Two New Biologically Active Triterpenoidal Saponins Acylated with Salicylic Acid from *Albizia adianthifolia*

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Two new oleanane-type triterpene saponins, adianthifoliosides A (**1**) and B (**2**), were isolated from a 95% ethanolic extract of roots of *Albizia adianthifolia*. Their structures were elucidated mainly by using a combination of 600 MHz 1D and 2D NMR techniques (COSY, NOESY, TOCSY, HSQC, and HMBC) and by FABMS and HRESIMS. Compounds **1** and **2** were characterized as glycosides of acacic acid acylated by an *o*-hydroxybenzoyl unit. The crude saponin mixture (CSM), compounds **1** and **2** together with **3** and **4** (prosapogenins obtained from the mild alkaline hydrolysate of the CSM), were evaluated for immunomodulatory activity on the Jurkat T cell line and for hemolytic property against sheep erythrocytes. Compound **2** and, to a lesser extent, **1** and **3** were found to exhibit a dose-dependent immunomodulatory effect in the concentration range 10^{-2}−10 μM, whereas **4** showed a lymphoproliferative activity in the same concentration range. Among the compounds tested, only **1** and **2** were found to be hemolytic.

The genus *Albizia* comprises about 150 species widely distributed in the tropics, with the greatest diversity in Africa and Central and South America.[1] *Albizia* species have been reported to contain alkaloids, steroids, triterpenoid saponins, and flavonols.[2] Some saponins such as julibrosides J_1, J_2, and J_9 from *Albizia julibrissin*[3,4] possess various biological effects, such as inhibitory activity against the KB cancer cell line in vitro.

In a previous contribution, we reported the isolation and structure determination of two prosapogenins (**3** and **4**) isolated from the butanol extract of the mild alkaline hydrolysate of the crude saponin fraction of the roots of *Albizia adianthifolia* (Schumach.) W. F. Wight (Mimosaceae).[5] The further investigation of the saponins of this plant, obtained as a complex mixture, afforded two new acylated triterpene saponins, adianthifoliosides A (**1**) and B (**2**), from the 95% ethanolic extract of the roots of *A. adianthifolia*. This paper deals with the isolation and structure elucidation of these new acylated triterpene glycosides (**1** and **2**) and the evaluation of the immunomodulatory activity of **1**−**4** (Chart 1) and the crude saponin mixture (CSM) on Jurkat T cell proliferation (human T cell leukemia) and the hemolytic activity of **1**−**4** on sheep erythrocytes.

Results and Discussion

The 95% ethanolic extract of the roots of *A. adianthifolia* was purified by precipitation with diethyl ether, yielding a crude saponin mixture, which was then dialyzed for 2 days. The powder obtained was submitted to column chromatography over Sephadex LH-20 and was separated by repeated medium-pressure liquid chromatography (MPLC) over normal Si gel, yielding compounds **1** and **2**. Their structures were elucidated mainly by 1D and 2D NMR spectroscopy (COSY, TOCSY, NOESY, HSQC, and HMBC) and by FABMS and HRESIMS.

Compound **1** was obtained as an amorphous powder. The HRESI mass spectrum (positive-ion mode) exhibited a quasimolecular ion peak at *m/z* 1714.7490 $[M + Na]^+$ (calcd 1714.7465), consistent with a molecular formula of $C_{79}H_{121}NO_{38}$. Upon acid hydrolysis with 2 N TFA at 120 °C, **1** afforded the aglycon **6**, which was identified as acacic acid lactone (the 21,28-lactone derivative of acacic acid obtained under the experimental conditions used), by comparison of its NMR data with literature values.[6] The native aglycon was characterized as acacic acid from the 2D NMR spectra from **1**. The sugars obtained from the saponin hydrolysate were identified as glucose, fucose, rhamnose, arabinose, and xylose by comparison with authentic samples. However, the presence of an *N*-acetamido group [IR 1639 and 1570 cm^{-1}; ^1H NMR δ_H 2.13 (3H, s, *Me*CO) and δ_H 8.91 (1H, d, J = 8.8 Hz, N*H*); ^{13}C NMR δ_C 22.9 and 171.9], together with the ^1H and ^{13}C NMR data of the C-2 of the Glc_1 (δ_H 4.40 and δ_C 56.8), suggested the presence of one 2-(acetylamino)-2-deoxyglucose (Glc_1NAc) unit. In addition, alkaline hydrolysis of **1** afforded salicylic acid (SA, *o*-hydroxybenzoic acid, **7**), identified by comparison of its ^1H and ^{13}C NMR data with those reported,[7] and a prosapogenin whose spectroscopic NMR data were in good agreement with those of **4**.[5] The above data suggested that **1** is a 21-acyl-3,28-bisdesmoside. This was confirmed by the observation of glycosylation- and acylation-induced shifts in the ^{13}C NMR spectrum at δ_C 88.7 (downfield shift of C-3), δ_C 76.5 (downfield shift of C-21), and δ_C 174.4 (upfield shift of C-28).

