Triterpenoid Saponin Anthranilates from *Albizia grandibracteata* Leaves Ingested by Primates in Uganda

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Received December 31, 2004

Three new oleanane-type triterpene saponins (**1**−**3**), named grandibracteosides A−C, were isolated from the methanolic extract of leaves of *Albizia grandibracteata,* a species consumed by primates in the Kibale National Park, Uganda. The structures of the saponins were established using 1D and 2D NMR experiments and mass spectrometry and confirmed by acid and alkaline hydrolysis. The crude extract and the pure compounds showed significant inhibitory activity against KB and MCF7 tumoral cell lines in vitro. The compounds are glycosides of acacic acid acylated by an *o*-aminobenzoyl unit. This is the first report of such ester saponins in dicotyledonous plants. Studies of the primate diet may provide a useful method for finding naturally occurring compounds of medicinal significance.

To understand diet selection by primates and in particular to investigate the potential for self-medication by chimpanzees (*Pan troglodytes*), we evaluated the bioactive properties of plant parts ingested by primates in the Kanyawara area of the Kibale National Park in Uganda.[1] We collected and screened 84 samples for antimicrobial activities and cytotoxicity. We previously reported that *Trichilia rubescens* leaves contained antimalarial compounds, which we isolated and identified as a result of observing the feeding behavior of chimpanzees.[1] Here we report the isolation and identification of new bioactive compounds from the leaves of *Albizia grandibracteata* Taub. (Fabaceae).

A. grandibracteata leaves contain high levels of saponins.[2] They are consumed frequently by red colobus monkeys (*Procolobus badius*), a species whose polygastric digestive system appears to allow efficient detoxification. By contrast, they are consumed so rarely and briefly by chimpanzees that they may be ingested for self-medication rather than nutrient maximization.

Various physiological effects of *A. grandibracteata* leaves have been described or suspected. *Cercopithecus* sp. monkeys dosed with aqueous or alcoholic extracts of leaves or bark developed a steady increase in spontaneous uterine activity, though the aqueous solution is without acute toxicity when injected in a quantity of 600 mg/kg body weight into *Cercopithecus* sp. over an 8 h period.[3] Likewise, abortion occurred in gravid rats, rabbits, and cats at any stage of gestation when these animals were treated with appropriate amounts of the extracts. *A. grandibracteata* is also sometimes used in traditional medicine. In the Democratic Republic of Congo the terminal bud decoction is used to treat wounds,[4] while the bark is used as treatment for parasitism and lumbago.[5,6] In Uganda, indigenous healers treat meteorism with the bark of this species.[7] We found that the methanolic extract of the leaves of *A. grandibracteata* exhibited significant in vitro anthelminthic activity against *Rhabditis* worms (the most active crude extract among the 84 plant extracts tested from Kibale Forest). It also showed cytotoxic activity against the KB cell line. Triterpenoid saponins are commonly described in this genus.[8,9] The related species, namely, *A. julibrissin*, *A. lebbek*, *A. procera,* and *A. adianthifolia*, are reported to contain various acacic acid glycosides.[10−18] Cytotoxic effects have been reported for julibrosides J1, J2, and J9 saponins isolated from *A. julibrissin*.[12,15]

We report herein the isolation and the structural elucidation of three new saponins in *A. grandibracteata* and the evaluation of their cytotoxic activity against KB and MCF7 tumor cells. Their structures were established through spectroscopic analysis as acacic acid glycoside anthranilates. Saponins esterified with *N*-methylanthranilic acid (avenacins) have been isolated only from oat root[19] and have been recognized to have antimicrobial properties,[20] in particular to confer protection against causative wheat fungus of take-all disease.[21] This is the first time that saponins esterified with anthranilic acid have been described in dicotyledonous plants.

Results and Discussion

Dried and powdered leaves of *A. grandibracteata* were macerated and percolated in petroleum ether and extracted by boiling in 90% MeOH. The aqueous MeOH extract was concentrated and precipitated in acetone. The filtered precipitate was dried over KOH in vacuo, yielding a crude saponin mixture, which was dialyzed for 3 days, and the solution contained in the tubes was freeze-dried. Purification of the saponin mixture by silica gel column chromatography followed by reversed-phase C-18 HPLC or normal-phase preparative TLC afforded compounds **1**−**3**. Their structures were mainly characterized on the basis of ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, ROESY, HSQC, and HMBC data and HRMS and ESIMS^{n} spectra.

The set of connectivities observed in the 2D-NMR spectra of the three saponins (**1**−**3**) and more particularly the presence of seven methyl groups, including three angular methyl groups, one trisubstituted ethylenic bond (H-12), and three oxymethine groups (C-3, C-16, C-21) (Table 1),

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10.1021/np049576i CCC: $30.25 © 2005 American Chemical Society and American Society of Pharmacognosy
Published on Web 06/03/2005

