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Two new prosapogenins from *Albizia adianthifolia*

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Two new triterpenoidal prosapogenins **1** and **2** were obtained from the mild alkaline hydrolysate of the crude saponin fraction of *Albizia adianthifolia* (Mimosaceae) roots. Their structures were mainly determined by spectral analyses as acacic acid 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-acetylamino-2-deoxy-β-D-glucopyranoside (**1**) and acacic acid 3-*O*-{β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-{6(*S*)-2-hydroxymethyl-6-methyl-6-*O*-(β-D-quinovopyranosyl)-2,7-octadienoyl} ester (**2**). Furthermore, the known julibroside A_3 was isolated from the crude saponin mixture. Compounds **1** and **2** did not show any ability to potentiate *in vitro* cisplatin cytotoxicity in a human colon cancer cell line.

1. Introduction

Albizia adianthifolia (Schumach.) W.F. Wight (Mimosaceae) is an umbrella-like crown tree of about 25 m high, common in tropical Africa, from South Africa to Ethiopia and Senegal. The roots are used as a fish poison and in the treatment of skin diseases, inflammatory conditions of eye and the stomach, and as a remedy for intestinal parasites and for snake bite [1]. There are no reports on either phytochemical or pharmacological works on this species. The investigation of the saponins of this plant, observed as a very complex mixture of highly polar compounds, afforded a known saponin, julibroside A_3, previously isolated from *Albizia julibrissin* [2]. However, the isolation of other pure saponins was very difficult. Thus, we decided to carry out an alkaline hydrolysis on the saponin fraction, yielding prosapogenins and monoterpene glycoside components. In this paper, we describe the isolation and structure elucidation of two new prosapogenins (**1** and **2**) obtained from the mild alkaline hydrolysate of the saponin fraction, together with julibroside A_3 (**3**) from the saponin mixture. Furthermore, the potentiation of cisplatin cytotoxicity on human colon cancer cells was investigated.

2. Investigations, results and discussion

The concentrated *n*-BuOH-soluble fraction of the MeOH extract of the roots of *Albizia adianthifolia* was purified by precipitation with Et_2O. The crude saponin mixture was further dialysed and subjected to multiple chromatographic steps over Sephadex LH-20 and medium pressure liquid chromatography (MPLC) over normal Si gel and ODS (RP-18), yielding a known saponin (**3**), which was identified as julibroside A_3 by comparison of its spectral data with those reported in the literature [2]. The *n*-BuOH-soluble fraction of the mild alkaline hydrolysate of the saponin fraction was fractionated by means of MPLC over normal Si gel and ODS (RP-18), yielding two new

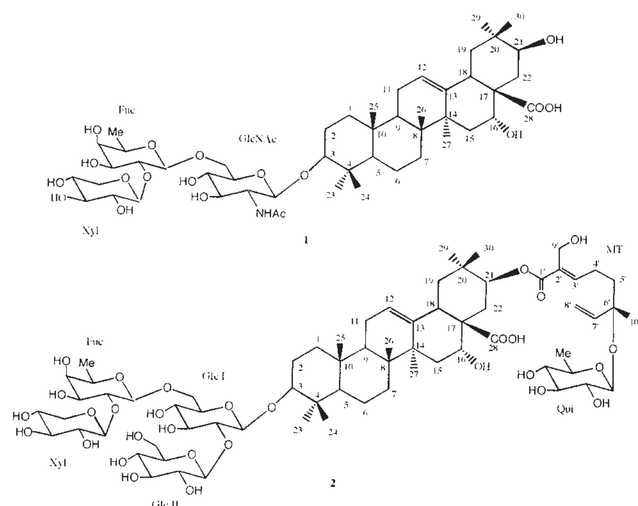

Table 1: ^{13}C NMR and ^{1}H NMR data of the aglycons of 1 and 2 (C_5D_5N)[a,b,c]

