.0
C(20)	–	30.2	–	30.6	–	30.3
CH_2(21)	[c]), [c])	33.7	1.86, [c])	33.8	1.72	33.6
CH_2(22)	[c]), 1.82	31.9	1.66, 1.84	31.9	1.64, 1.84	31.9
C(23)	–	185.3	–	185.8	–	185.5
Me(24)	1.90 (*s*)	14.6 (*s*)	1.88 (*s*)	15.0 (*s*)	1.83 (*s*)	14.6 (*s*)
Me(25)	1.49 (*s*)	17.3 (*s*)	1.44 (*s*)	17.4 (*s*)	1.40 (*s*)	17.0 (*s*)
Me(26)	1.10 (*s*)	18.6 (*s*)	1.06 (*s*)	18.6 (*s*)	1.00 (*s*)	18.5 (*s*)
CH_2(27)	3.84, 4.14	64.0	3.83, 4.13	64.3	3.76, 4.06	64.0
C(28)	–	176.5	–	176.8	–	176.5
Me(29)	0.79 (*s*)	32.6 (*s*)	0.82 (*s*)	32.6 (*s*)	0.73 (*s*)	32.6 (*s*)
Me(30)	0.81 (*s*)	23.5 (*s*)	0.80 (*s*)	23.8 (*s*)	0.75 (*s*)	23.4 (*s*)

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

Table 2. *^{13}C-NMR Data* (150 MHz, C_5D_5N) *of the Sugar Moieties of* **1**–**5** *from 1D- and 2D-NMR Experiments.* δ in ppm.

	1	**2**	**3**	**4**	**5**
3-*O*-Sugar					
Glc I					
C(1)	104.6	104.6	104.6	104.6	104.3
C(2)	74.0	74.0	74.2	74.2	74.8
C(3)	76.8	76.8	77.3	77.3	77.0
C(4)	70.9	70.9	71.2	71.2	70.9
C(5)	77.0	77.0	77.8	77.8	77.2
C(6)	62.6	62.6	62.5	62.5	61.9
28-*O*-Sugars					
Fuc					
C(1)	94.4	94.4	94.4	94.4	94.1
C(2)	71.2	71.2	71.3	71.0	72.0
C(3)	74.0	74.2	83.5	83.5	83.0
C(4)	75.0	74.8	74.3	74.1	74.0
C(5)	71.0	70.9	70.8	70.7	70.9
C(6)	16.5	16.5	16.6	16.6	16.7
Rha					
C(1)	101.4	100.8	101.6	101.4	100.8
C(2)	71.2	71.2	71.3	71.3	71.2
C(3)	72.0	72.0	72.0	71.9	71.7
C(4)	84.5	84.5	85.0	85.0	84.2
C(5)	67.8	67.8	68.0	68.0	68.0
C(6)	18.3	18.3	18.5	18.5	18.2
Xyl I					
C(1)	106.7	106.7	106.9	106.9	106.6
C(2)	75.5	75.5	75.6	75.6	75.4
C(3)	86.2	86.2	75.8	75.8	77.8
C(4)	70.9	70.9	77.0	77.0	70.3
C(5)	65.8	65.8	64.9	64.9	66.9
Glc II					
C(1)			105.4	105.4	104.3
C(2)			75.0	75.0	74.5
C(3)			85.0	85.0	77.4
C(4)			71.2	71.2	70.5
C(5)			78.0	78.0	77.2
C(6)			62.7	62.7	63.4
*Ac*O–C(6)					20.5, 171.0
Xyl II					
C(1)			104.5	104.5	
C(2)			74.9	74.9	
C(3)			77.4	77.4	
C(4)			70.0	70.0	
C(5)			65.0	65.0	
Ara					
C(1)	105.6	105.6	104.0	104.0	
C(2)	72.0	72.0	71.2	71.2	
C(3)	74.0	74.0	74.6	74.6	
C(4)	68.5	68.5	68.0	68.0	
C(5)	66.7	66.7	64.9	64.9	

Table 2 (cont.)