Chart 1

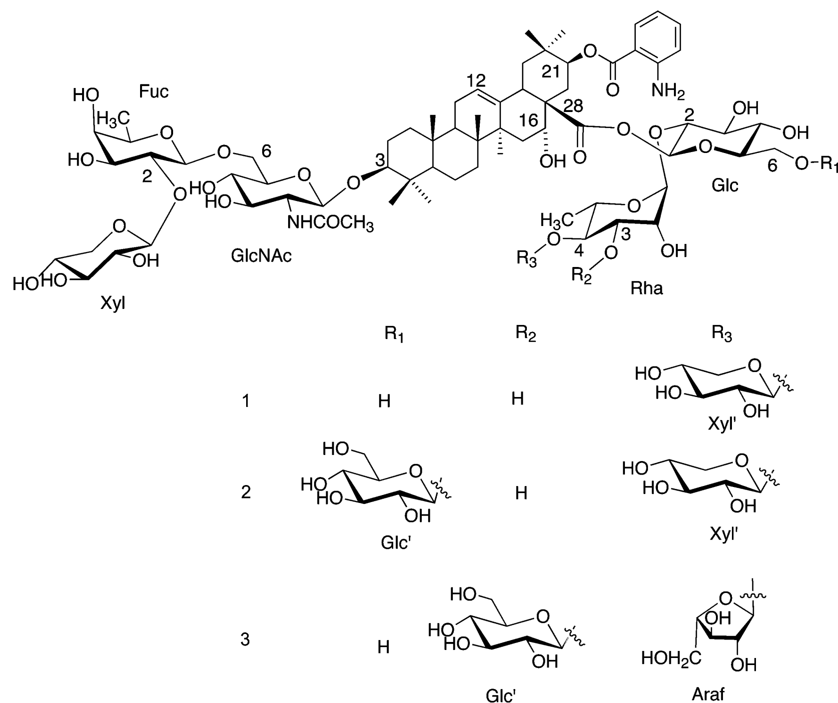

Table 1. ^{13}C and ^{1}H NMR Data of the Aglycones of **1**, **2**, and **3** in CD_3OD

	1				**2**				**3**			
position	δ_C	δ_H		J	δ_C	δ_H		J	δ_C	δ_H		J
1	38.4	1.11;1.64	m		38.2	1.11;1.64	m		38.3			
2	25.7	1.69;1.91	m		25.7	1.69; 1.90	t; m	(14.4)	nd	1.69;1.91	m	
3	88.3	3.31	m		88.4	3.31	m		88.4	3.31		
4	38.6				38.6				38.6			
5	55.6	0.83	brd	(8.0)	55.6	0.83	m		55.6	0.83	brd	(8.0)
6	18.0				nd				19.0			
7	32.9				32.9				33.5			
8	39.3				39.3				39.3			
9	46.6				46.6				46.6			
10	36.5				36.5				36.5			
11	23.1	1.96	m		23.0	1.96	m		nd	1.96	m	
12	122.6	5.39	t	(3.5)	122.6	5.39	t	(3.5)	122.6	5.39	t	(3.5)
13	142.3				142.3							
14	41.1				41.1				41.2			
15	34.7	1.54;1.64	m		34.6	1.54;1.64	brd;brd	(12.0−13.4)	34.7	1.56;1.64	brd;brd	(13.5)
16	72.7	4.54	m		72.8	4.54	m		72.8	4.54		
17	50.9				50.9				50.9			
18	40.2	3.04	dd	(4.6−14.6)	40.1	3.04	dd	(5.0−14.8)	40.2	3.04	dd	(4.2−14.1)
19	47.4	1.25;2.57	dd; t	(4−14; 13.9)	47.3	1.25;2.57	dd; t	(4−14; 13.8)	47.4	1.25;2.56	dd; t	(4.8−13.4; 14)
20	34.5				34.5				34.4			
21	76.1	5.64	dd	(11.1−5.4)	76.2	5.65	dd	(5.5−11.2)	76.2	5.65	dd	(5.4−11.1)
22	35.0	1.85;2.25	dd;dd	(11.3−13.2; 5.3−13.5)	36.5	1.82;2.23	dd;dd	(11.6−13.0; 5.4−13.3)	35.8	1.82;2.27	dd;dd	(11.5−13.6; 5.4−13.5)
23	27.1	1.01	s		27.1	1.01	s		27.1	1.01	s	
24	15.7	0.79	s		15.7	0.79	s		15.7	0.79	s	
25	14.8	0.99	s		14.8	0.99	s		14.8	0.98	s	
26	16.2	0.80	s		16.3	0.80	s		16.2	0.81	s	
27	25.9	1.47	s		25.9	1.47	s		26.0	1.48	s	
28	174.0				174.1				174.3			
29	28.0	0.94	s		28.0	0.93	s		28.0	0.94	s	
30	18.1	1.15	s		18.4	1.17	s		18.1	1.15	s	

and comparison with literature data indicated that the aglycone moiety of these compounds is acacic acid.[22] The ^{1}H NMR spectrum showed aromatic protons characteristic of an anthraniloyl (2-aminobenzoyl) ester residue, confirmed by alkaline hydrolysis, which afforded anthranilic acid identical by TLC and by ^{1}H and ^{13}C NMR with an authentic sample. The ^{13}C chemical shifts of C-28 between δ_C 174.0 and 174.3 and of C-3 at δ_C 88.3 or 88.4 of the aglycone moiety suggested that the glycosidic chains were connected to acacic acid at these positions, whereas the chemical shift of C-21 at δ_C 76.1 or 76.2 located the anthraniloyl ester moiety at this position. These data indicated that compounds **1**−**3** were 21-acyl-3,28-bidesmosides of acacic acid. The absolute configuration of the sugar residues obtained by acidic hydrolysis was determined as L-rhamnose, D-glucose, L-arabinose, D-fucose, and D-xylose from the measurement of optical rotations after purification.