The ^1H NMR spectrum of **1** displayed signals for seven anomeric protons at δ_H 6.06 (br s), 5.83 (d, J = 7.5 Hz), 5.77 (br s), 5.10 (d, J = 7.5 Hz), 4.91 (d, J = 7.5 Hz), 4.90 (d, J = 7.8 Hz), and 4.79 (d, J = 8.0 Hz), which correlated with the carbon signals at δ_C 109.8, 94.9, 101.2, 104.7, 103.8, 105.6, and 102.5, respectively, in the HSQC spectrum. Starting from the anomeric proton of each sugar unit, all the protons within each spin system were delineated using COSY with the aid of TOCSY and NOESY spectra. After assignments of the protons, the ^{13}C NMR resonances of each sugar unit were identified by HSQC and further confirmed by HMBC. The COSY and TOCSY spectra

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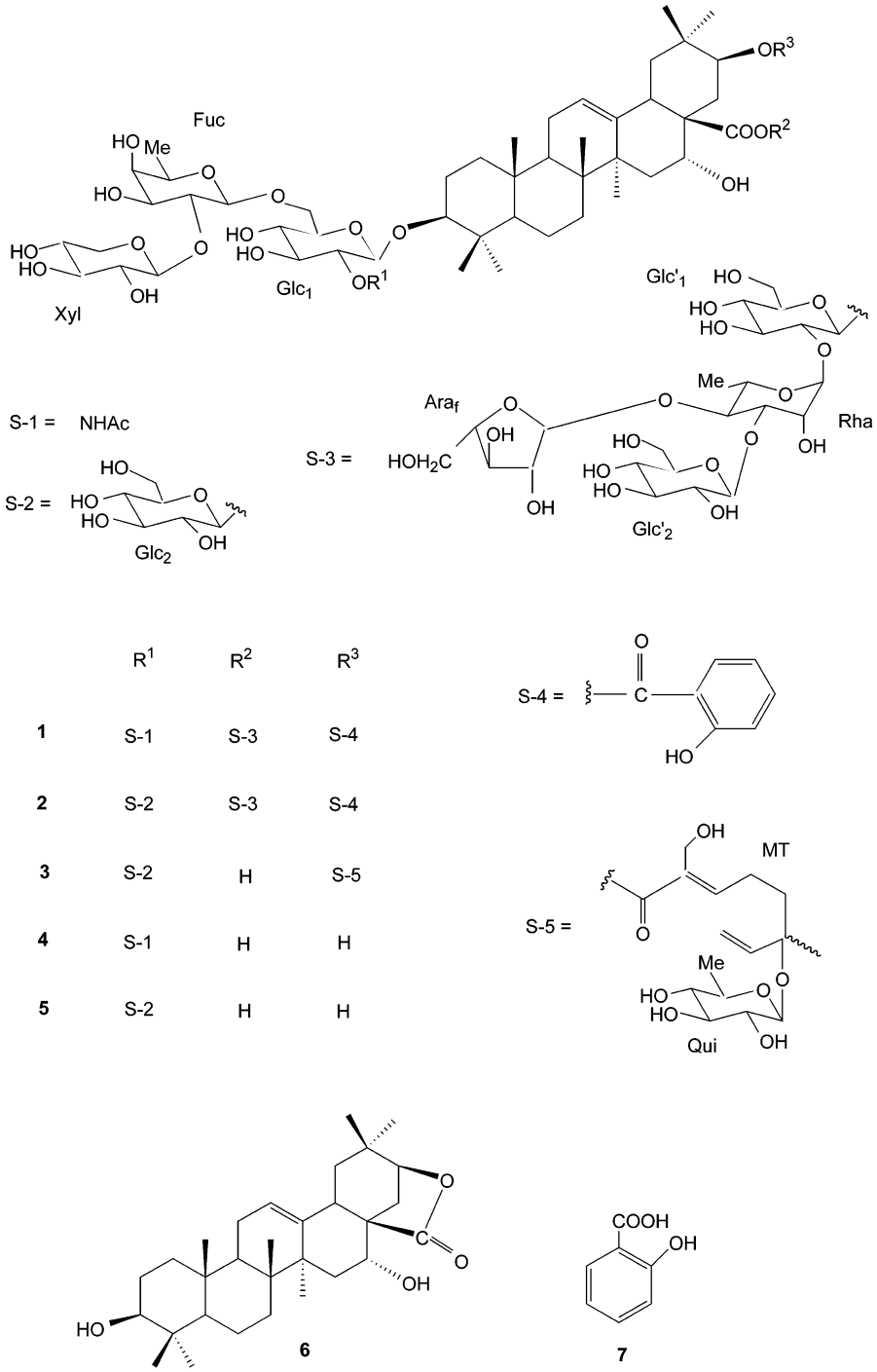
Chart 1