	1	**2**	**3**	**4**	**5**
DMC					
C(α)	167.5	166.3	167.9	166.8	166.4
C(β)	115.5	116.6	115.4	116.1	116.3
C(γ)	145.9	144.4	145.8	144.4	144.6
C(1)	127.2	127.5	127.5	127.9	127.6
C(2)	111.4	114.3	111.4	114.3	114.5
C(3)	149.5	148.6	149.2	148.3	148.6
C(4)	152.5	150.8	152.0	150.2	150.8
C(5)	111.9	110.7	111.7	110.7	111.1
C(6)	123.3	125.6	123.3	125.6	125.5
*Me*O–C(3)	56.0	56.0	56.0	56.0	55.8
*Me*O–C(4)	55.8	55.9	55.9	55.9	55.8

and 2D-NMR spectra, the relatively large ^3J(1,2) values of the glucose, fucose, xylose, and arabinose units in their pyranose form (6.9–8.3 Hz) indicated β-anomeric orientation for glucose, fucose, xylose, and α-anomeric orientation for arabinose. The large ^1J(H–(1),C(1)) value of the Rha (168 Hz), indicated α-anomeric orientation and confirmed that the anomeric H-atom was equatorial (α-anomeric pyranose) [5][14].

The positive-ion-mode HR-ESI-MS of libericosides A_1/A_2 (**1**/**2**) exhibited a quasimolecular-ion peak at *m*/*z* 1449.6308 ($[M+Na]^+$) consistent with the molecular formula $C_{69}H_{102}O_{31}$. Their negative-ion-mode FAB-MS displayed a quasimolecular-ion peak at *m*/*z* 1425 ($[M-H]^-$). Further fragment-ion peaks appeared at *m*/*z* 1293 ($[M-H-132]^-$) and 1103 ($[M-H-132-190]^-$), corresponding to the successive loss of one pentosyl and one 3,4-dimethoxycinnamoyl (DMC) unit, respectively (cinnamic acid = 3-phenylprop-2-enoic acid). The ^{1}H,^{1}H-COSY experiment of **1**/**2** permitted us to identify the *trans*-positioned olefinic H-atoms of the 3,4-dimethoxycinnamoyl moiety at δ(H) 6.62 (*d*, J = 16.1 Hz, H–C(β)) and 8.05 (*d*, J = 16.1 Hz, H–C(γ)), the *cis*-positioned olefinic H-atoms at δ(H) 6.00 (*d*, J = 13.8 Hz, H–C(α)) and 7.02 (*d*, J = 13.8 Hz, H–C(γ)), and the trisubstituted benzene ring H-atoms (*Table 3*). All the NMR data were in good agreement with literature data of the (*E*)/(*Z*)-3,4-dimethoxycinnamoyl (DMC) group [15]. These conclusions indicated that **1** and **2** are a mixture of (*E*)- and (*Z*)-3,4-dimethoxycinnamoyl-substituted triterpene glycosides, and all attempts to separate them were unsuccessful. The observed isomerization is due to the effect of light on the 4-methoxycinnamoyl group in aqueous MeOH solution. Under these conditions, the geometrically isomeric structures of the 4-methoxycinnamoyl groups in **1** and **2** showed tautomer-like behavior as already described for saponins from Polygalaceae [15]. The ^{1}H-NMR spectrum of **1**/**2**[1]) showed five anomeric H-atoms at δ(H) 6.59 (br. *s*, 1 H), 6.15 (*d*, J = 7.9 Hz, 1 H), 5.24 (*d*-like, 1 H), 5.17 (*d*, J = 7.5 Hz, 1 H), and 5.10 (*d*, J = 7.6 Hz, 1 H), which gave correlations, in the HSQC spectrum, with five anomeric C-atoms at δ(C) 101.4, 94.4, 106.7, 104.6, and 105.6, respectively, indicating the presence of five sugar units. From the extensive 1D-

[1]) For convenience, only the data of **1** of the mixture **1**/**2** and of **3** of the mixture **3**/**4** are given in the text (see *Tables 2* and *3*).

Table 3. *^{1}H-NMR Data* (600 MHz, C_5D_5N) *of the Sugar Moieties of* **1**–**5** *from 1D- and 2D-NMR Experiments*[a])[b]). δ in ppm, J in Hz.