Compound **1** gave in HR-MS (positive mode) an $[M + Na]^+$ peak at *m/z* 1551.6929 (calcd for $C_{73}H_{112}N_2O_{32}Na$, 1551.7096). The ^1H NMR of **1** displayed signals for six anomeric protons at δ_H 5.45, 5.35, 4.54, 4.53, 4.51, and 4.47 correlating with six anomeric carbons at δ_C 99.5, 93.8, 102.4, 105.4, 105.5, and 103.3, respectively (Table 2).

From the anomeric proton of each sugar unit, the other protons were assigned using COSY and TOCSY spectra, after which the glycosidic carbons were identified from analysis of HSQC spectra. Four of the glycosidic units were identified as two β-xylopyranosyl units, one β-glucopyranosyl unit, and one α-rhamnopyranosyl unit (Table 2). The fifth sugar was an *N*-acetylated aminoglycopyranosyl. The presence of an *N*-acetamido group was suggested by the detection of signals at δ_H 1.98 (3H, s, CH_3CONH), δ_C 21.7 (CH_3CONH) and 172 (CH_3*CONH)* as well as by the correlation observed in an HMBC experiment between *CO*NH (δ_C 172) and H-2 (δ_H 3.69) of the glycopyranosyl ring. The shielding of C-2 to δ_C 56.4 is characteristic of a 2-deoxy-2-acetamidoglycosyl unit. The large proton coupling constants indicated the *trans*-diaxial orientation of the protons of the 2-(acetylamino)-2-deoxy-β-D-glucopyranosyl moiety (Table 2). The final sugar was found to be a fucopyranosyl unit, as suggested by the presence of a three-proton doublet at δ_H 1.30 coupled with an axial proton at δ_H 3.63 (H-5) appearing as a quadruplet in an axial position (J = 6.5 Hz). This geometry indicated an α-equatorial position for H-4. In the HMBC experiment, the CH_3 is correlated with two carbons at δ_C 70.3 and 71.2 attributed to C-5 and C-4, respectively. These data were in good agreement with the presence of a β-D-fucose moiety. Finally, assignments of C-2 and C-3 were determined by comparison with literature data.[13,17] The sequencing of the glycosidic chains was achieved by analysis of HMBC and ROESY experiments and confirmed by LC-MS and MS-MS analysis. Connectivity in the ROESY spectrum was observed between H-3 of the aglycone and H-1 of the GlcNAc unit at δ_H 3.31/4.47. Moreover, the correlation observed in the HMBC spectrum between C-3 at δ_C 88.35 and H-1 of the Glc-NAc at δ_H 4.47 confirmed the location of this sugar residue at position 3 of acacic acid. The sequence of the chain linked to C-3 was determined from the HMBC correlations observed between C-1 of fucose and H-6 of GlcNAc at δ_C 102.4/δ_H 3.79−4.10 and between H-1 of xylose and C-2 of fucose at δ_H 4.51/δ_C 80.9. This sequence was partially confirmed by ROE correlations between H-1 of Glc-NAc and H-3 of acacic acid and between H-1 of Fuc and H-6 of GlcNAc. These data are consistent with a triglycosidic chain [β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-(acetamido)-2-deoxy-β-D-glucopyranosyl] linked to position C-3 of acacic acid. This oligosaccharide chain is common to compounds **1**−**3** and has previously been found in adianthifoliosides[17] and julibrosides.[10,12] In the HMBC spectrum, the correlation observed at δ_C 174/δ_H 5.35 revealed an ester linkage between the glucose and the aglycone (C-28/H-1 of Glc), supported by the upfield shift of C-28 compared to the shift of a free carboxylic acid.[17] HMBC interglycosidic correlations were evident between signals at δ_H 3.61 (H-2 of Glc ester) and δ_C 99.5 (C-1 of Rha) and between δ_H 3.60 (H-4 of Rha) and δ_C 105.4 (Xyl′-1). The cross-peaks observed in the ROESY experiment between H-2 of Glc ester and H-1 of Rha at δ_H 3.61/5.45 and between H-4 of Rha and H-1 of Xyl′ at δ_H 3.60/4.53 confirmed that the ester glycosidic chain was 28-*O*-[β-D-xylopyranosyl-(1−4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl].

Four aromatic protons between 6.6 and 7.81 ppm were detected in the ^1H spectrum of **1** characteristic of an *o*-disubstituted phenyl group for an anthraniloyl residue. In the HSQC experiment, these protons were linked to aromatic methine carbons resonating between 115.1 and 133.5 ppm. The aromatic protons were correlated in the HMBC spectrum with three quaternary carbons: one carboxylic ester at δ_C 167.9, one carboxyl-linked carbon at δ_C 110.3, and one N-substituted aromatic carbon at δ_C 151.3. A $^3J_{H-C}$ correlation between the carboxylic carbon and H-21 of acacic acid confirmed the presence of a 2-aminobenzoyl ester at this position.

The ESIMS (positive mode) of **1** displayed a molecular ion at *m/z* 1551.5 $[M + Na]^+$. The MS^2 spectrum gave a fragment at *m/z* 1111.4 $[M + Na - 440]^+$ accounting for the loss of the C-28 glycosidic chain. An MS^3 experiment on this peak gave fragments at *m/z* 979.5 $[1111.5 - 132]^+$ and 833.2 $[1111.4 - 132 - 146]^+$, corresponding to the successive losses of the terminal xylose and fucose of the C-3 glycosidic chain. A second MS^2 fragment was observed at *m/z* 1414.5 $[M + Na - 137]^+$, and in the MS^3 experiment a fragment was detected at *m/z* 974.5 $[1111.5 - 137]^+$ due to the loss of anthranilic acid. Thus, compound **1** is 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-21-*O*-[2-aminobenzoyl]-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]acacic acid, named grandibracteoside A.