indicated the presence of seven monosaccharide units, and one of them was an α-rhamnopyranosyl (Rha) from its typical pattern in the COSY-LR spectrum.[8,9] After interpretation of the ^{1}H and ^{13}C NMR signals, the other sugars were identified as two *β*-glucopyranosyl (Glc) units, one *β*-fucopyranosyl (Fuc) unit, one *β*-xylopyranosyl (Xyl) unit, one 2-(acetylamino)-2-deoxy-*β*-glucopyranosyl (GlcNAc) unit, and one arabinofuranosyl unit, respectively. The α-configuration of this latter sugar was clarified by observation of NOESY correlations between H-1, H-3, and H-5. The absolute configurations of these sugar residues were determined to be D-glucose, D-xylose, D-fucose, L-rhamnose, L-arabinose, and 2-(acetylamino)-2-deoxy-*β*-D-glucopyranose by GC analysis of chiral derivatives of sugars in the acid hydrolysate (see Experimental Section).[10]

The ^{1}H and ^{13}C NMR signals of **1** assigned from the 2D NMR experiments were almost superimposable with those of **4**, previously characterized as acacic acid 3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-2-(acetylamino)-2-deoxy-*β*-D-glucopyranoside, except for the presence of the additional salicylic acid unit[7] and an oligosaccharidic chain at C-28.

The assignments of the ^{1}H and ^{13}C NMR signals of **1** from the TOCSY, HSQC, and HMBC spectra showed that the four remaining sugars were a terminal Glc (Glc′$_2$), a disubstituted Glc (Glc-1,2)(Glc′$_1$), a terminal Ara*f*, and a trisubstituted Rha (Rha-1,3,4). A correlation in the HSQC spectrum at δ_C/δ_H 94.9/5.83 (d, J = 7.5 Hz) showed that the Glc′$_1$ residue is linked to the carboxylic group of the aglycon by an ester linkage. This conclusion was supported

by the upfield shift of C-28 at δ_C 174.4, in comparison with the free carboxylic acid observed in **4** at δ_C 180.0 ppm. The long-range correlations observed in the HMBC spectrum between the ^{1}H NMR signals at δ_H (Rha-1) 5.77 and the ^{13}C NMR signals at δ_C (Glc′$_1$-2) 76.9, between δ_H (Glc′$_2$-1) 5.10 and δ_C (Rha-3) 81.2, and between δ_H (Ara*f*-1) 6.06 and δ_C (Rha-4) 77.6 showed that the tetrasaccharide residue *O*-*a*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*a*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl was linked to the acacic acid unit at C-28.

The comparison of the ^{13}C NMR chemical shifts of **4** and **1** permitted the position of the linkage of the salicylic acid on the aglycon to be located. In **1**, when compared to **4**, the signals for C-20, C-21, and C-22 were displayed upfield by −2.1 ppm, downfield by + 2.6 ppm, and upfield by −6.5 ppm, respectively, as a consequence of the acylation at C-21.

From these data, compound **1** was assigned as 16α-hydroxy-21*β*-[(2-hydroxybenzoyl)oxy]-3*β*-[(*O*-*β*-D-xylopyranosyl-(1→2)-*O*-*β*-D-fucopyranosyl-(1→6)-2-(acetylamino)-2-deoxy-*β*-D-glucopyranosyl)oxy]olean-12-en-28-oic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester. Compound **1** is a new compound[11,17−25] and was given the trivial name adianthifolioside A.

