Three New Triterpene Saponins from Two Species of *Carpolobia*

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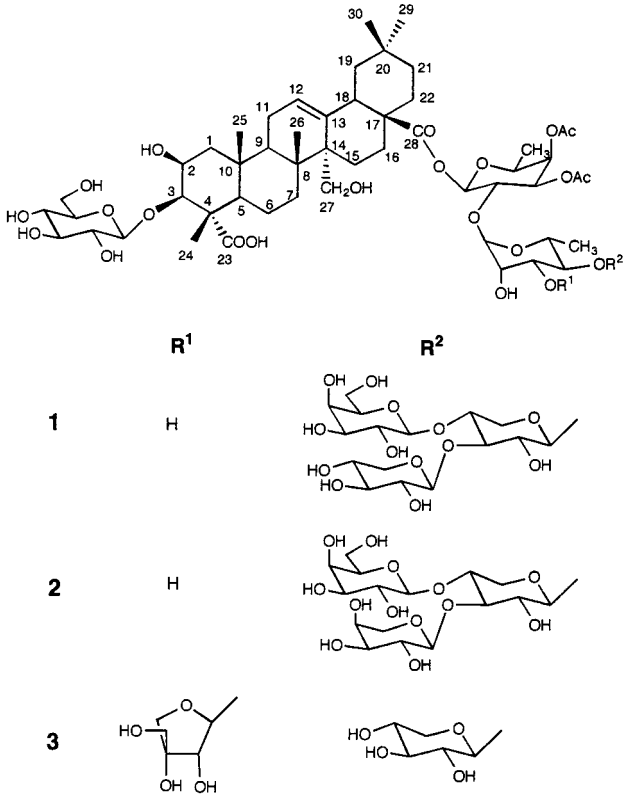
Three new acetylated triterpene saponins **1**−**3** were isolated from the roots of *Carpolobia alba* and *C. lutea*. Their structures were established mainly by 2D NMR techniques as 3-*O*-*β*-D-glucopyranosyl-presenegenin-28-*O*-*β*-D-galactopyranosyl-(1→4)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-*β*-D-fucopyranosyl ester, 3-*O*-*β*-D-glucopyranosylpresenegenin-28-*O*-*β*-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-*β*-D-fucopyranosyl ester, and 3-*O*-*β*-D-glucopyranosylpresenegenin-28-*O*-*β*-D-xylopyranosyl-(1→4)-[*β*-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-*β*-D-fucopyranosyl ester, respectively.

Carpolobia alba G. Don. and *C. lutea* G. Don. (Polygalaceae) are small trees distributed in West and Central areas of Tropical Africa. The roots of *C. alba* are used in traditional medicine as an aphrodisiac and vermifuge, and when mixed with other plants, they are utilized against miscarriage and poisoning and to preserve one from spirits and spells.[1] The roots of *C. lutea* are also used as vermifuge, and the decoction is employed to wash feverish and mental patients, to facilitate childbirth, and to treat sterility. The powdered roots are sternutatory and used to treat headaches and sleepiness.[1] The previous phytochemical studies on these *Carpolobia* species deal with one saponin possessing presenegenin (2*β*,3*β*,27α-trihydroxy-12-oleanene-23,28-dioic acid) as the aglycon. Six sugars were identified by acid hydrolysis as glucose, galactose, arabinose, xylose, fucose, and rhamnose, but no spectroscopic analysis was performed.[2,3] We describe in this paper the isolation and structural elucidation of three new triterpene saponins, **1** and **2** from *C. alba* and **1**−**3** from *C. lutea*. The influence of these new compounds on the potentiation of cisplatin cytotoxicity in human colon cancer cells was also investigated.

Results and Discussion

A concentrated MeOH-soluble fraction of the EtOH 80% extract of the roots of *C. alba* was purified by precipitation with diethyl ether and treatment with charcoal[4] and subjected to multiple chromatographic steps over silica gel to yield compounds **1** and **2**. The latter were also isolated from the roots of *C. lutea* according to the same method, together with compound **3**. Their structures were elucidated mainly by 600 MHz NMR analysis, including 1D and 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, TOCSY, NOESY) spectroscopy.