Compound **2** showed in the positive HRMS an $[M + Na]^+$ peak at *m/z* 1713.7641 (calcd for $C_{79}H_{122}N_2O_{37}Na$, 1713.7623), i.e., 162 mass units higher than compound **1**, corresponding to an additional terminal hexose unit. Spectroscopic data for the aglycone part and the glycosidic chain at C-3 of **1** and **2** were superimposable, as confirmed by the same fragments detected at *m/z* 1111.5 and 974.5 in ESIMS experiments. These results suggested that the C-28 chain of **2** contained four sugar units instead of three as in compound **1** and consistent with the presence of seven anomeric protons at δ_H 5.44 (d, J = 1.7 Hz), 5.33 (d, J = 7.9 Hz), 4.55 (m), 4.54 (d, J = 7.5 Hz) 4.51 (d, J = 7.4 Hz), 4.46 (d, J = 8.3 Hz), and 4.32 (d, J = 7.8 Hz), which correlated with the methine carbons at δ_C 99.3, 93.4, 102.4, 105.5, 105.4, and 103.3 (2C), respectively, in the HSQC spectrum. The additional hexose was determined to be a terminal glucopyranose (Glc′) according to the values of $^3J_{H-H}$ and carbon chemical shifts, which were in agreement with data observed for terminal glucose.[12] The ROESY correlations between H-6 of Glc ester and H-1 of Glc′ at δ_H 3.74−4.10/4.32 confirmed the location of this glucose unit at C-6 of the first glucose of the ester chain. Other correlations observed in the ROESY confirmed that sequencing of the chains was similar to that observed in **1**. Consequently, the structure of **2** is 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-21-*O*-[2-aminobenzoyl]-28-*O*-β-D-glucopyranosyl-(1→6)-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosylacacic acid, named grandibracteoside B.

Compound **3**, giving an $[M + Na]^+$ peak at *m/z* 1713.7 in ESIMS, was an isomer of compound **2**. HRMS for compound **3** showed an $[M + Na]^+$ peak at *m/z* 1713.7632 (calcd for $C_{79}H_{122}N_2O_{37}Na$, 1713.7623). As in compound **2**, the ^1H NMR spectrum displayed signals for seven anomeric protons correlating with seven anomeric carbons in the HSQC spectrum. The chemical shifts of the anomeric signals were at δ_C/δ_H: 103.3/4.46, 105.5/4.50, 102.4/4.50, 93.9/5.35, 109.5/5.36, 104.3/4.52, and 99.9/5.36. Comparison

Table 2. ^{1}H NMR (CD$_3$OD, 500 MHz) and ^{13}C NMR (CD$_3$OD, 125 MHz) of Glycosidic and Acidic Moieties of Compounds **1**, **2**, and 3