	1	**2**	**3**	**4**	**5**
3-*O*-Sugar					
Glc I					
H–C(1)	5.17 (*d*, *J* = 7.5)	5.17 (*d*, *J* = 7.5)	5.06 (*d*, *J* = 7.2)	5.06 (*d*, *J* = 7.2)	4.97 (*d*, *J* = 7.6)
H–C(2)	4.08	4.08	3.95	3.95	3.84
H–C(3)	4.38	4.38	4.24	4.24	4.19
H–C(4)	4.30	4.30	4.04	4.04	3.96
H–C(5)	4.03	4.03	3.91	3.91	3.85
CH_2(6)	4.27, 4.46	4.27, 4.46	4.16, 4.22	4.16, 4.22	4.10, 4.29
28-*O*-Sugars					
Fuc					
H–C(1)	6.15 (*d*, *J* = 7.9)	6.15 (*d*, *J* = 7.9)	6.07 (*d*, *J* = 7.9)	6.04 (*d*, *J* = 7.8)	6.00 (*d*, *J* = 8.3)
H–C(2)	4.91	4.91	4.79	4.76	4.75
H–C(3)	4.40	4.36	4.58	4.56	4.45
H–C(4)	5.90	5.87	6.10	6.02	5.87
H–C(5)	4.30	4.33	4.26	4.23	4.20
Me(6)	1.40 (*d*, *J* = 6.1)	1.38 (*d*, *J* = 6.1)	1.28 (*d*, *J* = 5.5)	1.24 (*d*, *J* = 5.7)	1.30 (*d*, *J* = 5.9)
Rha					
H–C(1)	6.59 (br. *s*)	6.59 (br. *s*)	6.43 (br. *s*)	6.38 (br. *s*)	6.39 (br. *s*)
H–C(2)	4.86	4.86	4.79	4.76	4.71
H–C(3)	4.65	4.65	4.54	4.54	4.50
H–C(4)	4.32	4.32	4.17	4.17	4.14
H–C(5)	4.34	4.34	4.44	4.42	4.38
Me(6)	1.82 (*d*, *J* = 5.6)	1.82 (*d*, *J* = 5.6)	1.72 (*d*, *J* = 5.2)	1.72 (*d*, *J* = 5.2)	1.65 (*d*, *J* = 5.5)
Xyl I					
H–C(1)	5.24 (*d*-like)	5.24 (*d*-like)	4.83 (*d*, *J* = 7.3)	4.83 (*d*, *J* = 7.3)	4.87 (*d*-like)
H–C(2)	4.06	4.06	4.04	4.04	3.99
H–C(3)	4.35	4.35	4.05	4.05	3.99
H–C(4)	4.49	4.49	4.08	4.08	4.09
CH_2(5)	3.34 (*t*, *J* = 10.2), 4.52	3.34 (*t*, *J* = 10.2), 4.52	3.43 (*t*, *J* = 10.4), 4.34	3.43 (*t*, *J* = 10.4), 4.34	3.42 (*t*, *J* = 9.8), 4.07
Glc II					
H–C(1)			5.10 (*d*, *J* = 6.9)	5.10 (*d*, *J* = 6.9)	5.02 (*d*, *J* = 7.6)
H–C(2)			3.93	3.93	3.89
H–C(3)			4.17	4.17	4.10
H–C(4)			3.94	3.94	3.92
H–C(5)			3.97	3.97	3.87
CH_2(6)			4.22, 4.51	4.22, 4.51	4.63, 4.90
AcO					2.02 (*s*)
Xyl II					
H–C(1)			5.05 (*d*, *J* = 7.2)	5.05 (*d*, *J* = 7.2)	
H–C(2)			3.94	3.94	
H–C(3)			4.26	4.26	
H–C(4)			4.30	4.30	
CH_2(5)			3.46 (*t*, *J* = 10.4), 4.10	3.46 (*t*, *J* = 10.4), 4.10	
Ara					
H–C(1)	5.10 (*d*, *J* = 7.6)	5.10 (*d*, *J* = 7.6)	4.91 (*d*, *J* = 7.6)	4.91 (*d*, *J* = 7.6)	
H–C(2)	4.68	4.68	4.45	4.45	
H–C(3)	4.08	4.08	4.09	4.09	

Table 3 (cont.)