Compound **1**, a white amorphous powder, showed in FABMS (negative-ion mode) a quasimolecular ion peak [M − H]$^-$ at *m*/*z* 1481, indicating a molecular weight of 1482, compatible with a molecular formula of $C_{68}H_{106}O_{35}$. Other



fragment ion peaks were observed at *m*/*z* 1319 [(M − H) − 162]$^-$, 1187 [(M − H) − 162 − 132]$^-$, and 1157 [(M − H) − 162 − 162]$^-$, which revealed the elimination of one terminal hexosyl, one terminal pentosyl, and another terminal hexosyl moieties. Its APIES (negative-ion mode) showed a quasimolecular ion peak [M − H]$^-$ at *m*/*z* 1481, which confirmed the proposed molecular weight. On acid hydrolysis, an artifactual aglycon was obtained. Glucose, galactose, xylose, fucose, and rhamnose were identified by comparison on TLC with authentic samples.

The spectroscopic NMR data of the prosapogenin of **1** obtained by alkaline hydrolysis were in good agreement with those of tenuifolin (3-*O*-*β*-D-glucopyranosylpresenegenin), commonly encountered in the Polygalaceae family.[5]

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Table 1. ^{13}C NMR and ^{1}H NMR Data of the Aglycons of Compounds **1**−**3** in Pyridine-d_5 (δ ppm)[a,b]

		1		**2**		**3**	
no.	mult[a]	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H
1	CH_2	44.0	1.33, 2.20	43.9	1.31, 2.20	43.9	1.34, 2.20
2	CH	70.2	4.57	70.2	4.58	70.1	4.58
3	CH	87.2	4.43 d (2.5)	87.2	4.44 d (2.5)	87.2	4.45 d (2.5)
4	C	53.1		53.1		53.1	
5	CH	51.9	2.40	52.1	2.36	52.1	2.13
6	CH_2	21.5	1.77, nd	21.5	1.75, nd	20.8	1.82, 1.95
7	CH_2	33.6	1.00, 1.26	33.5	1.00, 1.27	33.4	0.99, 1.14
8	C	40.8		40.8		40.5	
9	CH	49.1	2.34	49.0	2.30	48.7	2.15
10	C	36.3		36.3		36.3	
11	CH_2	23.0	1.88, nd	22.8	1.86, 2.12	22.9	1.82, 2.08
12	CH	127.5	5.80 t-like	127.5	5.80 t-like	127.4	5.75 t-like
13	C	138.7		138.7		139.4	
14	C	47.9		47.9		47.6	
15	CH_2	24.3	2.05, nd	24.4	2.04, nd	24.0	nd
16	CH_2	24.1	nd	24.2	nd	24.0	nd
17	C	46.9		46.9		46.8	
18	CH	41.9	3.13 dd (13.6, 3.0)	41.9	3.12 dd (13.6, 3.0)	41.2	3.08 dd (13.6, 3.0)
19	CH_2	44.8	1.30, 1.51	44.8	1.26, 1.66	45.2	1.18, 1.58
20	C	30.3		30.3		30.3	
21	CH_2	33.6	1.83, 2.27	33.5	1.80, 2.21	34.1	1.71, 2.27
22	CH_2	32.0	1.70, 1.80	32.0	1.69, 1.79	31.9	1.60, 1.80
23	COOH	185.5		186.0		185.5	
24	CH_3	14.3	1.84 s	14.3	1.84 s	14.5	1.86 s
25	CH_3	17.1	1.43 s	17.1	1.43 s	16.9	1.40 s
26	CH_3	18.6	1.01 s	18.5	1.00 s	18.6	0.98 s
27	CH_2	63.9	3.79, 4.16	64.1	3.78, 4.12	64.2	3.86, 4.08
28	COO	176.1		176.1		176.1	
29	CH_3	32.7	0.76 s	32.7	0.76 s	32.6	0.75 s
30	CH_3	23.3	0.74 s	23.3	0.74 s	23.2	0.74 s