	1				**2**				**3**			
	δ_C	δ_H		J	δ_C	δ_H		J	δ_C	δ_H		J
21-*O*-acyl												
1′	167.9				167.9				167.8			
2′	110.3				110.3				110.5			
3′	151.3				151.3				151.3			
4′	116.4	6.77	dd	1.0, 8.3	116.5	6.77	dd	1.0, 8.3	116.4	6.77	dd	1.0, 8.3
5′	133.5	7.25	ddd	1.6, 7.0, 8.4	134.5	7.26	ddd	1.6, 7.0, 8.5	133.5	7.25	ddd	1.6, 7.0, 8.5
6′	115.1	6.60	ddd	1.0, 7.0, 8.1	115.3	6.62	ddd	1.0, 7.1, 8.3	115.2	6.60	ddd	1.0, 7.1, 8.1
7′	130.5	7.81	dd	1.5, 8.1	130.0	7.82	dd	1.5, 8.0	130.5	7.81	dd	1.5, 8.1
3-*O*-sugar GlcNAc												
1	103.3	4.47	d	8.5	103.3	4.46	d	8.3	103.3	4.46	d	8.5
2	56.4	3.69	dd	10.3, 8.3	56.3	3.68	dd	8.6, 10.3	56.4	3.67	t	8.3
3	74.2	3.48	dd	8.8, 10.3	74.3	3.47	dd	8.7, 10.2	74.2	3.47	dd	8.5, 10.0
4	70.7	3.35	dd	8.8, 9.0	70.7	3.36	t	8.7	70.7	3.34	t	9.5
5	75.6	3.50	m		75.6	3.50	m		75.6	3.49	m	
6	68.4	3.79; 4.10	dd;dd	6.4, 11.5; 1.5,	68.2	3.79;4.10	dd;dd	6.4, 11.9; 1.5, 10.1	68.4	3.79; 4.10	dd;dd	5.5, 12.0; 1.5, 12.0
NHCOCH$_3$	172.0				171.9				172.0			
NHCOCH$_3$	21.7	1.98	s		21.7	1.97	s		21.7	1.97	s	
Xyl												
1	105.5	4.51	d	7.4	105.4	4.51	d	7.4	105.5	4.50	d	7.4
2	74.7	3.33	t	8.2	74.6	3.31	t	9.4	74.7	3.32	t	8.0
3	76.0	3.40	t	8.9	76.0	3.39	t	9.0	76.7	3.39	t	8.7
4	69.6	3.53	m		69.6	3.53	m		69.6	3.53	m	
5	65.8	3.29; 4.01	t;dd	11.0–5.3, 11.5	65.8	3.29;4.01	t;dd	10.8; 5.3, 11.5	65.8	3.29; 4.00	dd;dd	10.3, 11.5; 5.3, 11.5
Fuc												
1	102.4	4.54	d	5.8	102.4	4.55	m		102.4	4.55	m	
2	80.9	3.66	m		80.9	3.65	m		80.9	3.65	m	
3	73.5	3.66	m		73.5	3.65	m		73.5	3.65	m	
4	71.2	3.66	m		71.2	3.65	m		71.2	3.65	m	
5	70.3	3.63	q	6.5	70.2	3.62	q	6.5	70.3	3.62	q	6.5
6	15.3	1.30	d	6.5	15.3	1.29	d	6.4	15.3	1.30	d	6.5
28-*O*-sugar Glc (ester position)												
1	93.8	5.35	d	7.9	93.9	5.33	d	7.9	93.9	5.35	d	7.1
2	74.7	3.61	t	8.0	74.7	3.62	t	8.1	74.8	3.57	t	8.0
3	77.9	3.55	t	8.7	77.7	3.55	t	8.6	77.5	3.53	t	9.0
4	69.6	3.38	dd	9.0, 10.0	69.5	3.44	dd	8.6, 9.5	69.3	3.37	t	8.5
5	77.1	3.32	m		76.2	3.49	dm	9.5	77.0	3.35	m	
6	60.6	3.67; 3.8	dd;dd	3.5, 12.0; 2.0,	67.9	3.74;4.10	dd;dd	11.9, 4.8; 10.1, 1.5	60.8	3.67; 3.80	dd;dd	4.0, 10.0; 2.0, 10.0
Rha (at Glc C-2)												
1	99.5	5.45	d	1.7	99.3	5.44	d	1.7	99.9	5.36	d	1.6
2	70.3	3.94	dd	1.8, 3.3	70.4	3.94	dd	1.8, 3.3	69.6	4.25	dd	1.9, 3.1
3	70.7	3.86	dd	3.3, 9.5	70.7	3.86	dd	3.3, 9.4	81.1	3.92	dd	3.1, 10.0
4	82.4	3.6	t	9.5	82.3	3.60	t	9.4	77.1	3.68	t	8.5
5	67.4	3.86	m		67.4	3.85	m		67.7	3.92	m	
CH$_3$	16.8	1.33	d	6.2	16.8	1.30	d	6.3	17.2	1.36	d	6.3
Xyl′ (at Rha C-4)												
1	105.4	4.53	d	7.5	105.5	4.54	d	7.5				
2	76.5	3.31	dd	7.9, 9.0	74.7	3.31	t	9.4				
3	76.7	3.36	t	9.0	76.4	3.36	t	9.1				
4	69.6	3.65	m		69.6	3.53	m					
5	65.8	3.91; 3.23	dd;t	5.4, 11.4; 11.0	65.8	3.91;3.23	dd;t	5.4, 11.4; 11.0				
					Glc′ (at Glc C-6)				Glc′ (at Rha C-3)			
1					103.3	4.32	d	7.8	104.3	4.52	d	7.5
2					73.6	3.19	dd	7.8, 9.2	73.8	3.32	t	8.0
3					76.5	3.37	t	9.1	76.0	3.39	t	8.5
4					70.0	3.29	t	9.5	69.6	3.37	t	9.0
5					76.7	3.24	m	10.0	76.1	3.32	m	
6					61.2	3.84,3.68	dd;dd	2.1, 11.8; 3.5, 12.0	60.8	3.75; 3.85	dd;dd	5.0, 11.5; 2.2, 11.5
									Ara (at Rha C-4)			
1									109.5	5.36	d	1.9
2									82.5	4.12	dd	1.7, 3.7
3									77.0	3.91	dd	4.0, 6.7
4									84.0	4.05	ddd	3.0, 5.3, 6.0
5									61.5	3.77; 3.66	dd;dd	3.0, 11.5; 5.0, 11.5

Table 3. Biological Activities of the Crude Extract and Compounds **1**−**3**[a]

	KB cells cytotoxicity	MCF7 cytotoxicity	*Rhabditis pseudoelongata*	*Plasmodium falciparum*
reference products	adriblastine: 0.1 μM	adriblastine: 0.1 μM	ivermectine: 0.38 μg/mL	chloroquine: 0.1 μM
methanolic crude extract	95% at 10 μg/mL	NT	LD_{50} 25 μg/mL	IC_{50} > 100 μg/mL
	IC_{50} (μM)	IC_{50} (μM)		
1	1.3	0.4	NT	NT
2	2.3	1.7	NT	NT
3	2.3	1.7	NT	NT

[a] NT: not tested, IC_{50}: concentrations inhibiting parasite or cell growth by 50%, LD_{50}: lethal dose 50%.