Compound **2** was obtained as an amorphous powder. HRESIMS (positive-ion mode) exhibited a quasimolecular ion peak at *m*/*z* 1835.7700 [M + Na]$^+$ (calcd 1835.7727), consistent with a molecular formula of $C_{83}H_{128}O_{43}$. Upon acid hydrolysis with 2 N TFA at 120 °C, **2** afforded the aglycon **6**, again identified as acacic acid lactone by comparison of its ^{1}H and ^{13}C NMR data with those reported.[6] The sugars obtained from the saponin hydrolysate were identified as glucose, fucose, rhamnose, arabinose, and xylose, by comparison with authentic samples on TLC. Alkaline hydrolysis of **2** afforded salicylic acid (**7**), identified by comparison of its ^{1}H and ^{13}C NMR data with those reported,[7] and a prosapogenin (**5**), whose NMR data were almost similar to those of the prosapogenin previously isolated from the alkaline hydrolysate of the *Albizia cortex* saponin mixture.[12] The above data suggested that **2** should be a 21-acyl-3,28-*O*-bisdesmoside. This was confirmed by the observation of glycosylation- and acylation-induced shifts in the ^{13}C NMR spectrum at δ_C 88.8 (downfield shift of C-3), δ_C 76.1 (downfield shift of C-21), and δ_C 174.5 (upfield shift of C-28).

The ^{1}H NMR spectrum of **2** displayed signals for eight anomeric protons at δ_H 6.09 (br s), 5.85 (d, J = 7.5 Hz), 5.72 (br s), 5.30 (d, J = 7.5 Hz), 5.10 (d, J = 7.5 Hz), 4.90 (d, J = 7.8 Hz), 4.80 (d, J = 8.0 Hz), and 4.72 (d, J = 7.5 Hz), which correlated with the ^{13}C NMR signals at δ_C 109.8, 94.8, 101.3, 103.8, 104.7, 105.6, 102.5, and 104.2, respectively, in the HSQC spectrum. Evaluation of spin−spin couplings and chemical shifts and interpretation of extensive 2D NMR allowed the identification of four *β*-glucopyranosyl units, one *β*-fucopyranosyl unit, one *β*-xylopyranosyl unit, one α-rhamnopyranosyl unit, and one α-arabinofuranosyl unit, respectively. The absolute configuration of sugar residues was determined to be D-glucose, D-xylose, D-fucose, L-rhamnose, and L-arabinose by GC analysis of chiral derivatives of sugars in the acid hydrolysate (see Experimental Section).[10]

Almost all NMR signals of **2** (Tables 1 and 2) were superimposable on those of **1** except for those of the oligosaccharidic moiety linked at C-3. The signals of this chain assigned from 2D NMR experiments were almost superimposable with those of the 3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranose moiety of **3** and **5**. Thus, compound **2** was concluded to be 16α-hydroxy-21*β*-[(2-hydroxybenzoyl)-oxy]-3*β*-[(*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*O*-*β*-D-xylopyranosyl-(1→2)-*O*-*β*-D-fucopyranosyl-(1→6)]-*β*-D-glucopyranosyl)oxy]-olean-12-en-28-oic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester. As a new compound,[11,17−25] **2** was given the trivial name adianthifolioside B. It is interesting to note that **1** and **2** contain a salicilyl substituent, not reported before among plant triterpene glycosides.

Table 1. ^{13}C NMR and ^{1}H NMR Data of the Aglycons of **1** and **2** (C_5D_5N)[a,b]

		1		**2**	
position	DEPT	δ_C	δ_H	δ_C	δ_H
1	CH_2	38.0	0.94, 1.52	38.0	0.94, 1.51
2	CH_2	26.2	nd[c], nd		
3	CH	88.7	3.29	88.8	3.30
4	C	39.0		39.6	
5	CH	55.8	0.84	55.1	0.77
6	CH_2	18.2	1.33, nd	18.3	nd, nd
7	CH_2	33.3	1.66, nd	32.8	1.57, nd
8	C	40.1		39.5	
9	CH	46.9	1.78	46.1	1.73
10	C	36.8		37.0	
11	CH_2	23.3	1.95, nd	22.8	1.94, nd
12	CH	122.7	5.57	122.7	5.57
13	C	143.0		142.7	
14	C	41.2		41.5	
15	CH_2	35.1	1.91, 2.09	34.8	1.90, 2.11
16	CH	73.1	5.07	73	5.08
17	C	51.4		51.2	
18	CH	40.3	3.27	41.3	3.38
19	CH_2	47.3	1.33, 2.82	47.3	1.30
20	C	35.2		34.8	
21	CH	76.5	6.09	76.1	6.10
22	CH_2	35.7	2.13, 2.68		
23	CH_3	27.5	1.08	27.5	1.13
24	CH_3	16.5	0.88	16.7	1.06
25	CH_3	15.2	0.82	15.3	0.87
26	CH_3	16.8	1.02	16.9	1.02
27	CH_3	26.7	1.73	26.7	1.73
28	COOH	174.4		174.5	
29	CH_3	28.6	0.88	28.7	0.85
30	CH_3	18.7	1.02	18.7	1.03