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

The ^{1}H NMR spectrum of **1** displayed signals for six anomeric protons at δ 6.03 (d, J = 8.4 Hz), 5.76 (br s), 4.94 (d, J = 7.7 Hz), 4.89 (d, J = 7.3 Hz), 4.86 (d, J = 7.7 Hz), and 4.80 (d, J = 7.7 Hz), which gave correlations, in the HSQC spectrum, with carbon signals at δ 93.8, 100.9, 104.7, 105.7, 105.8, and 102.6, respectively. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the COSY, TOCSY, HSQC, and HMBC experiments (Table 3). Evaluation of spin−spin couplings and chemical shifts allowed the identification of one β-fucopyranose (Fuc), one α-rhamnopyranose (Rha), one β-glucopyranose (Glc), two β-xylopyranoses (Xyl), and one β-galactopyranose (Gal) unit. The common D-configuration for Fuc, Glc, Xyl, and Gal and the L-configuration for Rha were assumed to be those of the most commonly encountered analogues in the plant kingdom. Correlations observed in the HMBC spectrum between signals at δ_H(Glc-1) 4.94 (d, J = 7.7 Hz) and δ_C(Agly-C-3) 87.2, and in the NOESY spectrum between δ_H(Glc-1) 4.94 (d, J = 7.7 Hz) and δ_H(Agly-H-3) 4.43 (d, J = 2.5 Hz), confirmed the substitution at the C-3 position of the presenegenin by a 3-*O*-β-D-glucopyranose. After subtraction of the anomeric signals of the glucosyl moiety, the signals of five sugars linked to the aglycon by an ester linkage remained and will be assigned. In the HMBC spectrum, a correlation between signals at δ_H(Fuc-1) 6.03 (d, J = 8.4 Hz) and δ_C(Agly-C-28) 176.1 proved a glycosidic ester linkage to the C-28 of the aglycon. A correlation between signals at δ_C(Rha-1) 100.9 and δ_H(Fuc-2) 4.68 (t, J = 9.0 Hz), and a reverse correlation between δ_C(Fuc-2) 69.5 and δ_H(Rha-1) 5.76 (br s), revealed the (1→2) linkage between these two sugars. The signals at δ_H(Fuc-3) and δ_H(Fuc-4) were shifted downfield at δ_H 5.59 (dd, J = 9.5, 3.0 Hz) and δ_H 5.53, respectively, and the presence of two acetyl methyl signals at δ_H 2.00 (s) and δ_H 2.02 (s) suggested the Fuc-3 and Fuc-4 substitution by two acetyl groups. At this stage, according to the observation in the HMBC spectrum of a correlation between signals at δ_H(Rha-4) 4.17 and δ_C(Xyl-1) 105.8 and a reverse correlation between δ_H(Xyl-1) 4.86 (d, J = 7.7 Hz) and δ_C(Rha-4) 85.0, we concluded that saponin **1** presented the sequence 3-*O*-β-D-glucopyranosylpresenegenin-28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-β-D-fucopyranosyl ester, which has been already encountered in *Polygala fallax* (Polygalaceae).[6] The difference from the already described saponins was located in the other part of the oligosaccharide moiety. The assignments of the ^{1}H and ^{13}C NMR signals of a xylose attached to the Rha by a (1→4) linkage were deduced from the TOCSY, HSQC, HMBC, and NOESY spectra. Cross-peaks in the HMBC spectrum between the deshielded carbon at δ_C(Xyl-3) 86.4 and an anomeric signal at δ_H 4.89 (d, J = 7.3 Hz) and between a signal at δ_C(Xyl-4) 70.2 and another anomeric signal at δ_H 4.80 (d, J = 7.7 Hz) suggested an unusual 3, 4 substitution of this xylose by one terminal xylose (T-Xyl) and one terminal galactose (T-Gal), respectively. These substitutions are confirmed by a reverse correlation between δ_C(T-Xyl-1) 105.7 and δ_H(Xyl-3) 3.84 and in the NOESY spectrum by a correlation between δ_H(Xyl-4) 4.32 and δ_H(T-Gal-1) 4.80 (d, J = 7.7 Hz). The ^{1}H and ^{13}C NMR signals of T-Xyl and T-Gal were comparable to those previously described in terminal position.[6] The structure of **1** was thus established as 3-*O*-β-D-glucopyranosylpresenegenin-28-*O*-β-D-galactopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→3)]-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-β-D-fucopyranosyl ester, a new natural compound.