of the ^{1}H and ^{13}C NMR spectra of **3** with those of **1** and **2** revealed that they have a common sugar substitution pattern at C-3 and the same acyl substitution pattern at C-21 (Tables 1 and 2). ESIMS gave two fragments corresponding to the loss of the glycosidic units at *m/z* 1232.4 and at *m/z* 1111.5 at C-3 and C-28, respectively. Concerning the oligosaccharidic moiety linked to C-28, the glucose ester (Glc) possessed the same chemical shifts as in compounds **1** and **2**, and correlations from the HMBC spectrum confirmed the ester linkage to the carboxylic group of the aglycone. The β-D-glucose ester was substituted at C-4 by an α-L-rhamnose moiety. The deshielding of C-3 at δ_C 81.1 and C-4 at δ_C 77.1 of this rhamnose suggested a disubstitution by two sugar units. A terminal β-D-glucose (Glc′) was revealed from analysis of COSY and TOCSY spectra, and the HMBC correlation observed between its H-1 and C-3 of rhamnose, permitted its location at this position. The small values of $^{3}J_{H-H}$ and the correlations in COSY and HSQC experiments suggested an α-L-arabinose moiety for the fourth sugar of the ester chain. The correlation between its H-1 (5.36 ppm) and C-4 (84 ppm) in the HMBC experiment indicated an α-L-arabinofuranosyl unit. In the ROESY experiment the correlation between H-1 of arabinofuranose (δ_H 5.36) and H-4 of rhamnose (δ_H 3.68) confirmed that the tetrasaccharide chain located at C-28 of the acacic acid was α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl, common to several *Albizia* saponins such as adianthifoliosides, julibrosides, and proceosides. Thus, saponin **3** is 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl]-21-*O*-[2-aminobenzoyl]-28-*O*-(α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosylacacic acid, or grandibracteoside C.

Since triterpene saponins from the *Albizia* genus have been reported to be cytotoxic on tumoral cell lines,[15,23,24] the crude extract was tested for anthelminthic properties and cytotoxicity on KB cells as part of our investigation of potential bioactivities of plants ingested by chimpanzees. Because the methanolic crude extract had a significant activity on KB cells (95% cytotoxicity at 10 μg/mL), compounds **1**−**3** were tested in vitro against two tumor cell lines from nasopharyngeal carcinoma and breast adenocarcinoma, KB and MCF7, respectively (Table 3). Furthermore, the avicins isolated from *Acacia victoriae* (Bentham) and showing structural similarities with the present compounds have been found to have several pharmacological properties against tumor cells. The avicins have the ability to induce apoptosis in the Jurkat human T cell line[25] (IC_{50} from 0.72 to 6.50 μg/mL) and may inhibit chemically induced mouse skin carcinogenesis.[26] Induction of apoptosis has also recently been reported for adianthifoliosides isolated from *Albizia adianthifolia,* cytotoxic activity being detected at 0.1 μM for adianthifolioside D, the most cytotoxic product.[24] Avicins, adianthifoliosides, and the present saponins share the following structural features: they possess the same aglycone unit (acacic acid) substituted by an oligosaccharide moiety at C-28 and C-3 and esterified at C-21. The glycosidic chains are similar in grandibracteoside C (**3**), avicins D and G, and adianthifoliosides A and D. Grandibracteoside A (**1**), with a trisaccharidic chain at C-28, showed an IC_{50} at 0.4 and 1.3 μM against MCF7 and KB cell lines, respectively, while grandibracteosides B and C (**2** and **3**), which possess a tetrasaccharide chain at C-28, displayed similar weaker cytotoxicity, i.e., IC_{50} at 1.7 and 2.3 μM against MCF7 and KB cell lines. These results are in good agreement with previous observations pointing out the crucial role of acacic acid with the hydroxy group at C-16 together with the presence of an ester substituent at C-21 and an *N*-acetylglucosamine at C-3 for mediating high cytotoxicity.[12,27] The structural similarities of grandibracteosides with avicins and adianthifoliosides and their significant cytotoxicity suggested that an apoptotic mechanism is likely to be involved.[24] Moreover the crude methanolic extract was inactive against *Plasmodium falciparum* development at a concentration <100 μg/mL, suggesting that, at these concentrations, the red cells were intact and enable parasite survival (Table 3). Since adianthifolioside A, a salicylate ester, was less cytotoxic (5 μM) than grandibracteosides anthranylate type,[17,24] it will be interesting to investigate the potential role of anthranilic acid in tumor cell toxicity and other biological properties.

Observations of primate feeding behavior prompted the isolation and structural determination of these new bioactive compounds. Further investigation on the feeding behavior of different species of primates under free-ranging conditions may highlight potential ecological adaptation of animal species to the secondary metabolite content of plant and possible effects of the identified compounds on health or parasite loads. In addition to traditional methods used for plant collection in pharmacognosy, this approach is an alternative and promising way for discovering new natural products.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 500, at 500 and 125 MHz, respectively. Two-dimensional NMR experiments were performed using standard Bruker microprograms (XwinNMR software). ESIMS and MS-MS experiments were recorded on a Finnigan LCQ deca quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA). Direct infusion at a flow rate of 5 μL/min was used on samples in MeOH solution. HR mass spectra were obtained on a MALDI-TOF Voyager-DE STR Perseptive Biosystems instrument. Optical rotations were recorded in MeOH for saponins and H_2O for monosaccharides, with a Perkin-Elmer 241 polarimeter. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, a P580 pump, a UVD 340S diode array detector, and Chromeleon software. A C-18 Dionex column (201SP510, 250 × 10 mm, 5 μm, Jouy-en-Josas, France) was used for semipreparative HPLC with MeCN−H_2O at a flow rate of 3 mL/min. CC was carried out on Merck Kieselgel 60 (63−200 mesh) or Merck Lichroprep RP-18 (40−63 μm).