Position	DEPT	1		2	
		δ^{13}C	δ^{1}H	δ^{13}C	δ^{1}H
1	CH_2	38.8	1.12, 1.55	38.9	nd, 1.55
2	CH_2	26.8	1.88, 2.26	27.0	nd, nd
3	CH	88.8	3.46	88.8	3.45
4	C	39.5		39.9	
5	CH	56.1	0.89	56.1	0.85
6	CH_2	18.7	1.31, 1.42	18.3	nd, nd
7	CH_2	33.7	1.32, 1.60	33.7	1.28, 1.55
8	C	40.0		40.0	
9	CH	47.4	1.90	47.4	1.88
10	C	36.9		37.2	
11	CH_2	24.0	1.97, 2.07	24.0	1.40, 2.10
12	CH	123.2	5.60	122.4	5.60
13	C	145.5		145.5	
14	C	42.2		42.1	
15	CH_2	36.1	1.75, 2.40	36.1	1.73, 2.45
16	CH	73.9	4.95	74.0	4.11
17	C	52.2		52.2	
18	CH	41.4	3.72	41.4	nd
19	CH_2	48.8	1.50, 2.96	48.8	1.52, nd
20	C	36.9		35.5	
21	CH	73.9	4.10	75.0	5.30
22	CH_2	42.2	2.50, 2.80	36.8	nd, nd
23	CH_3	28.3	1.21	28.3	1.26
24	CH_3	17.3	0.98	17.0	1.09
25	CH_3	15.7	0.85	15.8	0.89
26	CH_3	17.4	0.96	17.4	1.01
27	CH_3	27.4	1.90	27.4	1.88
28	COOH	180.0		180.0	
29	CH_3	30.3	1.33	30.3	1.30
30	CH_3	18.7	1.42	18.8	1.40

[a] Multiplicities were assigned from DEPT spectra. [b] assignments were based on the HMBC, HSQC, and DEPT experiments (125 MHz for ^{13}C and 500 MHz for ^{1}H NMR). [c]nd: not determined.

triterpene glycosides **1** and **2**. Compound **1** was obtained as an amorphous powder. The negative FABMS of **1** showed a quasimolecular ion peak at *m/z* 968 $[M-H]^-$ indicating a molecular weight of 969, compatible with the molecular formula $C_{49}H_{79}NO_{18}$. Other significant ion peaks visible at *m/z* 836 $[(M-H)-132]^-$, 469 $[(M-H)-132-367]^-$, corresponded to the successive loss of one pentosyl unit, one deoxyhexosyl and one acetylamino-2-deoxy-hexosyl moieties, respectively. The ^{13}C NMR signals of the aglycon in **1** (Table 1) were almost superimposable with those of acacic acid [3], except the C-3 showing a glycosylation shift (+10 ppm) suggesting that **1** was an acacic acid-3-*O*-glycoside. Compound **1** was shown to contain three sugar residues from the HSQC spectrum. The anomeric ^1H NMR signals at δ_H 5.02 (d, $J = 7.0$ Hz), 5.01 (d, $J = 8.0$ Hz) and 4.95 (d, $J = 8.2$ Hz) gave correlations with ^{13}C NMR signals at δ_C 106.9, 104.9 and 103.5, respectively. Complete assignments of each sugar proton system were achieved by considering TOCSY and ^1H-^1H COSY spectra, while the carbons were assigned from HSQC and HMBC spectra. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one β-xylopyranosyl (Xyl), one β-fucopyranosyl (Fuc), and one acetylamino-2-deoxy-β-glucopyranosyl (GlcNAc) units, respectively. The common D-configuration for Xyl, Fuc and GlcNAc was assumed, according to those most encountered among plant glycosides in each case. The cross peaks observed in the HMBC spectrum between δ_H 5.01 (d, $J = 8.0$ Hz) (GlcNAc-1) and δ_C 88.8 (Agly C-3), between δ_H 4.95 (d, $J = 8.2$ Hz) (Fuc H-1) and δ_C 70.0 (GlcNAc C-6) as well as between δ_H 5.02 (d, $J = 7.0$ Hz) (Xyl H-1) and δ_C 82.2 (Fuc C-2) together with reverse correlations observed between δ_H 4.31 (GlcNAc H-6) and δ_C 103.5 (Fuc C-1), δ_H 4.39 (Fuc H-2) and δ_C 106.9 (Xyl C-1) allowed the sequencing of the oligosaccharidic part linked at C-3 of the aglycon.

Based on the above results, the structure of **1** was represented as acacic acid 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-acetylamino-2-deoxy-β-D-glucopyranoside, a new natural compound [2, 3, 8–13].