Compound **2** was obtained as a white, amorphous powder. The FABMS (negative-ion mode) and APIES (negative-ion mode) spectra showed the same quasimo-

Table 2. ^{13}C NMR Data of the Sugar Moieties of Compounds **1**−**3** in Pyridine-d_5 (δ ppm)[a,b]

position	**1**		**2**		**3**
3-*O*-sugar					
Glc 1	*104.7*	Glc1	*104.6*	Glc1	*104.1*
2	74.9	2	74.8	2	74.7
3	77.1	3	77.0	3	76.9
4	70.9	4	70.9	4	70.8
5	77.5	5	77.5	5	77.4
6	61.9	6	61.9	6	61.8
28-*O*-sugars					
Fuc 1	*93.8*	Fuc 1	*93.8*	Fuc1	*94.1*
2	*69.5*	2	*70.0*	2	*70.1*
3	*75.7*	3	*75.7*	3	*75.3*
4	*71.2*	4	*71.2*	4	*71.2*
5	69.9	5	70.0	5	69.9
6	15.7	6	15.7	6	15.6
Ac at C-3	20.4	Ac at C-3	20.4	Ac at C-3	20.4
	170.1		170.1		170.1
Ac at C-4	20.1	Ac at C-4	20.1	Ac at C-4	20.1
	170.9		170.9		170.9
Rha 1	*100.9*	Rha 1	*101.0*	Rha 1	*101.2*
2	70.9	2	70.9	2	71.2
3	71.9	3	71.9	3	*81.2*
4	*85.0*	4	*85.1*	4	*77.4*
5	67.8	5	67.8	5	67.8
6	18.0	6	18.0	6	18.1
Xyl 1	*105.8*	Xyl 1	*106.1*	T-Api 1	*110.9*
2	75.4	2	75.5	2	78.2
3	*86.4*	3	*85.7*	3	79.1
4	*70.2*	4	*70.6*	4	74.0
5	65.9	5	65.8	5	64.6
T-Xyl 1	*105.7*	T-Ara 1	*105.0*	T-Xyl 1	*104.2*
2	75.0	2	72.3	2	74.9
3	77.2	3	73.9	3	77.4
4	69.1	4	68.3	4	70.4
5	66.8	5	66.6	5	66.4
T-Gal 1	*102.6*	T-Gal 1	*102.6*		
2	69.5	2	69.6		
3	74.0	3	73.9		
4	69.3	4	69.3		
5	77.0	5	76.8		
6	61.7	6	61.7		

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

lecular ion peak $[M - H]^-$ at *m/z* 1481 as **1**, which suggested an identical molecular formula of $C_{68}H_{106}O_{35}$. The other fragment ion peaks at *m/z* 1319 $[(M - H) - 162]^-$, 1187 $[(M - H) - 162 - 132]^-$, and 1157 $[(M - H) - 162 - 162]^-$ were equally observed. Acid hydrolysis of **2** afforded the same artifactual aglycon as for **1** with glucose, galactose, xylose, fucose, rhamnose, and arabinose as sugars. Tenuifolin was also characterized as the prosapogenin by alkaline hydrolysis. The signals of **2**, assigned from the 1D and 2D NMR spectra, were almost superimposable on those of **1**, excepted for one sugar. Signals of a terminal α-L-arabinopyranose (T-Ara) instead of a T-Xyl were found (Tables 2 and 3) and compared with those described in the literature.[7] Signals of a trisubstituted xylose (1,3,4) were once again determined. An examination of the HMBC spectrum confirmed the (1→4) linkage between Xyl-1 and Rha-4 by a correlation between signals at δ_H(Xyl-1) 4.82 (d, $J = 7.0$ Hz) and δ_C(Rha-4) 85.1 and a reverse correlation between δ_H(Rha-4) 4.16 and δ_C(Xyl-1) 106.1. A cross-peak between signals at δ_H(T-Gal-1) 4.78 (d, $J = 7.7$ Hz) and δ_C(Xyl-4) 70.6 suggested a (1→4) linkage between T-Gal-1 and Xyl-4, confirmed by a correlation in the NOESY spectrum between δ_H(T-Gal-1) 4.78 (d, $J = 7.7$ Hz) and δ_H(Xyl-4) 4.30. The substitution of the Xyl-3 position at δ_C 85.7 by the previously identified T-Ara is revealed by the observation in the HMBC spectrum of a cross-peak between δ_H(Xyl-3) 3.87 and δ_C(T-Ara-1) 105.0. On the basis of the above results, the structure of **2** was established as 3-*O*-β-D-glucopyranosylpresenegenin-28-*O*-β-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-β-D-fucopyranosyl ester, a new natural saponin.