	1	**2**	**3**	**4**	**5**
H–C(4)	4.34	4.34	4.44	4.44	
CH_2(5)	3.56 (br. *d*, *J* = 4.25)	3.56 (br. *d*, *J* = 4.25)	[c]), 4.34	[c]), 4.34	
DMC					
H–C(β)	6.62 (*d*, *J* = 16.1)	6.00 (*d*, *J* = 13.8)	6.52 (*d*, *J* = 16.1)	5.92 (*d*, *J* = 13.8)	5.95 (*d*, *J* = 13.8)
H–C(γ)	8.05 (*d*, *J* = 16.1)	7.02 (*d*, *J* = 13.8)	7.90 (*d*, *J* = 16.1)	6.90 (*d*, *J* = 13.8)	6.93 (*d*, *J* = 13.8)
H–C(2)	7.04 (*s*)	8.00 (*s*)	7.04 (*s*)	7.87 (*s*)	7.94 (*s*)
H–C(5)	7.15 (*d*, *J* = 8.6)	7.09 (*d*, *J* = 8.6)	7.00 (*d*, *J* = 8.6)	6.96 (*d*, *J* = 8.6)	6.96 (*d*, *J* = 8.6)
H–C(6)	7.16 (*d*, *J* = 8.6)	7.62 (*d*, *J* = 8.6)	7.04 (*d*, *J* = 8.6)	7.50 (*d*, *J* = 8.6)	7.48 (*d*, *J* = 8.6)
*Me*O–C(3)	3.97 (*s*)	4.02 (*s*)	3.82 (*s*)	3.88 (*s*)	3.87 (*s*)
*Me*O–C(4)	3.97 (*s*)	4.02 (*s*)	3.82 (*s*)	3.88 (*s*)	3.87 (*s*)

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

Table 4. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; CD_3OD) *of* **7** *from 1D- and 2D-NMR Experiments*[a])[b]). δ in ppm, *J* in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
Glc			CH_2(6)	3.70 (*dd*, *J* = 12.0, 3.0),	62.3
H–C(1)	5.43 (*d*, *J* = 3.0)	93.6	Acid	3.80 (*dd*, *J* = 12.0, 6.0)	
H–C(2)	3.44 (*dd*, *J* = 9.7, 3.0)	73.2	C(α)	–	167.8
H–C(3)	3.68 (*d*, *J* = 9.7)	74.7	H–C(β)	5.87 (*d*, *J* = 12.9)	115.2
H–C(4)	3.39	71.4	H–C(γ)	6.89 (*d*, *J* = 12.9)	143.2
H–C(5)	3.40	73.2	C(1)	–	127.0
CH_2(6)	3.74, 3.83 (*dd*, *J* = 12.0, 2.0)	62.2	H–C(2)	7.14 (*d*, *J* = 1.9)	111.7
Fru			C(3)	–	150.0
CH_2(1)	3.57 (*d*, *J* = 12.2),	65.5	C(4)	–	151.8
	3.64 (*d*, *J* = 12.2)		H–C(5)	6.32 (*d*, *J* = 7.8)	114.2
H–C(2)	–	105.3	H–C(6)	7.03 (*dd*, *J* = 7.8, 1.9)	124.2
H–C(3)	5.45 (*d*, *J* = 7.9)	80.0	*Me*O–C(3)	3.86 (*s*)	56.5
H–C(4)	4.35 (*t*, *J* = 7.9)	73.8			
H–C(5)	3.72–3.76 (*m*)	83.8			

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

and 2D-NMR experiments, these sugar units were identified as β-glucopyranosyl (Glc I), β-fucopyranosyl (Fuc), α-rhamnopyranosyl (Rha), α-arabinopyranosyl (Ara), and β-xylopyranosyl (Xyl I). It was concluded that **1**/**2** were bisdesmosidic saponins with one sugar located at C(3) of the aglycone (downfield shift at δ(C) 87.0) and the four other monosaccharides linked at C(28) (upfield shift at δ(C) 176.5) through an ester bond. A correlation in the HMBC spectrum between δ(H) 6.15 (*d*, *J* = 7.9 Hz, Fuc H–C(1)) and δ(C) 176.5 (Agly C(28)) showed that Fuc was linked to the COOH group of the aglycon by an ester linkage. The location of the DMC at C(4) of Fuc (δ(H) 5.90) was determined by the TOCSY and COSY experiments, starting from the anomeric H-atoms at δ(H) 6.15 (Fuc H–C(1)). The downfield shifts observed in the HSQC