Compound **2** was obtained as an amorphous white powder. The negative FABMS of **2** showed a quasimolecular ion peak at *m/z* 1417 $[M-H]^-$ indicating a molecular weight of 1418, compatible with the molecular formula $C_{69}H_{110}O_{30}$. Other significant ion peaks visible at *m/z* 1089 $[(M-H)-328]^-$, 957 $[(M-H)-328-132]^-$, 811 $[(M-H)-328-132-146]^-$, 487 $[(M-H)-328-132-146-324]^-$, corresponded to the successive loss of one $C_{16}H_{24}O_7$ unit, one pentosyl , one deoxyhexosyl and two hexosyl moieties. The NMR spectral data for the aglycon moiety in **2** (Table 1) were in good agreement with those of acacic acid moiety possessing glycosyl and acyl groups at C-3 and C-21, respectively [4]. Compound **2** was shown to contain five sugar residues from the HSQC spectrum. The anomeric ^1H NMR signals at δ_H 5.39 (d, $J = 7.5$ Hz), 5.03 (d, $J = 7.1$ Hz), 4.95 (d, $J = 8.2$ Hz), 4.85 (d, $J = 7.6$ Hz) and 4.74 (d, $J = 7.7$ Hz) gave correlations with ^{13}C NMR signals at δ_C 106.0, 107.2, 103.6, 105.2 and 99.5, respectively. Complete assignments of each sugar proton system were achieved by considering ^1H-^1H COSY, HMQC and HMBC spectra. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one β-xylopyranosyl (Xyl), one β-fucopyranosyl (Fuc), two β-glucopyranosyl (Glc) and one β-quinovopyranosyl (Qui) units, respectively. The common D-configuration for Xyl, Fuc, Glc and Qui was assumed. The cross peaks observed in the HMBC spectrum between δ_H 4.85 (Glc I H-1) and δ_C 88.8 (Agly C-3), between δ_H 5.39 (Glc II C-1) and δ_C 82.7 (Glc I C-2), δ_H 4.95 (Fuc H-1) and δ_C 69.8 (Glc I C-6), as well as between δ_H 5.03 (Xyl H-1) and δ_C 82.4 (Fuc C-2) together with reverse correlations observed between δ_H 4.26 (Glc I H-2) and δ_C 106.0 (Glc II C-1), between δ_H 4.43 (Fuc H-2) and δ_C 107.2 (Xyl C-1), showed that the tetrasaccharide moiety 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl was linked to the C-3 hydroxyl group of acacic acid. Moreover, one group of ^1H and ^{13}C signals was very similar to those of one monoterpenyl moiety in julibroside J_2 [5], except for the resonances of ^1H and ^{13}C belonging to the aglycon and sugar moieties, indicating the presence of one unit of 6(*S*)-2-hydroxymethyl-6-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoic acid (MT) moiety in **2**. Correlations in the HMBC spectrum between δ_H 4.74 (d, $J = 7.7$ Hz) (Qui-1) and δ_C 80.0 (MT-6) showed that the quinovose unit was involved in a glycosidic linkage with the C-6 of the MT, and signals observed in the ^{13}C NMR spectrum of **2** at δ_C 75.0 (downfield shift of C-21 of the aglycon, +1.1 ppm), δ_C 35.5 and δ_C 36.8 (upfield shifts of C-20: −1.4 ppm, and C-22: −5.4 ppm, respectively) in the ^{13}C NMR spectrum of **2** showed that the MT unit must be bound to the C-21 of the aglycon by an ester linkage. Thus the moiety linked at C-21 by an ester linkage was characterized as 6(*S*)-2-hydroxymethyl-6-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoic acid. Based on the above results, the structure of compound **2** was represented as acacic acid 3-*O*-{β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-{6(*S*)-2-hydroxymethyl-6-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoyl} ester, a new natural compound [2, 3, 8–13].

The known compound **3** was identified by comparing its MS, ^1H and ^{13}C NMR data obtained from 2D NMR experiments with published data as julibroside A_3 [2].

Since triterpene saponins have been reported to potentiate the cytotoxicity of cisplatin in human colon cancer cells [6], the prosapogenins **1** and **2** were tested in an *in vitro* cytotoxicity assay according to the previously described technique [7]. No significant effect could be found in this bioassay with these two compounds.