Compound **3**, a white amorphous powder, showed in FABMS (negative-ion mode) a quasimolecular ion peak $[M - H]^-$ at *m/z* 1319, indicating a molecular weight of 1320, compatible with a molecular formula of $C_{62}H_{96}O_{30}$. Other fragment ion peaks at *m/z* 1157 $[(M - H) - 162]^-$ and 1025 $[(M - H) - 162 - 132]^-$ suggested the elimination of one terminal hexosyl and one terminal pentosyl moieties. On acid hydrolysis, an artifactual aglycon was obtained. Glucose, apiose, xylose, fucose, and rhamnose were also identified by comparison on TLC with authentic samples. Like for **1** and **2**, tenuifolin was characterized after an alkaline hydrolysis. The examination of the 1D and 2D NMR spectra showed the presence of the sequence 3-*O*-β-D-glucopyranosylpresenegenin-28-*O*-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-β-D-fucopyranosyl ester, already encountered in **1** and **2**. The signals at δ_C(Rha-3) 81.2 and δ_C(Rha-4) 77.4 suggested a 1,3,4-trisubstituted Rha. In the HSQC spectrum, signals of two sugars possessing anomers at δ_H 5.19 (d, $J = 7.7$ Hz) and δ_H 5.90 (d, $J = 3.7$ Hz) which correlated with signals at δ_C 104.2 and δ_C 110.9, respectively, remained to be determined. Assignments of the ^{1}H and ^{13}C NMR signals of **3** from the TOCSY, HSQC, HMBC, and NOESY spectra showed that the latter were one terminal xylose (T-Xyl) and one terminal apiose (T-Api). The cross-peaks between signals at δ_H 5.19 (d, $J = 7.7$ Hz) and δ_C(Rha-4) 77.4 observed in the HMBC spectrum and signals at δ_H 5.19 (d, $J = 7.7$ Hz) and δ_H(Rha-4) 4.36 in the NOESY spectrum revealed the (1→4) linkage between T-Xyl and Rha. From the NOESY spectrum, the correlation between δ_H(Api-1) 5.90 (d, $J = 3.7$ Hz) and δ_H(Rha-3) 4.36 confirmed the (1→3) linkage between T-Api and Rha. After comparison of the ^{1}H and ^{13}C NMR signals of **3** and similarly molecules isolated from *Polygala fallax* and *P. reinii*,[6,8] we concluded that saponin **3** was 3-*O*-β-D-glucopyranosylpresenegenin-28-*O*-β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-(3,4-di-*O*-acetyl)-β-D-fucopyranosyl ester, a new natural compound.

Saponins possess the ability to interact with plasma cell membranes, inducing rearrangement of the phospholipid bilayer and the pore formation.[9] It was recently reported that esterification of the saponins might be a structural condition for the assumed pore-forming capacity.[10] The potentiating effect of saponins esterified by *p*-methoxycinnamoyl groups on the cytotoxicity of the antineoplasic drug cisplatin in HT 29 colon cancer cells[11] corroborated the influence of the acylation of the saponins on the membrane permeability. As the three molecules isolated from the two *Carpolobia* species were acetylated, we have tested the influence of **1**, **2**, and **3** on the potentiation of the cisplatin cytotoxicity in the human cancer colon HT-29 cell line.[12] However, no significant effect was found in this bioassay underlying the importance of the nature of the acyl group in the molecules.