spectrum for the Fuc H–C(4)/C(4) resonances at δ(H)/δ(C) 5.90/75.0, as well as an HMBC cross-peak δ(H) 5.90 (Fuc H–C(4))/δ(C) 167.5 (DMC C(α)) established the secondary-alcohol function at C(4) of Fuc to be acylated. The HMBC cross-peaks δ(H) 5.10 (*d*, $J = 7.6$ Hz, Ara H–C(1))/δ(C) 86.2 (Xyl I C(3)), δ(H) 5.24 (*d*-like, Xyl I H–C(1))/δ(C) 84.5 (Rha C(4)), and δ(H) 6.59 (br. *s*, Rha H–C(1))/δ(C) 71.2 (Fuc C(2)) established the sequence of the sugar chain at C(28) to be 28-[*O*-α-L-arabinopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl], with an additional substitution at C(4) of Fuc by DMC. This sequence was confirmed by the NOESY correlations δ(H) 4.35 (Xyl I H–C(3))/δ(H) 5.10 (Ara H–C(1)), δ(H) 4.91 (Fuc H–C(2))/δ(H) 6.59 (Rha H–C(1)), and δ(H) 4.32 (Rha H–C(4))/δ(H) 5.24 (Xyl I H–C(1)). Thus, libericosides A_1/A_2 (**1**/**2**) were elucidated as 3-*O*-β-D-glucopyranosylpresenegenin 28-{*O*-α-L-arabinopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]-β-D-fucopyranosyl} ester (**1**) and its (*Z*)-isomer **2**.

Compounds **3**/**4** showed a quasimolecular-ion peak at *m*/*z* 1743.7250 ($[M + Na]^+$) in the HR-ESI-MS, indicating the molecular formula $C_{80}H_{120}O_{40}$. Their negative-ion-mode FAB-MS displayed a quasimolecular-ion peak at *m*/*z* 1719 ($[M - H]^-$). Other significant fragment-ion peaks were observed at *m*/*z* 1455, 1293, and 1103, indicating the successive loss of two pentosyl, one hexosyl, and one DMC units. The molecular mass was 294 mass units higher than that of **1**/**2**, which indicated the presence of two additional hexosyl and pentosyl units. The ^{1}H-NMR spectrum of **3**/**4**[1]) showed seven anomeric H-atoms at δ(H) 6.43 (br. *s*), 6.07 (*d*, $J = 7.9$ Hz), 5.10 (*d*, $J = 6.9$ Hz), 5.06 (*d*, $J = 7.2$ Hz), 5.05 (*d*, $J = 7.2$ Hz), 4.91 (*d*, $J = 7.6$ Hz), and 4.83 (*d*, $J = 7.3$ Hz), which were correlated in the HSQC spectrum with δ(C) 101.6, 94.4, 105.4, 104.6, 104.5, 104.0, and 106.9, respectively, indicating the presence of seven sugar units. Evaluation of chemical shifts and coupling constants from the extensive 2D-NMR analysis allowed the identification of one Rha, one Fuc, two Glc (Glc I and Glc II), two Xyl (Xyl I and Xyl II), and one Ara moiety. Comparison of the 2D-NMR spectra of **3**/**4** with those of **1**/**2** revealed that most of the signals of **3**/**4** were superimposable with those of **1**/**2**, except for those of Fuc and Xyl I. In the HMBC spectrum, the long-range correlations (^3J) δ(H) 5.10 (Glc II H–C(1))/δ(C) 83.5 (Fuc C(3)), δ(H) 5.05 (Xyl II H–C(1))/δ(C) 85.0 (Glc II C(3)), δ(H) 4.83 (Xyl I H–C(1))/δ(C) 85.0 (Rha C(4)), δ(H) 6.43 (Rha H–C(1))/δ(C) 71.3 (Fuc C(2)), and δ(H) 4.91 (Ara H–C(1))/δ(C) 77.0 (Xyl I C(4)) established the sugar chain to be 28-{*O*-α-L-arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)]-β-D-fucopyranosyl}, with an additional substitution at C(4) of Fuc by DMC. This was confirmed by the NOESY cross-peaks δ(H) 4.91 (Ara H–C(1))/δ(H) 4.08 (Xyl I H–C(4)), δ(H) 4.83 (Xyl I H–C(1))/δ(H) 4.17 (Rha H–C(4)), δ(H) 6.43 (Rha H–C(1))/δ(H) 4.79 (Fuc H–C(2)), δ(H) 5.10 (Glc II H–C(1))/δ(H) 4.58 (Fuc H–C(3)), and δ(H) 5.05 (Xyl II H–C(1))/δ(H) 4.17 (Glc II H–C(3)). Thus, the structures of libericosides B_1/B_2 (**3**/**4**) were established as 3-*O*-β-D-glucopyranosylpresenegenin 28-{*O*-α-L-arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)]-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]-β-D-fucopyranosyl} ester (**3**) and its (*Z*)-isomer **4**. The substitution of Fuc at C(3) by a disaccharide chain is rare in the Polygalaceae family [4].