3. Experimental

3.1. General procedures

The spectra were recorded with the following instruments. NMR: Bruker avance DRX 500 FT, 500 MHz and 125 MHz for ^1H and ^{13}C, respectively, in pyridine-d_5. FAB-MS: JEOL SX 102 (glycerol matrix). IR: Perkin Elmer 281 in KBr disc. Optical rotations: Perkin-Elmer 241 polarimeter. TLC and HPTLC employed percoated 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 (6 : 3.5 : 1, lower phase); for sapogenins (c) toluene-Me_2CO (8: 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 50% H_2SO_4; for the sugars: diphenylaminephosphoric acid reagent. Isolations: MPLC (Gilson pump M 303, head pump 25 SC, manometric module M 802, Büchi column (460 × 25 mm and 460 × 15 mm), Büchi precolumn (110 ×15 mm), Kieselgel 60, Merck (15–40 μm).

3.2. Plant material

The roots of *Albizia adianthifolia* were collected in 1990 in Lamto, Ivory Coast. A voucher specimen (No 16–90) is deposited in the Herbarium of the Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Burgundy, France.

3.3. Extraction and isolation

Dried powdered roots of *Albizia adianthifolia* (335 g) were defatted in a Soxhlet with *n*-hexane (3 × 2.5 l) and extracted successively with $CHCl_3$

Table 2: ^{1}H and ^{13}C NMR data of sugar and acid moieties of compounds 1 and 2 (C_5D_5N)^{a,b}

Position		1		Position		2	
3-*O*-GlcNAc	1	5.01 (d, $J = 8.0$ Hz)	104.9	3-*O*-Glc I	1	4.85 (d, $J = 7.6$ Hz)	105.2
	2	4.54	58.0		2	4.26	82.7
	3	4.33	75.8		3	4.21	77.1
	4	4.08	72.5		4	3.98	71.8
	5	4.01	76.8		5	3.96	77.0
	6	4.31, 4.71	70.0		6	4.30, 4.68	69.8
	$NH\underline{C}OCH_3$	8.93	170.9				
	$NHCO\underline{C}H_3$	2.21	23.8				
Fuc	1	4.95 (d, $J = 8.2$ Hz)	103.5	Glc II	1	5.39 (d, $J = 7.5$ Hz)	106.0
	2	4.39	82.2		2	4.11	75.4
	3	4.15	75.3		3	4.21	77.9
	4	4.03	72.4		4	4.19	71.5
	5	3.73	71.5		5	4.08	77.9
	6	1.46 (d, $J = 6.3$ Hz)	17.3		6	3.98, nd	62.8
Xyl	1	5.02 (d, $J = 7.0$ Hz)	106.9	Fuc	1	4.95 (d, $J = 8.2$ Hz)	103.6
	2	4.04	75.9		2	4.43	82.4
	3	4.08	77.5		3	4.01	75.2
	4	4.12	70.8		4	4.03	72.3
	5	3.55, 4.42	67.2		5	3.74	71.5
					6	1.47 (d, $J = 6.4$ Hz)	17.4
				Xyl	1	5.03 (d, $J = 7.1$ Hz)	107.2
					2	4.04	76.0
					3	4.07	77.6
					4	4.08	70.9
					5	3.58, 4.41	67.5
				21-*O*-MT	1		169.0
					2		135.5
					3	nd	144.0
					4	2.57	24.0
					5	1.78	42.3
					6		80.0
					7	nd	145.5
					8	5.12, 5.25	115.6
					9	4.65	58.0
					10	1.88	27.4
				Qui	1	4.74 (d, $J = 7.7$ Hz)	99.5
					2	4.01	75.6
					3	3.90	78.4
					4	4.07	77.3
					5	4.01	72.6
					6	1.54	19.0

^{a} ^{13}C and ^{13}H chemical shifts of substitued residues are italicized. ^{b}The assignments were based on the COSY, TOCSY, NOESY, HSQC, and HMBC experiments (125 MHz for ^{13}C and 500 MHz for ^{1}H NMR). Multiplicities were assigned from DEPT spectra. Coupling constants in Hz. Overlapped signals are reported without designated multiplicities.