[a] Multiplicities were assigned from DEPT spectra. [b] Assignments were based on the HMBC, HSQC, and DEPT experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR). [c] nd: not determined.

Since triterpene saponins from plants have been reported to exert immunostimulatory activities,[11,13] the CSM and compounds **1**−**4** were tested in an in vitro lymphocyte proliferation assay.[14] The cellular proliferation was measured by ^{3}H-thymidine incorporation in Jurkat tumor cell lines (human T cell leukemia). Compound **2** showed a concentration-dependent immunomodulatory effect (Figure 1). At a concentration of 10^{-2} μM, compound **2** exerted a weak but significant lymphoproliferative activity with a stimulation index (SI) of 1.21, and at 1 μM it showed an inhibition of this effect. Furthermore, **1** and the CSM displayed a cytotoxic effect to lymphocytes in culture (from 1 μM for **1**). Compound **3** showed a weak cytotoxic effect from 5 μM, whereas **4** exhibited a lymphoproliferative effect in the concentration range 1−10 μM and no cytotoxic effect at all tested concentrations.

In addition, the hemolytic activities of the CSM and compounds **1**−**4** were evaluated on sheep erythrocytes according to a procedure adapted from the litterature.[15] Strong hemolytic activity is considered to be a typical characteristic of saponins, although some saponins show only weak or no hemolytic effect at all. This activity is often

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

	1			**2**			**1**		**2**	
position	δ_H	δ_C		δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
3-*O*-sugar						28-*O*-sugar				
Glc_1NAc-1	*4.91* (d, *J* = 7.5 Hz)	*103.8*	Glc_1-1	*4.72* (d, *J* = 7.5 Hz)	*104.2*	Glc′$_1$-1	*5.83* (d, *J* =7.5 Hz)	*94.9*	*5.85* (d, *J* = 7.5 Hz)	*94.8*
2	*4.40*	*56.8*	2	4.17	80.3	2	*3.90*	*76.9*	*3.89*	*75.5*
3	4.30	74.5	3	3.88	77.9	3	3.83	78.1	3.87	77.0
4	3.93	73.1	4	3.91	71.0	4	3.91	70.5	3.92	70.6
5	3.90	75.0	5	4.04	77.1	5	3.84	78.9	3.84	78.2
6	*4.19*	*69.0*	6	*4.20*	*68.7*	6	4.00	61.2	4.00	61.6
	4.50			*4.49*			4.23		4.21	
N*HC*OCH$_3$	8.91 (d, *J* = 8.8 Hz)	171.9								
NHCO*CH*$_3$	2.13	22.9								
Fuc-1	*4.79* (d, *J* = 8.0 Hz)	*102.5*	Glc_2-1	*5.30* (d, *J* = 7.5 Hz)	*103.8*	Rha-1	*5.77* (*brs*)	*101.2*	*5.72 (brs)*	*101.3*
2	*4.25*	*80.8*	2	3.92	76.3	2	4.98	69.8	4.99	69.9
3	*3.90*	74.5	3	3.83	78.1	3	*4.65*	*81.2*	*4.69*	*81.2*
4	3.92	71.4	4	4.06	70.1	4	*4.33*	*77.6*	*4.33*	*77.7*
5	3.65	70.7	5	4.07	77.1	5	4.32	68.6	4.34	68.7
6	1.37	16.4	6	4.08	61.2	6	1.65	18.2	1.66	18.4
				4.30						
Xyl-1	*4.90* (d, *J* = 7.8 Hz)	*105.6*	Fuc-1	*4.80* (d, *J* = 8.0 Hz)	*102.5*	Ara$_f$-1	*6.06* (brs)	*109.8*	*6.09* (brs)	*109.8*
2	4.01	74.1	2	*4.26*	*80.9*	2	4.79	83.1	4.80	83.2
3	4.03	77.1	3	4.08	74.1	3	4.58	77.4	4.55	77.6
4	4.08	70.7	4	3.98	73.0	4	4.56	84.4	4.56	84.4
5	3.48	66.2	5	3.66	71.0	5	4.10	61.4	4.10	61.7
	4.33		6	1.37	16.4		4.32		4.35	
			Xyl-1	*4.90* (d, *J* = 7.8 Hz)	*105.6*	Glc′$_2$-1	*5.10* (d, *J* = 7.5 Hz)	*104.7*	*5.10* (d, *J* = 7.5 Hz)	*104.7*
			2	3.89	74.4	2	3.89	74.5	3.90	74.4
			3	4.03	77.3	3	4.32	79.9	4.30	79.3
			4	4.05	69.8	4	4.34	70.3	4.10	70.6
			5	3.50	66.1	5	3.81	77.4	4.05	77.1
				4.16		6	4.05	61.3	4.07	61.5
							4.30		4.33	
						21-*O*- acyl				
						1		110.1		110.2
						2		151.7		151.8
						3	6.62	115.4	6.62	115.4
						4	7.24	134.2	7.25	134.1
						5	7.03	117.0	7.04	116.8
						6	7.83	130.9	7.86	131.0
						7		167.9		167.9