Plant Material. The leaves of *A. grandibracteata* were collected in September 2003 in the Kibale National Park, in western Uganda. A specimen of the plant has been identified by Annette Hladik at the Laboratoire de Phanérogamie of the Museum National d'Histoire Naturelle in Paris by comparison to the reference herbarium, and a voucher specimen (Ug17) is stored in the herbarium of MNHN.

Cytotoxicity Bioassays against Tumor Cells. The semiautomated colorimetric test using neutral red was used for the in vitro assay of cytotoxicity in KB cells[29,30] and MCF7 cells.[31]

Antiparasite Bioassays. Antimalarial activity was tested against intra-erythrocytic asexual forms of the human malaria parasite *P. falciparum*, following the method of Desjardin et al.[32] For anthelminthic bioassays, we used *Rhabditis pseudoelongata* isolated from wild rabbit feces and maintained on sterilized rabbit feces. They were recuperated from a 10-day-old culture by the Baerman method. Worms isolated were larvae I, II, III, and IV stage and male and female adults. Two hundred worms were deposited in each of the 24 wells of the plate. Extracts in DMSO were added, and the wells were filled up to 500 μL with sterile water. Motile and nonmotile worms were counted and compared with the control wells.

Extraction and Isolation. Air-dried powdered leaves (1 kg) were macerated in 15 L of petroleum ether for 10 h and percolated during 10 h. The solvent was evaporated and the leaf powder dried, then macerated for 48 h with 15 L of 90% aqueous MeOH and further refluxed for 4 h. After cooling, the solution was filtered and evaporated and the residue suspended in methanol (2 L). The solution was poured into 10 L of acetone, and the precipitate was filtered and dried over KOH in vacuo (65 g). A portion of the precipitate (31 g) was dissolved in H_2O and dialyzed against H_2O in seamless cellulose tubing under agitation for 72 h. Freeze-drying of the tube content yielded 4.2 g of saponin mixture (yield 0.88%). The saponin mixture (4 g) was fractionated on a silica gel VLC (vacuum liquid chromatography) using a gradient of $CHCl_3$−MeOH (50:50 to 0:100). Fraction 16 (1 g) was purified on an RP-18 column using a gradient of MeOH−H_2O (50:50 to 0:100). Fractions [E−F] (19 mg) were purified by preparative TLC in $CHCl_3$−MeOH−H_2O−AcOH (60:32:6.5:0.5), yielding compound **2** (5.9 mg). Fractions [M−N] were chromatographed on silica gel with a gradient of $CHCl_3$−MeOH−H_2O (from 80:20:0 to 70:30:5) to give compound **1** (25 mg) and a fraction that was purified by HLPC using 33% of acetonitrile−0.025% aqueous TFA, to afford compound **3** (6.5 mg).

Acid Hydrolysis of the Saponin Mixture. An aliquot of the saponin mixture (4.3 g) was dissolved in 100 mL of 0.02N H_2SO_4−6.5% $HClO_4$ (1:1) and refluxed for 4 h. The precipitate was filtered, and the aqueous acid solution was neutralized with 1 N KOH and freeze-dried. The sugar mixture was purified by preparative TLC, and the sugars isolated were compared with standard sugars in EtOAc−Me_2CH_2OH−Me_2CO−H_2O (20:10:7:6) for rhamnose, glucose, and xylose or in $CHCl_3$−MeOH−H_2O (8:5:1) for fucose and arabinose. The optical rotations of the purified sugars were measured for L-rhamnose $[\alpha]^{25}_D$ −1.54 to +7.1 (*c* 0.39, H_2O), D-glucose $[\alpha]^{25}_D$ +13.5 (*c* 0.13, H_2O), D-xylose $[\alpha]^{25}_D$ +23.3 to +6.7 (*c* 0.30, H_2O), D-fucose $[\alpha]^{25}_D$ +75.8 (*c* 0.48, H_2O), and L-arabinose $[\alpha]^{25}_D$ +73.6 (*c* 0.13, H_2O).

Alkaline Hydrolysis of the Saponin Mixture. Fraction L (100 mg) from the RP-18 column was refluxed with 5% aqueous KOH (200 mL) for 2 h. The solution was adjusted to pH 6 with HCl and extracted with Et_2O (2 × 150 mL) and H_2O-saturated n-BuOH (2 × 100 mL) The Et_2O extract was washed with H_2O (2 × 150 mL), dried over Na_2SO_4, and evaporated. The extract (8.5 mg) was purified on 800 mg of silica gel using $CHCl_3$−MeOH (1:0 to 10:1) to afford 3 mg of anthranilic acid, identified by comparison with an authentic sample by TLC in $CHCl_3$−MeOH (10:1) and ^1H NMR data ($CDCl_3$): δ 6.74 (2H, m,), 7.37 (1H, ddd, $J = 8.4, 7.1, 1.6$ Hz), 7.98 (1H, dd, $J = 8.2, 1.6$ Hz).