The positive-ion-mode HR-ESI-MS of libericoside C_2 (**5**) showed the quasimolecular-ion peak at *m/z* 1521.6510 ($[M+Na]^+$), leading to the molecular formula $C_{72}H_{106}O_{33}$. Its FAB-MS displayed the quasimolecular-ion peak at *m/z* 1497 ($[M-H]^-$). The fragment-ion peak at *m/z* 1293 ($[M-H-204]^-$) in the FAB-MS revealed the loss of an acetylated hexosyl unit. All the ^{1}H- and ^{13}C-NMR signals assigned from extensive 2D-NMR analysis of **5** were in good agreement with those of securioside A (**6**) [8], except for those of the olefinic C- and H-atoms of (*Z*)-DMC (*Tables 2* and *3*). On the basis of the above evidence, the structure of libericoside C_2 (**5**) was established as 3-*O*-*β*-D-glucopyranosylpresenegenin 28-{*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→3)]-4-*O*-[(*Z*)-3,4-dimethoxycinnamoyl]-*β*-D-fucopyranosyl} ester (**5**). Its (*E*)-isomer **6** was isolated a few years ago by *Kuroda et al.* from *Securidaca inappendiculata* (Polygalacae) [6].

Compound **7** was obtained as an amorphous powder. The molecular formula $C_{22}H_{30}O_{14}$ was deduced from the HR-ESI-MS which gave a quasimolecular-ion peak at *m/z* 541.1539 ($[M+Na]^+$). Its FAB-MS displayed the quasimolecular-ion peak at *m/z* 517 ($[M-H]^-$). The 2D-NMR data of **7** showed the presence of a (*Z*)-feruloyl group and sucrose (=*β*-D-fructofuranosyl *α*-D-glucopyranoside) (Fru, *α*-Glc) signals (*Table 3*). The position of the (*Z*)-feruloyl group was determined by HMBC and NOESY experiments. The ^3J correlation in the HMBC spectrum was observed between an olefinic H–C(*γ*) at *δ*(H) 6.89 (*d*, *J* = 12.9 Hz) and an ester C=O group at *δ*(C) 167.8, and between *δ*(H) 5.45 (*d*, *J* = 7.9 Hz, Fru H–C(3)) and the C=O signal at *δ*(C) 167.8, ascertaining the position of the (*Z*)-feruloyl residue at C(3) of Fru. These data led us to assign the structure of atroximoside (**7**) as 3-*O*-[(*Z*)-feruloyl]-*β*-D-fructofuranosyl *α*-D-glucopyranoside.

Since triterpene saponins of the Polygalaceae have been reported to present cytotoxicity [4], we evaluated some of the compounds **1**–**6** and **8**/**9** against two human-colorectal-cancer cell lines (HCT 116 and HT-29), by using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [16], with docetaxel and paclitaxel as positive controls. In this assay, compounds **3**/**4** and **8**/**9** showed a moderate cytotoxicity (IC_{50} = 11.5 μM (HCT 116) and 12.7 μM (HT-29) for **3**/**4**, and IC_{50} = 17.0 μM (HCT 116) and 17.3 μM (HT-29) for **8**/**9**). Compounds **1**/**2** with five sugar units showed a lower cytotoxicity with IC_{50} = 22.8 (HCT 116) and 51.3 μM (HT-29). Compounds **5**/**6** were inactive against HCT 116 ($IC_{50} > 100$ μM) but showed weak cytotoxicity when tested against HT-29 cells (IC_{50} = 40.4 μM). The IC_{50} values were three orders of magnitude higher than the positive controls paclitaxel (IC_{50} = 2.40 and 4.27 nM against HCT 116 and HT-29, resp.), and docetaxel (IC_{50} = 0.43 and 0.72 nM against HCT 116 and HT-29, resp.). These results corroborated those previously obtained on acylated presenegenin glycosides from *Muraltia heisteria* [11].