(2.5 l) and MeOH (2.5 l). After removal of the solvent by evaporation, the MeOH extract was obtained (20 g). This extract was suspended in H_2O (400 ml) and submitted to a partition with *n*-BuOH saturated with H_2O (3×200 ml). After evaporation under reduced pressure of the solvent, 7 g of the *n*-BuOH extract was obtained. This *n*-BuOH extract was solubilized in MeOH (10 ml) and precipitated in Et_2O (3×250 ml) yielding 4.2 g of a crude saponin fraction of which 1 g was hydrolysed with alkali (0,5 N NaOH, 60 h at room temperature). The hydrolysate was neutralized with 10% HCl and extracted successively with EtOAc and *n*-BuOH. The *n*-BuOH layer was subjected to successive column chromatography on Si gel 60 (15–40 μm) using as eluent $CHCl_3$–MeOH–H_2O (8 : 5 : 1) and ODS (RP-18, 25–40 μm, MeOH–H_2O, linear gradient 40–80%), giving compounds **1** (8 mg) and **2** (25 mg). Furthermore, 500 mg of the crude saponin fraction was submitted to successive MPLC on Si gel 60 (15–40 μm) and ODS (RP-18) with the same eluents as above, giving compound **3** (7 mg).

3.4. Compound 1

Acacic acid 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-2-acetylamino-2-deoxy-β-D-glucopyranoside. White amorphous powder (25 mg). $[\alpha]_D^{20}$ +8.6° (MeOH, c 0.1). R_f 0.33 (system a) blue spot by spraying with Komarowsky reagent. ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; long range correlations in HMBC spectrum used for defining the aglycon, as previously reported [3]. IR (ν_{max}, KBr, cm^{-1}) 3398 (OH), 2928 (CH), 1735 (C=O ester), 1718 (C=O carboxylic acid) cm^{-1}, 1570 et 1639 (CO–NH). Negative FABMS (rel. int%) at m/z: 968 $[M-H]^-$ (37), 836 $[(M-H)-132]^-$ (7), 469 $[(M-H)-132-367]^-$ (4).

3.5. Compound 2

Acacic acid 3-*O*-{β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-21-*O*-{6(*S*)-2-hydroxymethyl-6-methyl-6-*O*-(β-D-quinovopyranosyl)-2,7-octadienoyl} ester. White amorphous powder (8 mg). $[\alpha]_D^{20}$ −7.0° (MeOH, c 0.1). R_f 0.23 (system b) blue spot by spraying with Komarowsky reagent. IR (ν_{max}, KBR, cm^{-1}) 3400 (OH), 2930 (CH), 1735 (C=O ester), 1718 (C=O carboxylic acid). ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1, 2 and 3; long range correlations in HMBC spectrum used for defining the aglycon, as previously reported [3]. Negative FABMS (rel. int%) at m/z: 1417 $[M-H]^-$ (3), 1089 $[(M-H)-328]^-$ (28), 957 $[(M-H)-328-132]^-$ (5), 811 $[(M-H)-328-132-146]^-$ (4), 487 $[(M-H)-328-132-146-324]^-$ (4).

3.6. Compound 3

Acacic acid lactone 3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-fucopyranosyl-(1 → 6)-2-acetylamino-2-deoxy-β-D-glucopyranoside. The spectral data were in full agreement with previously published data [2].

3.7. Acid hydrolysis

A solution of each compound (3 mg) in 2N aqueous CF_3COOH (5 ml) was 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 (co-TLC with an authentic sample, solvent c). After repeated evaporations to dryness of the aqueous layer with MeOH until neutral, the sugars were analysed by Si gel TLC by comparison with standard sugars (solvent system d).

3.8. Mild alkaline hydrolysis

The crude saponin fraction (1 g) was hydrolyzed with 0,5N NaOH (40 ml) in MeOH (5 ml) for 60 h at room temperature. The mixture was neutralized with 10% HCl and extracted successively with EtOAc and *n*-BuOH. The EtOAc extract gave a mixture of monoterpene glycosides. The *n*-BuOH residue (600 mg) was subjected to successive MPLC column chromatography on Si gel (15–40 μm) using as eluent $CHCl_3$–MeOH–H_2O (8 : 5 : 1 and 6.5 : 4 : 1) and ODS (RP-18, 25–40 μm, MeOH–H_2O, linear gradient 40–80%), giving compounds **1** (8 mg) and **2** (25 mg).

3.9. Bioassay

The potentiation of the in vitro cisplatin cytotoxicity in human colon cancer cell line was evaluated according to the method of Assem et al. [7].

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