[a] Assignments were based on COSY, TOCSY, NOESY, HSQC, and HMBC experiments (150 MHz for ^{13}C and 600 MHz for ^{1}H NMR). Multiplicities were assigned from DEPT spectra. Coupling constants in Hz. Overlapped signals are reported without designated multiplicities. [b] ^{1}H and ^{13}C chemical shifts of substituted residues are italicized.

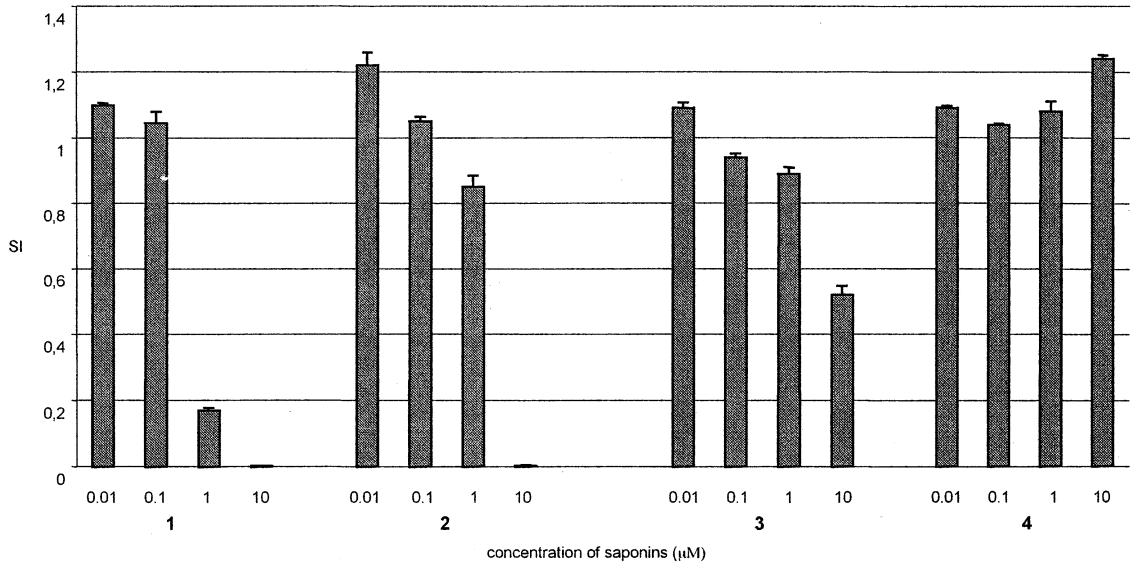


Figure 1. Effect of compounds **1**−**4** on the cellular proliferation of Jurkat cells measured by ^{3}H-thymidine incorporation (see Experimental Section). SI = stimulation index. Each column represents the mean ± SE (*n* = 4).