Compound 1: $[\alpha]^{23}_D$ −20.7 (*c* 0.1 MeOH); ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive) *m/z* 1551.5 [M + Na]$^+$, 1111.4 [M + Na − Xyl − Rha − Glc]$^+$, 974.4 [1111.4 − anthranilic acid]$^+$; MS/MS (1551.5) *m/z* 1414.5 [M + Na − anthranilic acid]$^+$, 1111.4 [M + Na − Xyl − Rha − Glc]$^+$; MS^3 (1111.5) *m/z* 1067.6 [1111.5 − CO_2]$^+$, 979.5 [1111.5 − 132]$^+$, 974.5 [1111.5 − anthranilic acid]$^+$, 833.2 [1111.4 − 132 − 146]$^+$, 789.3 [833.2 − 44]$^+$, 652.3 [789.3 − anthranilic acid]$^+$; MS^4 (979.4) *m/z* 935.5 [979.4 − CO_2]$^+$, 917.4 [935.4 − H_2O]$^+$, 842.4 [979.4 − anthranilic acid]$^+$, 798.4 [842.4 − CO_2]$^+$; MS^5 (935.4) *m/z* 917.4 [935.4 − H_2O]$^+$, 798.2 [935.4 − anthranilic acid]$^+$, 789.4 [935.5 − 146]$^+$; ESIMS (negative) *m/z* 1527.5 [M − H]$^-$, 1087.5 [1527.5 − Xyl − Rha − Glc]$^-$; MS/MS (1527.3) *m/z* 1407.2 [M − H − 120]$^-$, 1087.5 [1527.5 − Xyl − Rha − Glc]$^-$; MS^3 (1087.4) *m/z* 1043.4 [1087.4 − CO_2]$^-$, 950.3 [1087.4 − anthranilic acid]$^-$; MS^4 (950.4) *m/z* 906.4 [950.4 − CO_2]$^-$, 818.3 [950.3 − 132]$^-$, 671.8 [818.3 − 146]$^-$; HRMS positive mode *m/z* 1551.6929 [M + Na]$^+$ (calcd for $C_{73}H_{112}N_2O_{32}Na$, 1551.7096).

Compound 2: $[\alpha]^{23}_D$ 27.2 (*c* 0.1 MeOH); ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive) *m/z* 1713.7 [M + Na]$^+$, 1111.5 [M + Na − saccharide chain at C-28]$^+$, 974.5 [1111.5 − anthranilic acid]$^+$; MS/MS (1713.7) *m/z* 1576.5 [M + Na − 137]$^+$, 1111.5 [M + Na − saccharide chain at C-28]$^+$; MS^3 (1111.5) *m/z* 1067.6 [1111.5 − CO_2]$^+$, 979.6 [1111.5 − 132]$^+$, 974.5 [1111.5 − anthranilic acid]$^+$; MS^4 (979.6) *m/z* 935.5 [979.6 − CO_2]$^+$, 798.4 [935.4 − anthranilic acid]$^+$, 917.4 [935.4 − H_2O]$^+$; MS^5 (935.4) *m/z* 798.2 [935.4 − anthranilic acid]$^+$, 789.4 [935.5 − 146]$^+$; HRMS (positive) *m/z* 1713.7641 [M + Na]$^+$ (calcd for $C_{79}H_{122}N_2O_{37}Na$, 1713.7623).

Compound 3: $[\alpha]^{23}_D$ −19.8 (*c* 0.1 MeOH); ^1H NMR and ^{13}C NMR, see Tables 1 and 2; ESIMS (positive) *m/z* 1713.7 [M + Na]$^+$, 1581.5 [M + Na − 132]$^+$, 1435.5 [1581.5 − 146]$^+$, 1232.4 [1435.5 − sugars in C-3]$^+$, 1111.5 [M + Na, − saccharide chain at C-28]$^+$, 974.5 [1111.5 − anthranilic acid]$^+$, 868.4 [M + 2Na]$^{2+}$, 625 [saccharide chain at C-28+Na]$^+$; MS/MS (1713.7) *m/z* 1576.5 [M + Na − 137]$^+$, 1111.5 [M + Na − saccharide chain at C-28]$^+$; MS^3 (1111.5) *m/z* 1067.6 [1111.5 − CO_2]$^+$, 979.5 [1111.5 − 132]$^+$, 974.5 [1111.5 − anthranilic acid]$^+$; MS^4 (979.4) *m/z* 935.4 [979.4 − CO_2]$^+$, 798.4 [935.4 − anthranilic acid]$^+$; MS^5 (935.4) *m/z* 917.4 [935.4 − H_2O]$^+$, 798.4 [935.4 − anthranilic acid]$^+$, 652.5 [798.5−146]$^+$; HRMS (positive) *m/z* 1713.7632 [M + Na]$^+$ (calcd for $C_{79}H_{122}N_2O_{37}Na$, 1713.7623).

Acknowledgment. This research was financially supported by the Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, and the Muséum National d'Histoire Naturelle, France. We acknowledge C.-M. Hladik for his important contribution to the primatological survey, A. Hladik for her collaboration in species identification, and the Kibale Chimpanzee Project team and J.-M. Krief for their assistance in the field. We are most grateful to C. Bories for assistance with anthelminthic bioassays, to P. Grellier for antimalarial tests, to C. Chapman for helpful discussions, and to R. Dodd for his careful reading of the manuscript. We are also grateful to the Uganda Wildlife Authority and to the Uganda National Council for Science and Technology for permission to work in the Kibale National Park and to Makerere University Biological Field Station for providing facilities.

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NP049576I