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Experimental Part

General. Column Chromatography (CC), medium-pressure liquid chromatography (MPLC), and vacuum liquid chromatography (VLC): silica gel *60* (SiO_2; *Merck*, 15–40 μm), *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: SiO_2 plates *60* F_{254} (*Merck*); solvent systems $CHCl_3$/MeOH/H_2O 80:20:2,

70:30:5, and 60:32:7; detection by vanillin reagent (2% mixture of 1% vanillin in EtOH and conc. H_2SO_4 soln.). GC: *Thermoquest* gas chromatograph, with a *DB-1701* cap. column (30 m × 0.25 mm (i.d.); *J & W Scientific*); detection by FID; detector temp. 250°, injection temp. 230°; initial column temp. 80° for 5 min, then at the rate of 15°/min up to 270°; carrier gas He. Optical rotation: *AA-OR* automatic polarimeter. 1D- and 2D-NMR Spectra: *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; δ in ppm, J in Hz; C_5D_5N (δ(C)) 150.3, 135.9, and 123.9) or CD_3OD solns. (δ(C)) 49.0). FAB-MS (neg., glycerol matrix): *Jeol-SX-102* mass spectrometer; in *m/z*. HR-ESI-MS (neg.): *Q-TOF-1-Micromass* spectrometer; in *m/z*.

Plant Material. The roots of *A. liberica* STAPF were collected in July 2009 in the village Bangoua near the city Bangangté located in the Ndé division of the Western Highlands of Cameroon, and identified by *P. Nana*, botanist at the National Herbarium of Cameroon (NHC), Yaoundé, where a voucher specimen (7176/SRF/Cam) was deposited.

Extraction and Isolation. Dried and finely powdered roots of *A. liberica* (90 g) were extracted 3 × under reflux by MeOH/H_2O 7:3 (3 × 1 l) for 3 h. After evaporation of the solvent, a dark residue (12 g) was obtained. A portion (4 g) of this extract was submitted to VLC (*RP-18* SiO_2 (75–200 μm), MeOH/H_2O gradient (each eluent 250 ml): *Fractions 1–10*. *Frs. 7–10* (MeOH; 750 mg) were fractionated by successive MPLC (SiO_2 60 (15–40 μm), $CHCl_3$/MeOH/H_2O 80:20:2, 70:30:5, and 60:32:7), then *RP-18* SiO_2, gradient MeOH/H_2O 40:60 → 100:0): **1/2** (24 mg), **3/4** (15 mg), **5/6** (10 mg), and 28-{*O*-β-D-xylopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-4-*O*-[(*E*)-3,4-dimethoxycinnamoyl]}-β-D-fucopyranosyl}ester **8** and its (*Z*)-isomer **9** (11 mg). Separation of *Frs. 2* and *3* (MeOH/H_2O 1:1; 1250 mg) under the same conditions furnished **7** (10 mg), 6-*O*-feruloyl-3′-*O*-sinapoylsucrose (10 mg), 3′-*O*-feruloyl-6-*O*-sinapoylsucrose (10 mg), 3′,6-di-*O*-sinapoylsucrose (8 mg), sibiricose A5 (15 mg), and 3′,6-di-*O*-feruloylsucrose (15 mg).

*(2β,3β,4α)-3-(β-D-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-α-L-Arabinopyranosyl-(1 → 3)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-6-deoxy-4-*O*-[(2E)-3-(3,4-dimethoxyphenyl)-1-oxoprop-2-en-1-yl]-β-D-galactopyranosyl} Ester* (**1**) *and Its (Z)-Isomer* **2** (= *Libericosides A_1/A_2*; **1/2**): White amorphous powder. $[\alpha]_D^{20} = -11.7$ ($c = 0.10$, MeOH). ^{1}H- and ^{13}C-NMR (C_5D_5N): *Tables 1–3*. HR-ESI-MS (pos.): 1449.6308 ($[M+Na]^+$, $C_{69}H_{102}NaO_{31}^+$; calc. 1449.6303). FAB-MS (neg.): 1425 ($[M-H]^-$), 1293 ($[M-H-132]^-$), 1103 ($[M-H-132-162]^-$).