related to the amphiphilic character of the saponin and is influenced by the affinity of the aglycon to cholesterol in cell membranes, allowing the formation of pores. In this test, since the CSM was shown to exhibit a good hemolytic activity (HC_{50} = 12 μg/mL), we have tested the pure compounds. Among them, **1** exhibited the highest hemolytic activity (HD_{50} = 17.5 μg/mL), followed by **2**, which was less active than **1** (HD_{50} = 48 μg/mL), whereas **3** and **4** were not hemolytic at the concentration tested (HD_{50} > 400 μg/mL). These data suggested the importance of the *N*-acetamido group in **1** for the hemolytic activity compared with **2**. The results of this above test correlated well with

those of the cytotoxicity observed for **1** and **2** against Jurkat cells in the concentration range 1−10 μM. Moreover, concerning **3**, which is not hemolytic up to 400 μg/mL (282 μM) but cytotoxic from 10 μM, it could be thought that another mechanism than the membrane effect is at the origin of the cytotoxicity. Since avicins (closely related to **3**) were shown to induce inhibition of the Jurkat cell proliferation by induction of apoptosis,[16] we are currently investigating this possible mechanism involved in the cytotoxic effect of **3** against Jurkat T cells.

Experimental Section

General Experimental Procedures. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. IR spectra (KBr disks) were recorded on a Perkin-Elmer 281 spectrophotometer. The 1D and 2D NMR spectra (^{1}H−^{1}H COSY, TOCSY, NOESY, HSQC, and HMBC) were performed using a UNITY-600 spectrometer at the operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz for ^{1}H and 150 MHz for ^{13}C spectra). Conventional pulse sequences were used for COSY, HSQC, and HMBC. TOCSY spectra were acquired using the standard MLEV17 spin-locking sequence and 90 ms mixing time. The mixing time in the NOESY experiment was set to 500 ms. The carbon type (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 150.3, 155.9, 123.9). The fast-atom bombardment mass spectrum (FABMS) (negative-ion mode, thioglycerol matrix) was obtained on a JEOL SX 102 mass spectrometer. HRESIMS was carried out on a Q-TOF 1 micromass spectrometer. GC analysis was carried out on a Termoquest gas chromatograph using a DB-1701 capillary column (30 m × 0.25 mm, i.d.) (J & W Scientific); detection, FID; detector temperature, 250 °C; injection temperature, 230 °C; initial temperature was maintained at 80 °C for 5 min and then raised to 270 °C at the rate of 15 °C/min; carrier gas, He. TLC and HPTLC employed precoated Si gel plates 60 F_{254} (Merck). The following TLC solvent systems were used: for saponins (a) $CHCl_3$−MeOH−AcOH−H_2O, 15:8:3:2, (b) $CHCl_3$−MeOH−H_2O, 65:40:10; for sapogenins (c) toluene−Me_2CO, 4:1; for monosaccharides (d) $CHCl_3$−MeOH−H_2O, 8:5:1. Spray reagents for the saponins were Komarowsky reagent, a mixture (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and H_2SO_4 50%; for the sugars, diphenylaminephosphoric acid reagent. Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Gilson pump M 305, head pump 25 SC, manometric module M 805, Autoinjector 234, Büchi column (460 × 25 mm and 460 × 15 mm), Büchi precolumn (110 × 15 mm)].

Plant Material. The roots of *Albizia adianthifolia* (Schumach.) W. F. Wight (Mimosaceae) were collected in April 1990 at Lamto, Ivory Coast, and identified by Mr. N. Konan, Tropical Ecology Station, Lamto. A voucher specimen (No. 16-90) is deposited in the Herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Burgundy, France.

Extraction and Isolation. Dried, powdered roots (200 g) were macerated for 4 h with 3 L of 95% ethanol and further refluxed for 24 h (3 × 3 L). After cooling, the ethanolic solution was filtrated and evaporated to dryness, and the 95% ethanolic extract was obtained (20 g). This extract was suspended in H_2O (400 mL) and submitted to a partition against *n*-BuOH saturated with H_2O (3 × 400 mL). After evaporation under reduced pressure of the solvent, 7 g of a *n*-BuOH extract was obtained. This was solubilized in MeOH (10 mL) and precipitated in Et_2O (3 × 250 mL), yielding 2.5 g of a crude saponin fraction. This resulting mixture was suspended in water, dialyzed for 2 days, and lyophilized, yielding a crude saponin mixture (CSM). An aliquot (1.8 g) of this mixture was fractionated by column chromatography over Sephadex LH-20 and submitted to repeated MPLC column chromatography on Si gel 60 (15−40 μm) using as eluent $CHCl_3$−MeOH−H_2O (8:5:1 and 6:4:1), affording compounds **1** (13 mg) and **2** (14 mg).