Experimental Section

General Experimental Procedures. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. IR spectra (KBr disk) were recorded on a Perkin-Elmer 281 spectrophotometer. The 1D and 2D NMR spectra ($^{1}H-^{1}H$ COSY, TOCSY, HSQC, and HMBC) were performed using a UNITY-600 spectrometer at an operating frequency of 600 MHz on a

Table 3. ^{1}H NMR Data of the Sugar Moieties of Compounds **1**−**3** in Pyridine-d_5 (δ ppm, J in Hz)[a,b]

position	**1**		**2**		**3**
3-*O*-sugar					
Glc 1	*4.94 d (7.7)*	Glc1	*4.95 d (7.0)*	Glc1	*5.01 d (7.3)*
2	3.87	2	3.88	2	3.87
3	4.17	3	4.19	3	4.18
4	3.99	4	3.98	4	3.98
5	3.82	5	3.83	5	3.85
6	4.13, 4.30	6	4.12, 4.28	6	4.11, 4.30
28-*O*-sugars					
Fuc 1	*6.03 d (8.4)*	Fuc 1	*6.03 d (8.4)*	Fuc1	*5.96 d (8.1)*
2	*4.68 t (9.0)*	2	*4.66 t (9.0)*	2	*4.63 t (9.0)*
3	*5.59 dd (9.5, 3.0)*	3	*5.59 dd (9.5, 2.9)*	3	*5.57 dd (9.5, 3.0)*
4	*5.53*	4	*5.53*	4	*5.51*
5	4.18	5	4.18	5	4.18
6	1.12 d (6.2)	6	1.13 d (6.2)	6	1.12 d (6.2)
Ac at C-3	2.02 s	Ac at C-3	2.02 s	Ac at C-3	2.02 s
Ac at C-4	2.00 s	Ac at C-4	2.01 s	Ac at C-4	2.00 s
Rha 1	*5.76 br* s	Rha 1	*5.75 br s*	Rha 1	*5.74 br s*
2	4.46	2	4.45	2	4.60
3	4.41	3	4.38	3	*4.36*
4	*4.17*	4	*4.16*	4	*4.36*
5	4.37	5	4.36	5	4.36
6	1.66 d (5.9)	6	1.66 d (6.2)	6	1.63 d (5.5)
Xyl 1	*4.86 d (7.7)*	Xyl 1	*4.82 d (7.0)*	T-Api 1	*5.90 d (3.7)*
2	3.93	2	3.90	2	4.65
3	*3.84*	3	*3.87*	3	
4	*4.32*	4	*4.30*	4	4.14, 4.35
5	3.42 br t (9.5), 4.38	5	3.42 br t (10.4), 4.34	5	4.03
T-Xyl 1	*4.89 d (7.3)*	T-Ara 1	*4.94 d (6.6)*	T-Xyl 1	*5.19 d (7.7)*
2	3.98	2	4.51	2	3.83
3	4.00	3	3.95	3	4.08
4	4.01	4	4.20	4	4.01
5	3.42 br t (9.5), 4.17	5	3.53 br d (11.7), 4.24	5	3.44 br t (10.3), 4.08
T-Gal 1	*4.80 d (7.7)*	T-Gal 1	*4.78 d (7.7)*		
2	4.44	2	4.43		
3	3.98	3	3.99		
4	4.35	4	4.33		
5	3.88	5	3.87		
6	4.17, 4.20	6	4.17, 4.23		

[a] 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, 600 MHz for ^{1}H NMR). Overlapped signals are reported without designated multiplicity.

Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz for ^{1}H and 150 MHz for ^{13}C 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 carbon type (CH_3, 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_C 149.3, 135.8, 123.5). Fast-atom bombardment (FAB) mass spectra (negative-ion mode, glycerol matrix) were obtained on a JEOL SX 102 mass spectrometer and atmospheric pressure ionization electrospray (APIES) mass spectra (negative-ion mode) on a Micromass Quattro LS instrument. TLC and HPTLC employed precoated $60F_{254}$ (Merck) silica gel plates. The following TLC solvent systems were used: for saponins (a) $CHCl_3$−MeOH−AcOH−H_2O (15:8:3:2); for sapogenins (b) CH_2Cl_2−MeOH (19:1); for monosaccharides (c) $CHCl_3$−MeOH−H_2O (8:5:1). The spray reagent for saponins and sapogenins was Komarowsky reagent, a mixture (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and ethanolic H_2SO_4 (50%), and for the sugars, aqueous H_2SO_4 (50%). Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Gilson pump M 303, head pump 25 SC, manometric module M 802, Rheodyne 7125 injector, Büchi column (460 × 25 mm and 460 × 15 mm), Büchi precolumn (110 × 15 mm)].