*(2β,3β,4α)-3-(β-D-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-α-L-Arabinopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-*O*-[*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)]-6-deoxy-4-*O*-[(2E)-3-(3,4-dimethoxyphenyl)-1-oxoprop-2-en-1-yl]-β-D-galactopyranosyl} Ester* (***3***) *and Its (Z)-Isomer* **4** (= *Libericosides B_1/B_2*; **3/4**): White amorphous powder. $[\alpha]_D^{20} = -5.3$ ($c = 0.10$, MeOH). ^{1}H- and ^{13}C-NMR (C_5D_5N): *Tables 1–3*. HR-ESI-MS (pos.): 1743.7250 ($[M+Na]^+$, $C_{80}H_{120}NaO_{40}^+$; calc. 1743.7254). FAB-MS (neg.): 1719 ($[M-H]^-$), 1455 ($[M-H-2\times132]^-$), 1293 ($[M-H-2\times132-162]^-$), 1103 ($[M-H-2\times132-162-190]^-$).

*(2β,3β,4α)-3-(β-D-Glucopyranosyloxy)-2,27-dihydroxyolean-12-ene-23,28-dioic Acid 28-{*O*-β-D-Xylopyranosyl-(1 → 4)-*O*-6-deoxy-α-L-mannopyranosyl-(1 → 2)-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)]-6-deoxy-4-*O*-[(2Z)-3-(3,4-dimethoxyphenyl)-1-oxoprop-2-en-1-yl]-β-D-galactopyranosyl} Ester* (= *Libericoside C_2*; **5**): White amorphous powder. $[\alpha]_D^{20} = -1.8$ ($c = 0.10$, MeOH). ^{1}H- and ^{13}C-NMR (C_5D_5N): *Tables 1–3*. HR-ESI-MS (pos.): 1521.6510 ($[M+Na]^+$, $C_{72}H_{106}NaO_{33}^+$; calc. 1521.6514). FAB-MS (neg.): 1497 ($[M-H]^-$), 1293 ($[M-H-162-42]^-$).

*3-*O*-[(3Z)-3-(4-Hydroxy-3-methoxyphenyl)-1-oxoprop-2-en-1-yl]-β-D-fructofuranosyl α-D-Glucopyranoside* (= *Atroximoside*; **7**): White amorphous powder. $[\alpha]_D^{20} = -23.4$ ($c = 0.10$, MeOH). UV (MeOH): 203 (4.38), 232 (4.27), 321 (4.53). ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 4*. HR-ESI-MS (pos.): 541.1539 ($[M+Na]^+$, $C_{22}H_{30}NaO_{14}^+$; calc. 541.1533). FAB-MS (neg.): 517 ($[M-H]^-$), 341 ($[M-H-176]^-$).

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 to dryness with MeOH until neutral, and then analyzed by TLC (SiO_2, $CHCl_3$/MeOH/H_2O 8:5:1) by

comparison with authentic samples: glucose (R_f 0.23), arabinose (R_f 0.45), xylose (R_f 0.47), rhamnose (R_f 0.51), and fucose (R_f 0.54). Furthermore, the residue of sugars was dissolved in anh. pyridine (100 µl), and L-cysteine methyl ester hydrochloride (0.06M) 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 and H_2O (0.1 ml each), and the hexane layer (1 µl) was analyzed by GC [17]. D-Glucose, D-xylose, L-rhamnose, D-fucose, and L-arabinose were detected for **1/2** and **3/4** by co-injection of the hydrolyzate with standard silylated samples giving peaks at t_R 18.60, 13.47, 13.15, 12.15, and 11.89 min, resp. D-Glucose, D-xylose, D-fucose, and L-rhamnose were detected for **5**.

MTT Cytotoxicity Assay. The bioassay was carried out according to the method described by *Carmichael et al.* [16] with two human colorectal cancer cell lines (HCT 116 and HT-29) provided by the *Oncodesign Society*, Dijon, France. The antiproliferative effect of the test compound was monitored with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT). HCT 116 and HT-29 cells were seeded at an initial density of 5000 or 10000 cells/well in 96-well plates and treated with medium containing various concentrations of the test compound. DMSO Controls (0.1%) did not affect cell proliferation. After 96 h, MTT soln. (20 µl; 5 mg/ml in PBS) was added to the culture medium, and the mixture was incubated at 37° in a 5% CO_2 atmosphere for 4 h. The MTT soln. was aspired, and DMSO (200 µl) was added. The optical density (*OD*) was measured spectrophotometrically at 570 nm. The results are expressed as concentration of compound producing 50% toxicity (IC_{50} value). The experiments were repeated twice. Paclitaxel and docetaxel (*Sigma*; purity 99.5%) were used as pos. controls, and exhibited IC_{50} values of 2.40 and 0.43 nM against HCT 116, 4.27 and 0.72 nM against HT-29, resp.

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