Adianthifolioside A (1): white amorphous powder; $[\alpha]^{20}_D$ −20° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3500−3300 (OH), 2928 (CH), 1735 (C=O ester), 1718 (C=O carboxylic acid), 1570 and 1639 (CO-NH), 1580, 1260, 1090 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; long-range correlations in the HMBC spectrum used for defining the aglycon, as previously reported;[5] negative FABMS (glycerol matrix) *m/z* 1690 $[M - H]^-$, 1088 $[(M - H) - 162 - 132 - 146 - 162]^-$; HRESIMS positive mode *m/z* 1714.7490 $[M + Na]^+$ (calcd for $C_{79}H_{121}NO_{38}Na$, 1714.7465); TLC R_f 0.29 (system b), brown spot by spraying with Komarowsky reagent.

Adianthifolioside B (2): white amorphous powder; $[\alpha]^{20}_D$ −20° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3500−3300 (OH), 2927 (CH), 1735 (C=O ester), 1718 (C=O carboxylic acid), 1580, 1260, 1090 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; long-range correlations in the HMBC spectrum used for defining the aglycon, as previously reported;[5] negative FABMS (glycerol matrix) *m/z* 1811 $[M - H]^-$; HRESIMS positive mode *m/z* 1835.7700 $[M + Na]^+$ (calcd for $C_{83}H_{128}O_{43}Na$, 1835.7727); TLC R_f 0.2 (system b), brown spot by spraying with Komarowsky reagent.

Prosapogenin 3. The spectral data were in full agreement with previously published data for this compound.[5]

Prosapogenin 5. The spectral data were in full agreement with previously published data for this compound.[12]

Determination of Sugar Components. A solution of **1** and **2** (7 mg of each) in H_2O (2 mL) and 2 N aqueous CF_3-COOH (5 mL) were refluxed on a water bath for 3 h. After this period, the reaction mixture was diluted with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 extracts were washed with H_2O and then evaporated to dryness in vacuo. Evaporation of the solvent gave acacic acid lactone **6** (1.5 mg) (co-TLC with an authentic sample, solvent c). After repeated evaporations to dryness of the aqueous layer with MeOH until neutral, the sugars were analyzed by Si gel TLC by comparison with standard sugars (solvent system d); for sugars of **1** and **2**: TLC R_f(glucose) 0.30, R_f(rhamnose) 0.50, R_f(arabinose) 0.56, R_f(xylose) 0.45, and R_f(fucose) 0.49. Furthermore, the residue of sugars was dissolved in anhydrous pyridine (100 μL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60 °C for 1 h, then 150 μL of HMDS-TMCS (hexamethyldisilazane−trimethylchlorosilane, 3:1) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under a N_2 stream. The residue was partitioned between *n*-hexane and H_2O (0.1 mL each), and the hexane layer (1 μL) was analyzed by GC. D-Glucose, D-xylose, D-fucose, L-rhamnose, L-arabinose, and 2-(acetylamino)-2-deoxy-β-D-glucopyranose for **1** were detected in each case (**1** and **2**) by co-injection of the hydrolysate with standard silylated samples, giving single peaks at 18.64, 13.46, 12.14, 13.13, 11.88, and 21.55 min, respectively. In the same manner, identification of D-glucose, D-xylose, D-fucose, L-rhamnose, and L-arabinose was carried out for **2**, giving single peaks at 18.60, 13.46, 12.12, 13.13, and 11.90 min, respectively.

Alkaline Hydrolysis. Compounds **1** and **2** (5 mg of each) were refluxed with 5% aqueous KOH (10 mL) for 2 h. The reaction mixture was adjusted to pH 6 with dilute HCl and extracted successively with Et_2O (3 × 10 mL) and H_2O-saturated *n*-BuOH (3 × 10 mL). The combined Et_2O extracts were washed (H_2O). Evaporation of the Et_2O layer yielded salicylic acid **7** (1 mg) (TLC, ^{13}C NMR), identified by comparison with literature data,[7] whereas evaporation of the *n*-BuOH extract yields the prosapogenin **4** (3 mg) (TLC, ^{13}C NMR) from **1**, which was identified by comparison of its spectral data with those reported in the literature,[5] and a prosapogenin **5** (2.5 mg) from **2**, previously isolated from the alkaline hydrolysate of the crude saponin mixture of *Albizia cortex* by comparison of its spectral data with those reported in the literature.[12]

Proliferation Assay. The effect of **1**−**4** on the cell proliferation in Jurkat cells was evaluated according to a method previously described in the literature.[14]

Hemolysis Assay. The hemolytic activity of CSM and **1**−**4** was evaluated on sheep erythrocytes according to a procedure adapted from the literature.[15]

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