Plant Material. In June 1972, *C. alba* was collected in Congo, in the Eala Forest, and *C. lutea* in Ife, Nigeria. Two voucher specimens (C. Evrard 3608 and 6948, respectively) were deposited in the Herbarium of the National Botanical Garden of Brussels, Belgium.

Extraction and Isolation. A crude saponin mixture (9 g for *C. alba* and 8 g for *C. lutea*) was obtained according to a previously described method.[4] For *C. alba*, 500 mg of the saponin mixture was fractionated by column chromatography on silica gel 60 (15−40 μm) using as eluent $CHCl_3$−MeOH−H_2O (8:5:1) to give compounds **1** (6.4 mg) and **2** (1.8 mg). The yield of the MPLC (2.9%) is explained by the very low solubility of the crude saponin extract in the current solvents. For *C. lutea*, applying the same protocol, from 300 mg of the crude saponins, compounds **1** (34.0 mg), **2** (13.7 mg), and **3** (8 mg) were obtained.

Compound 1: white amorphous powder; $[\alpha]^{25}_D$ +8.8° (*c* 0.057, MeOH); IR (KBr) ν_{max} 3500−3400 (OH), 2926 (CH), 1750 (CO ester), 1707 (CO carboxylic acid), 1630, 1440, 1090 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1, 2, and 3; negative FABMS *m*/*z* 1481 [M − H]$^-$, 1319 [(M − H) − 162]$^-$, 1187 [(M − H) − 162 − 132]$^-$, 1157 [(M − H) − 162 − 162]$^-$, negative APIES *m*/*z* 1481 [M − H]$^-$; TLC R_f 0.46 (system a); pink-violet spot developed on spraying with Komarowsky reagent.

Compound 2: white amorphous powder; $[\alpha]^{25}_D$ −8.3° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500−3400 (OH), 2926 (CH), 1750 (CO ester), 1707 (CO carboxylic acid), 1630, 1440, 1090 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1, 2, and 3; negative FABMS *m*/*z* 1481 [M − H]$^-$, 1319 [(M − H) − 162]$^-$, 1187 [(M − H) − 162 − 132]$^-$, 1157 [(M − H) − 162−162]$^-$, negative APIES *m*/*z* 1481 [M − H]$^-$; TLC R_f 0.40 (system a); pink-violet spot developed on spraying with Komarowsky reagent.

Compound 3: white amorphous powder; $[\alpha]^{25}_D$ −5.6° (*c* 0.063, MeOH); IR (KBr) ν_{max} 3500−3400 (OH), 2930 (CH), 1750 (CO ester), 1707 (CO carboxylic acid), 1630, 1440, 1090 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1, 2, and 3; negative FABMS *m*/*z* 1319 [M − H]$^-$, 1157 [(M − H) − 162]$^-$, 1025 [(M − H) − 162 −

132]$^-$; TLC R_f 0.63 (system a); pink-violet spot developed on spraying with Komarowsky reagent.

Acid Hydrolysis of 1. A solution of each compound **1**, **2**, and **3** (3 mg) in 2 N aqueous CF_3COOH (5 mL) was heated on a water bath for 2 h. After extraction with $CHCl_3$, the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral and then analyzed by TLC on silica gel by comparison with standard sugars (solvent system c).

Alkaline Hydrolysis. Compound **1**, **2**, and **3** (3 mg of each) were refluxed with 5% aqueous KOH (10 mL) for 2 h. The reaction mixtures were adjusted to pH 7 with dilute HCl and then extracted with H_2O-saturated *n*-BuOH (3 × 10 mL). The combined *n*-BuOH extracts were washed (H_2O) and concentrated to dryness, each of the three compounds yielding a prosapogenin which was identified by comparison with an authentic sample of tenuifolin (3-*O*-*β*-D-glucopyranosylpresenegenin).

Bioassays. The potentiation of the in vitro cisplatin cytotoxicity in human colon cancer cell lines was evaluated according to the method of Assem et al.[12]

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